ANNUAL REPORT,
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE,
Division of Cancer Treatment
Volume I

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1981 through September 30, 1982

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ANNUAL REPORT

DIVISION OF CANCER TREATMENT

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

Personnel and Organization

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

A. Office of the Director

1. Dr. Jane Henney left to become Deputy Director, NCI.

2. Dr. Arthur Levine transferred from the Clinical Oncology Program to become Assistant Director for Science.

3. Dr. Abraham Goldin retired from the federal service. His responsibilities related to international activities were assumed by Dr. Levine.

B. Clinical Oncology Program (COP)

1. Dr. Samuel Broder replaced Dr. Arthur Levine as Acting Associate Director.

2. Dr. Philip Pizzo was appointed Chief of the Pediatric Oncology Branch.

C. Baltimore Cancer Research Program (BCRP)

The phase-out of the BCRP as an intramural program was completed during Fiscal Year 1982.

D. Cancer Therapy Evaluation Program (CTEP)

1. Dr. John Macdonald, Associate Director, left to enter private practice.

2. Dr. Daniel Kisner served as Acting Associate Director until July 1982, when he left for the University of Texas, San Antonio.

3. Dr. William DeWys was appointed Chief of the Clinical Investigations Branch.

4. The Radiotherapy Development Branch was transferred to the new Radiation Research Program.

5. The Biologics Evaluation Branch became a Section within the Investigational Drug Branch.

E. Radiation Research Program (RRP)

1. The Radiation Research Program was formally approved on May 17, 1982, with Dr. David Pistenmaa as Associate Director.

2. The Program consists of three Branches: Radiotherapy Development Branch (Dr. Pistenmaa, Acting Chief), transferred from CTEP; the Diagnostic Imaging Research Branch (Mr. Roger Powell, Acting Chief); and the Low-Level Radiation Effects Branch (Dr. Murray, Acting Chief), transferred from OD, NCI.

F. Developmental Therapeutics Program (DTP)

1. The Laboratory of Experimental Therapeutics and Metabolism was created and is headed by Dr. Michael Boyd (formerly in COP).

2. Dr. Richard Adamson left the Laboratory of Chemical Pharmacology to become Director, Division of Cancer Cause and Prevention. Dr. Richard Cysyk is serving as Acting Chief.

3. Dr. John Douros, Chief of the Natural Products Branch, left to enter private industry. Dr. Matthew Suffness is serving as Acting Chief.

4. Dr. Michael Lowe, Acting Chief, Toxicology Branch, has returned to laboratory activities. Search for a Branch Chief is underway.

5. Dr. Nicholas Bachur transferred to the Developmental Therapeutics Program from the Baltimore Cancer Research Program.

G. Biological Response Modifiers Program (BRMP)

1. The Laboratory of Immunodiagnosis (DCBD), headed by Dr. Ronald Herberman, moved to Frederick and was merged with the Biological Development Branch to form the new Biological and Research Therapy Branch, under Dr. Herberman.

2. Dr. Richard Smalley was appointed Chief of the Biological Resources Branch, replacing Dr. John Martin.

Program Highlights

More detailed reports of the individual program areas within DCT will be presented later in this report. The following summaries describe selected highlights and activities of each program during fiscal year 1982. In addition, activities within the Office of the Director will be briefly summarized.

Office of the Director

International Treatment Research

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

The U.S.-French Agreement has been particularly active, with 18 joint projects underway in the areas of clinical trials, clinical pharmacology, biological response modifiers, radiation therapy, and clinical epidemiology. Within the U.S.-Japan Agreement, major emphasis has been on the exchange of drug information, including both synthetic compounds and antibiotics. Japanese investigators now regularly participate in all NCI Phase I-II working group meetings, and a collaborative clinical trial in carcinoma of the stomach has been completed. Under the U.S.-Egypt Agreement, a Phase II trial in bladder cancer was recently completed, involving 12 drugs studied in about 400 patients. Several of the drugs demonstrated significant response rates in this disease. A new surgical adjuvant study is being initiated using the active drugs.

Scientific Information Branch (SIB)

The Scientific Information Branch consists of two Sections--Publications and Literature Research. The Publications Section is concerned primarily with publishing the journal, Cancer Treatment Reports (CTR). Although experiencing backlog problems previously, the journal has been published on schedule since mid-1981. During the past year a new section was added to the journal, presenting invited papers on current controversies in cancer management. Another important segment of the journal over the years has been the publication of important symposia, many held under DCT auspices. Although efforts to establish a separate symposium series have not been successful, several symposia have been published recently through other means.

The Literature Research Section continues to provide bibliographic services to the DCT, particularly in relation to drugs being developed for clinical trial. During the past year, the Section filled about 170 requests for information, including 37 bibliographies for five Decision Network meetings.

Developmental Therapeutics Program

This Program, which is managed by seven branches, has an extensive extramural component which is devoted to acquisition, antitumor evaluation, formulation, large-scale production, and toxicology studies of new antitumor drugs. In addition, the DTP has an intramural component of five laboratories which investigate basic mechanisms of tumor growth and its control by drugs.

During the past year, refinements have continued in the drug screening program so as to place it on a more rational footing; prescreening selection is now carried out on the basis of unique chemical structure and computerized analysis of possible reactive groups. Approximately 12,000 synthetic compounds of new structural types were acquired for antitumor screening, and about 7% of these new compounds were found to have reproducible P388 pre-screen activity. With regard to natural products, over 10,000 cultures were fermented with 120 *in vivo* active cultures discovered.

During the past year, almost 19,000 compounds were screened for the first time *in vivo*, and an analysis of the results for 1,100 compounds tested in all 8 tumor panel models was completed. Three models (L1210, B16 and the mammary xenograft) predicted 94% of the actives found using all eight panel tumors. Human tumor xenografts selected 26 compounds which had failed all of the mouse screens. A number of tumor panel modifications are planned based on this analysis.

Further progress was made in the development of the human tumor clonogenic assay as a screening tool, and a number of variables critical to scaling-up the procedure were determined, including elimination of tumor clumps in the counting procedure and establishment of appropriate pharmacological conditions.

With regard to Decision Network results, 11 new compounds passed the DN2 stage, five DN2B, eight DN3, 3 DN4, and 2 (uridine and sangivamycin) passed the DN "special" category. The structure was determined for Fredericamycin, a new and novel compound which is very active in the human tumor clonogenic assay. Two new natural products, homoharringtonine (plant) and echinomycin (fermentation), and five other compounds were entered in Phase I clinical trials.

Toxicology reports were completed, using the new toxicology protocol, for homoharringtonine, dihydro-5-azacytidine, Henkel compound, echinomycin and N-methylformamide. In all Phase I studies evaluated thus far, the new protocol has predicted a safe starting clinical dose. A study of the mouse as a possible subject for preclinical studies of the toxicity of anticancer drugs showed that the mouse predicts qualitative toxicities about equally as well as larger species if the sample time intervals are appropriately chosen.

Finally, the newly established Information Technology Branch (in the extramural program of DTP) has designed a modern drug information system which encompasses all of the critical current capabilities while greatly improving them.

In the intramural DTP program, attempts are underway to improve the cellular uptake of radiolabeled liposome-entrapped drugs into lymphocytes by using liposomes to which monoclonal antibodies are coupled. Where specific surface antigens are expressed on lymphocytes and liposomecoupled monoclonal antibody is employed, 50% of the I^{125} activity is demonstrable within the lymph nodes of C57 mice so treated, as opposed to 2% in the absence of specific antigens. Liposome studies were also undertaken with radiolabeled Ara-C, and clearance of Ara-C from the plasma was found to be much slower for liposome-entrapped drug than for the free drug. Moreover, tissue concentrations of Ara-C were higher in the spleen, liver, and tumors of monkeys receiving the liposome-entrapped drug than in corresponding tissues of animals given free Ara-C.

Anthracycline-induced cardiotoxicity continues to be the subject of intensive study, and a protein was isolated from rat heart cells that appears to be an intracellular receptor for anthracyclines. Identification of this high molecular weight, high affinity mitochondrial protein should provide further insight into the mechanism of cardiotoxicity of these drugs. Another major area of interest is the biochemical toxicology of the lung, with particular regard to alkylating agents which are carcinogenic as well as toxic. Using the furan derivative 4-ipomeanol, it was demonstrated that these agents do not cause lung toxicity in species devoid of the cytochrome P-450 system within the lung's Clara cells. Moreover, species which lack the Clara cell P-450 system only rarely develop lung tumors. Other studies with 4-ipomeanol have demonstrated the potential importance of pulmonary glutathione as a protective factor against lung damage by alkylating agents. BCNU and bleomycin, two antineoplastic agents responsible for most drug-induced lung disease in cancer patients, cause a marked depletion of this substance, suggesting its protective role. Toxicology studies were also initiated with animal models for drug-induced renal injury, and it was shown that Me-CCNU renal damage in rodents produces marked changes in certain easily measured biochemical parameters that closely parallel histopathological changes. These biochemical markers may lead to improved approaches for predicting clinical toxicity.

Several compounds which originated in the DTP intramural program are in various stages of clinical development, including AZQ, BZQ, spirohydantoin mustard, dihydro-5-azacytidine, and Ara-AC, a molecular hybrid between Ara-C and 5-aza-cytidine. The latter compound is active against all three xenografts in the DCT tumor panel. Progress continues to be made on the preparation of cytidine deaminase inhibitors for potential use in combination with the antitumor agents Ara-C and 5-azacytidine, whose clinical utility is presently limited by enzyme-catalyzed deamination *in vivo*. A new member of this inhibitor series (NSC-322096), when combined with Ara-C, is ten times more potent than the most active compound previously described (tetrahydrouridine). In studies of interferon, it was shown that the interferon-mediated induction of (2',5') oligo(A) synthetase, an enzyme which is unique to interferon-treated cells and is associated with antiviral activity, is related to the growth inhibitory properties of this agent as well. Finally, a tubulin-dependent assay was developed for a promising new drug, taxol. The assay is sensitive to 0.1 μM and is suitable for clinical pharmacokinetic studies.

In molecular pharmacology studies, it was shown that a particular DNA repair defect affecting the removal of O^6 alkyl-guanine lesions confers cellular sensitivity to chloroethylnitrosoureas. The expression of this enzyme-dependent defect was quite variable among a number of human glioblastoma cell lines examined. These differences in sensitivity appear to derive from differences in the ability to repair DNA-chloroethyl monoadducts prior to their converson to interstrand crosslinks. Interestingly, the potentials for crosslinking by cis-platinum II and by chloroethylnitrosourea were independent of each other. The possibility of employing these molecular events to predict clinical response is now being tested. In another study, a new type of DNA alteration was found in mammalian cells treated with DNA intercalating agents, consisting of characteristic protein-associated strand breaks. In the course of these studies, a previously undescribed uptake phenomenon was discovered and characterized for m-AMSA, and it was found that this drug behaves pharmacodynamically in a manner associated with some antimetabolites rather than with DNA-damaging agents. In accord with this idea, a clinical protocol has been designed in which m-AMSA is delivered as a continuous infusion.

In studies of human leukemia biology, T-cell growth factor (TCGF) was purified to homogeneity and a number of new neoplastic human T-cell lines were developed using this factor. The factor has been essential for stimulating the growth of lines which produce the newly isolated human T-cell leukemia virus (HTLV), 13 isolates of which have now been obtained from 8 different areas of the world. All isolates have been from adult T-cell leukemia or lymphoma patients. Antibodies to HTLV are found in the sera of a large number of T-cell leukemia/lymphoma patients and in some healthy normals, and the virus appears to be endemic in Japan, the West Indies, the southeastern United States, China, Alaska, certain regions of South America, and possibly in Israel. Molecular cloning of HTLV has been achieved, and it has also been found that the virus can directly transform human mature cord blood T cells, which then produce tumors in nude mice. ELISA assays for HTLV antibodies and antigens have been developed suitable for routine clinical laboratory testing. Using these assays, it was found that 1% of normal blood bank donors are HTLV positive.

In other human leukemia studies, four human onc genes were cloned and the first example of an amplified onc gene in man has been found, i.e., the myc gene in the human promyelocytic leukemia line, HL-60. Using the DNA transfection method, it was established that DNA from some fresh human lymphoid tumors can transform NIH 3T3 cells. Finally, myeloid cells and monocytes from normal donors have been successfully grown in long-term culture, and a permanent normal human monocyte cell line has been established. Using these lines as well as HL-60, it was found that retinoic acid, cAMP, and differentiation inducing factor (DIF), produced by T-cells, act synergistically in inducing terminal differentiation.

Cancer Therapy Evaluation Program

This Program is responsible for administering and evaluating the extramural clinical trials supported by the DCT. Its scope of activity includes clinical evaluation of new anticancer agents and the coordination of extramural clinical trials performed by the Clinical Cooperative Groups.

A clinical trials program has been set up in conjunction with the BRMP and Phase II trials for evaluating thymosin, pyran copolymer (MVE-2), and interferon have been initiated. A separate Phase I trial program for pediatric patients has been initiated as a result of new evidence that children in general show considerably enhanced tolerance to drugs.

Several surgical planning grant proposals have been developed and the initial support for these Surgical Oncology Programs is being provided. Several Regional Clinical Trial Groups have been established, and a transfer of all group funding to the Cooperative Agreement mechanism has occurred. Two chemoprevention programs in cervical and skin cancer are continuing. In the Cooperative Groups, new Phase II studies were initiated in breast and ovarian carcinoma. An RFA was issued for the purpose of studying patients with the Kaposi's sarcoma/acquired immunodeficiency/opportunistic infection syndrome. This RFA will also be carried out by the Cooperative Agreement mechanism, and will include studies of etiology, epidemiology, and treatment. NCI's distribution of THC under its Group C program continues, and more than 12,000 patients have already received this antiemetic.

The CTEP is expanding its efforts in the monitoring of clinical trials, and has now organized a program of periodic site visits to each institution performing trials on investigational drugs for the purpose of verifying the accuracy of the

clinical data. Progress was also made toward the implementation of an information system for CTEP, and the system is now expected to become operational in FY83. This system will offer a computerized information resource with which to develop forward planning in specific therapeutic areas.

New drugs introduced into Phase I clinical trials during the current year include tricyclic nucleoside, homoharringtonine, Henkel compound, CBDCA, echinomycin, N-methylformamide, and dihydroazacytidine. Two new drugs entered the commercial market and are now generally available for the treatment of cancer patients, streptozotocin and ifosphamide.

In addition to the 7 new investigational drugs introduced into Phase I clinical trials during the past year, 120 Phase II clinical trials were conducted under sponsorship of this program. Activity has been defined for the following drugs: mitoxantrone - breast, leukemias, lymphomas, hepatomas; AZQ - primary and secondary brain tumors; bisantrene - breast; Methyl-G - head and neck malignancy, lymphoma; 2'DCF - T-cell lymphomas and leukemias.

During this past year, more than 25,000 patients were entered into research clinical trials. Added to the 46,000 patients in followup from previous years, the CTEP followed more than 71,000 patients during the current fiscal year. Moreover, the CTEP continues to be responsible for providing input for treatment research activities of the DCT which involve International Agreements. The CTEP functions specifically as the project officer on a major contract with the Pan American Health Organization for cancer clinical trials, a contract which now supports approximately 20 active protocols.

Among the significant results of clinical trials or preclinical feasibility studies supported by CTEP in the past year are the following: It was demonstrated that an extramedullary tumor in mice induces a microenvironmental lesion in the bone marrow that inhibits blood cell production, and that this marrow failure is mediated by prostaglandin-E. Recognition of this mechanism may permit the design of less myelosuppressive chemotherapy regimens. In a clinical feasibility trial, a totally implanted hepatic arterial drug delivery system was developed, which, when combined with nuclide angiography for correct catheter placement, represents an extremely reliable method for producing a high response rate in treating hepatic metastases from colorectal cancer or carcinoid tumors. In nutrition studies, it was found that cancer patients deviate significantly from the normal metabolic response following protein ingestion. Moreover, cancer patients were found to be hypermetabolic when evaluated by indirect calorimetry.

The Gastrointestinal Tumor Study Group continues to demonstrate significant benefit from adjuvant therapy in the treatment of rectal (adjuvant radiation and chemotherapy) and gastric (adjuvant chemotherapy) carcinomas, and in the National Surgical Adjuvant Breast and Bowel Project, it was found that Tamoxifen adds to the efficacy of the combination of L-PAM plus 5-FU in the subset of postmenopausal women with positive estrogen receptors. Moreover, the Milan Cancer Institute, with CTEP support, has shown an improved overall survival for premenopausal women treated with CMF, regardless of the number of nodes involved. They also report that 12 months of CMF is no more effective than 6 months.

In a study of immunotherapy, a survival advantage was found for patients with acute myelocytic leukemia treated with chemotherapy plus allogeneic neuraminidase-treated myeloblasts over chemotherapy alone, although additional patient accrual

is needed to obtain statistical significance. However, another immunotherapy trial, in which intrapleural BCG is administered after primary surgery for lung cancer, was negative.

Clinical Oncology Program

This Program, which is managed by seven branches, conducts clinical and laboratory investigations into the etiology, diagnosis and treatment of human cancer. Significant research advances in the past year include a clinical trial of ProMACE-MOPP induction therapy in advanced diffuse large cell lymphoma. About 62% of all patients remain continuously disease-free after therapy, contrasted with a figure of 30-35% in previous studies. In another clinical study, second malignancies were sought in patients with non-Hodgkin's lymphoma after treatment, and it was found that such malignancies - specifically acute leukemia are increased significantly in patients who require long-term therapy to control their disease ("indolent" histologies). Paradoxically, patients with very aggressive lymphomas who are cured with intensive short-term therapy are not at increased risk. In other clinical trials, a new combination consisting of cis-platinum, velban, bleomycin, and VP-16 has produced an 89% complete remission rate in patients with poor prognosis advanced non-seminomatous testicular carcinoma. The best complete response rate previously in this group of patients has been about 60%. Another promising clinical trial is the CHIPS study in patients with advanced ovarian carcinoma; CHIPS consists of initial therapy with cyclophosphamide and hexamethylmelamine followed by total abdominal irradiation, intraperitoneal misonidazole, and systemic cis-platinum. The results, while preliminary, appear to represent an advance, and toxicity has so far been minimal. A clinical trial in patients with limited stage small cell lung cancer randomly compares combination chemotherapy with or without simultaneous chest radiotherapy; preliminary results suggest the benefit of the combined modality therapy. However, analysis of a very intensive regimen in small cell patients with extensive disease, in which combination chemotherapy is followed by high-dose chemo-radiotherapy and autologous bone marrow support, so far suggests that the benefits of intensification are small.

In a childhood acute lymphocytic leukemia study, the efficacy of a high-dose protracted intravenous methotrexate infusion has been compared with conventional cranial irradiation plus intrathecal methotrexate to achieve central nervous system prophylaxis. While it appears that the two arms are equivalent with respect to CNS prophylaxis, it has been found that the high-dose methotrexate arm may be very effective at inducing prolonged marrow remission. With 139 patients on the study, these results so far appear to be the best yet achieved in the management of high risk ALL. In another pediatric tumor, metastatic Ewing's sarcoma, a regimen of intensive combination chemotherapy has been employed together with total body irradiation and autologous bone marrow reconstitution. About 30% of a small group of patients so treated are now surviving free of disease, a better result than any previously achieved.

In a radiation therapy clinical trial, the radiosensitizer misonidazole has been studied in carcinoma of the esophagus. In a randomized study involving 26 patients, it was found that no major benefit accompanied the use of the sensitizer over that which can be obtained with radiotherapy alone. A prospective randomized trial in the soft tissue sarcomas was completed in which it was found that adjuvant chemotherapy offers a very significant benefit with respect to remission duration beyond that of resection alone. Limited surgery plus radiation was as effective as amputation in controlling sarcomas of the extremities.

One mechanism of tissue injury by chemotherapeutic agents involves the formation of free radicals; recently it has been found in laboratory studies that adriamycin forms iron chelates which are capable of catalyzing free radical injury. In other pharmacology studies, it was found that polyglutamate formation is a property of human cancer cells, that formation of the higher polyglutamates of methotrexate correlates with drug sensitivity, and that this finding appears to relate to the fact that higher polyglutamates do not efflux rapidly from cells. These results have implications for the dose and scheduling of methotrexate. With further respect to methotrexate, it was shown that methotrexate resistance in human breast and lung cancer cells is due to reduplication of the dihydrofolate reductase gene. Finally, tubulin, the major protein in the mitotic spindle, is being studied as a target for chemotherapeutic attack. A fluorescent antibody against tubulin has been produced and is being employed with the fluorescent activated cell sorter to study the cell cycle from the point of view of tubulin synthesis.

In other laboratory studies of human breast cancer, it was found that intranuclear estrogen receptors are changed over time; the "processed" receptor appears tightly bound to DNA and may be the proximate receptor form involved in gene regulation. These data provide further insight into the requirements for hormonal manipulation in breast cancer treatment. For example, it has been shown that ^{125}I 16-iodoestradiol can be used selectively to kill human breast cancer cells as a novel ligand with human estrogen receptors.

In laboratory studies of immune response regulation, it was found that antigen-specific T-cells synthesize high titers of gamma interferon upon exposure to the antigen for which they are specific. The amount of interferon produced parallels T-cell proliferation, and the affinity of the receptor for the antigen controls both the amount of proliferation and the amount of interferon production. An important laboratory study was also completed on the cytogenetics of small cell lung cancer. This study provided further evidence on the specificity of a chromosome 3 deletion marker for this tumor by demonstrating its presence in direct marrow preparations [3p(14-23)].

A new approach to the treatment of lung cancer may be immunotherapy with monoclonal antibodies; such antibodies with specificity for human lung cancer have now been produced and characterized within the Program. It was also learned that a series of APUD markers and peptide hormones specific for small cell cancer exist, and that these markers may be of great use for tumor typing, staging and for monitoring the response to therapy. Human lung cancer cells also produce peptide hormones such as bombesin which are required for their own growth in an autocrine fashion. This finding suggests another new approach to lung cancer treatment, i.e., hormonal manipulation.

The human clonogenic tumor stem cell assay has been modified so as to measure tritiated thymidine incorporation as an alternative to colony counting. With this new modification, it has been found that the drug sensitivity of human tumor cells from primary explants can be determined in 5 days as compared to 2-3 weeks with the usual counting assay.

Finally, attempts to develop cytotoxic T-cell clones have been successful, and it is planned to explore the use of expanded clones of such cells in treating certain tumors.

Radiation Research Program

This Program was formally launched in May, 1982, and includes the Radiotherapy Development Branch, the Diagnostic Imaging Research Branch, and the Low-Level Radiation Effects Branch. Initial efforts are directed toward staffing, and initiating the development, administration and evaluation of an extramural radiation research program. The RRP provides a focal point on radiation research within NIH for extramural investigators nationally and internationally.

The initial areas being supported by this new program include the further development and clinical evaluation of nuclear magnetic resonance imaging, the development of monoclonal antibodies coupled with radio-emitters in diagnostic imaging and tumor therapy, low LET treatment planning, the further development of interstitial radiotherapy, improvements in non-ionizing diagnostic imaging techniques, and studies on radiation carcinogenesis.

An evaluation of the emerging technology of nuclear magnetic resonance imaging is being accomplished by comparing the clinical application of NMR imaging with the results of examinations using other modalities such as ultrasound, positron emission tomography, and CAT scanning.

Ongoing studies of the effects of exposure to low doses of ionizing radiation are concerned with biological responses at low dose rates when exposures are protracted, and extrapolation of data from experimental animals to man. Other studies are concerned with the genetic and late somatic effects resulting from long-term ingestion of low concentrations of tritiated water, using the mouse as a model. A clinical study of particular interest is a followup of patients who had I^{131} Iodine and other diagnostic procedures during childhood and adolescence; the goal of the study is to determine if there is an increased risk of thyroid neoplasia in such people. A study in mice is concerned with the dose-response relationships and the mechanisms of neutron radiation-induced myelogenous leukemia, and a study in man of particular importance is a detailed reassessment of possible long-term effects of radioactive fallout resulting from atmospheric weapons testing at the Nevada Test Site between 1950 and 1962.

In the area of radiotherapy development, studies are concerned with the identification of new and/or improved radiosensitizers and radioprotectors, and with their screening in cell and animal models. With regard to equipment development, the Clinical Neutron Therapy Program is well underway with construction of a facility at Fox Chase Cancer Center completed in October, 1981, and the treatment of patients to begin there in August, 1982. UCLA's facility should be completed in November 1982, and patient treatment should begin there in late 1983. The facility at the University of Washington will be completed this winter, and patient treatment will start there in Spring of 1983.

Other studies being initiated currently include an evaluation of hyperthermia equipment and its clinical results, an investigation of the role of intraoperative radiotherapy in the treatment of intraabdominal malignancies, and an evaluation of treatment planning for particle beam radiotherapy utilizing state-of-the-art imaging and computer treatment planning systems. Studies will also be initiated for dose calculations using radioactively labelled monoclonal antibodies; low LET treatment planning; and the use of interstitial irradiation alone or in conjunction with external beam radiotherapy or hyperthermia.

Biologic Response Modifiers Program

The BRMP, in its extramural and intramural components, develops and brings to clinical trial potential therapeutic agents which may alter the biological responses important in the control of tumor growth and metastases. The agents investigated include immunomodulating and immunorestorative agents, interferons, lymphokines, antigrowth factors, thymic factors, antitumor antibodies, antitumor cells, and differentiation factors. The staffing reached full strength, and the intramural BRMP was merged with the NCI's Laboratory of Immunodiagnosis (Dr. R. Herberman).

In the intramural program, early clinical trials of highly purified recombinant and non-recombinant alpha interferon were completed. In escalating dose studies, it was shown that recombinant leukocyte A interferon could be administered safely in doses up to 118 x 10^6 units three times weekly. The toxicities resemble those previously reported for non-recombinant leukocyte interferon, suggesting that these toxicities are a direct result of the interferon molecule *per se*. Antitumor effect was seen in 9/81 patients (partial remissions) with non-Hodgkin's and Hodgkin's lymphoma, chronic lymphocytic leukemia, breast cancer and melanoma. Most patients had no change or a decrease in their natural killer cell mediated cytotoxic activity, and lymphocyte blastogenesis was uniformly decreased. However, approximately 80% of the patients demonstrated increased monocyte function. In a clinical trial of lymphoblastoid interferon, the toxicities seen in 39 patients were similar to those described for recombinant leukocyte A interferon. Antitumor effects (partial responses) were seen in three patients. Clinical trials of thymosin are nearing completion, and the Northern California Oncology Group, in a trial of thymosin fraction 5 sponsored by the BRMP, reports several partial responses in patients with renal cell carcinoma.

In laboratory studies, a murine monoclonal antibody was developed against a high molecular weight antigen expressed on the surface membrane of cells from a guinea pig hepatocellular carcinoma line. This antibody was conjugated to doxorubicin and the conjugate was toxic to 100% of the hepatoma cells *in vitro*. When the antibody was conjugated to diptheria toxin, the resulting conjugate also demonstrated dramatic inhibition of tumor growth *in vivo*. Two mouse monoclonal antibodies were developed against human non-small cell carcinoma of the lung; these antibodies react specifically with that tissue but not with appropriate controls. The antibodies are being labelled with drugs, toxins, and radioisotopes and will be employed in imaging and therapy studies. A monoclonal antibody was also developed against a high molecular weight glycoprotein expressed by human melanoma cells, and will be employed in a Phase I study in patients with this tumor.

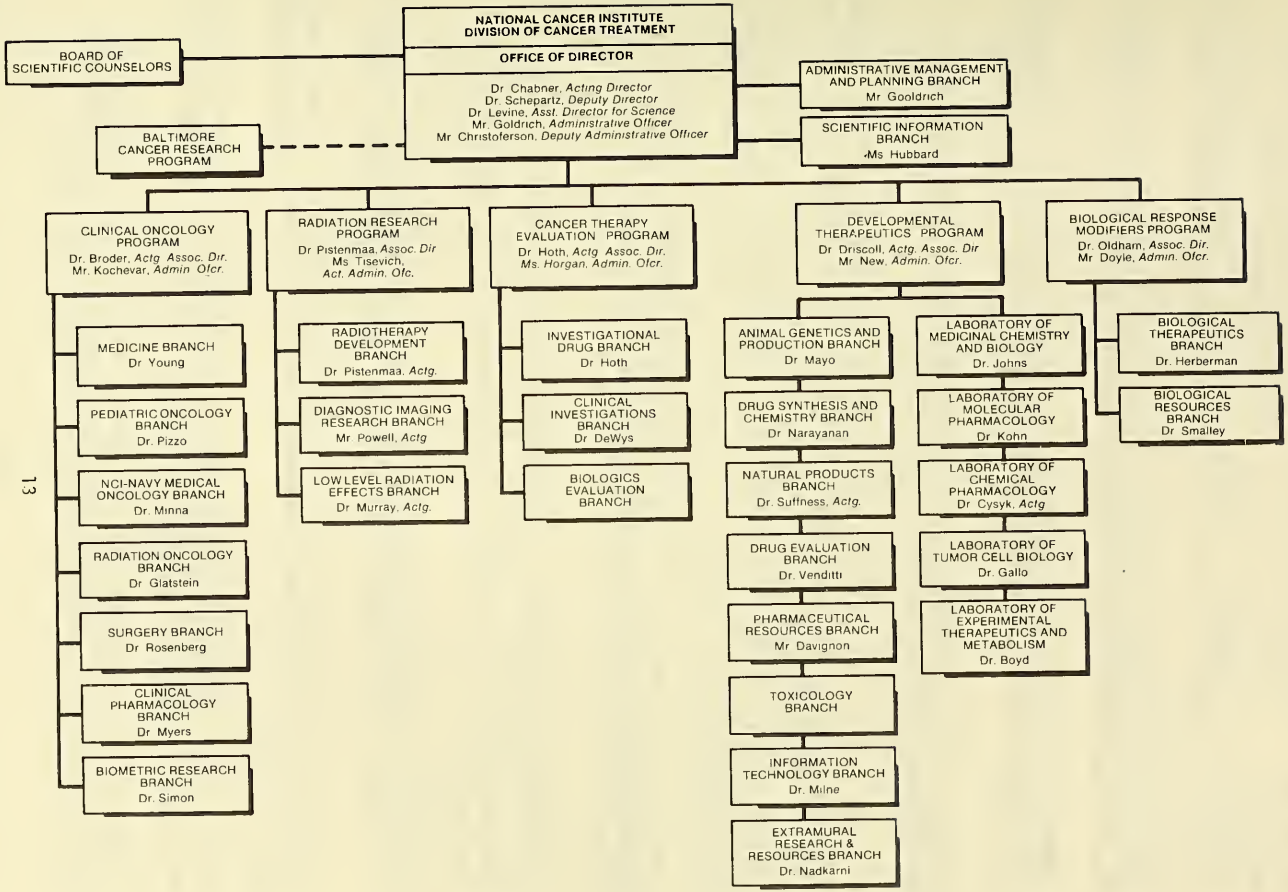
A study of the effects of lymphokines on macromolecular synthesis by macrophages has shown that activation for cytotoxicity is associated with an inhibition of macrophage RNA synthesis, suggesting that the decreased rate of RNA synthesis and the acquisition of cytotoxic activity may be causally related. The role of macrophages was also shown to be very important in *in vitro* immunization, and the effects of lymphokines on macrophages during these reactions to antigens appear to be crucial for augmentation or suppression of the antibody response. An important observation is that the human lymphoblastoid cell line RPMI-1788 constitutively produces MIF, MAF and chemotactic factors, greatly simplifying production and study of these lymphokines. It was also found that Namalva cells produce abundant quantities of Macrophage Activation Factor (MAF). MAF has the

ability to induce resting macrophages to become cytotoxic tumor cells, and therapeutic trials with this factor are anticipated. Macrophage growth factor (MGF) has also been produced within the Program, and is being employed for the in vitro propagation of cell lines and for the expansion of human monocytes for potential therapeutic use. Peptide growth factors have also been studied during the past year, and it was found that both small cell and non-small cell lung cancer cells produce soft agar growth factors. These factors are related to both the epidermal growth factor and transforming growth factor. Moreover, acid-stable growth factors of low molecular weight have been found in the urine in cancer patients and are being characterized with respect to their biological activity and their utility as a marker.

A major advance in the characterization of human NK cells has come from the finding that they are probably identical to large granular lymphocytes (LGL), and a similar association was made between mouse NK cells and LGL. It was also found that highly purified populations of LGL maintain rapid growth when cultured in the presence of interleukin 2. Moreover, LGL tumors were found in a high percentage of aged rats, and a large percentage of these leukemia-like cell lines demonstrate very high cytolytic potential. LGL had significant reactivity against most primary tumors in vitro. Twelve species of human leukocyte interferon, as well as beta and gamma interferons, were shown to augment significantly the cytotoxic reactivity of both NK cells and monocytes, and it was possible specifically to reconstitute the cytolytic function of animals treated by agents which depress NK activity by the transfer of LGL but not T-cells or monocytes. Using a mammary adenocarcinoma model, it was also found that transfer of LGL can significantly reduce the number of metastasizing lung colonies, providing the first direct evidence for a role of LGL (NK cells) in the control of metastases. It was also found, in two primary carcinogenesis models, that NK cells play an important role in resistance against initial development of tumors.

Finally, in immunopharmacology studies, it was found that the interferon inducers MVE_2 and Poly ICLC are effective adjuvants in combination with chemotherapy in treating a mouse mammary adenocarcinoma.

Figure 1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Act. Admin. Ofc.
RADIOTHERAPY DEVELOPMENT BRANCH
Dr Pistenmaa, Actg.
DIAGNOSTIC IMAGING RESEARCH BRANCH
Mr. Powell, Actg
LOW LEVEL RADIATION EFFECTS BRANCH
Dr Murray, Actg.
CANCER THERAPY EVALUATION PROGRAM
Dr Hoth, Actg Assoc. Dir.
Ms. Horgan, Admin. Ofcr.
INVESTIGATIONAL DRUG BRANCH
Dr Hoth
CLINICAL INVESTIGATIONS BRANCH
Dr DeWys
BIOLOGICS EVALUATION BRANCH
DEVELOPMENTAL THERAPEUTICS PROGRAM
Dr Driscoll, Actg. Assoc. Dir
Mr New, Admin. Ofcr.
ANIMAL GENETICS AND PRODUCTION BRANCH
Dr Mayo
DRUG SYNTHESIS AND CHEMISTRY BRANCH
Dr Narayanan
NATURAL PRODUCTS BRANCH
Dr. Suffness, Actg.
DRUG EVALUATION BRANCH
Dr. Venditti
PHARMACEUTICAL RESOURCES BRANCH
Mr Davignon
TOXICOLOGY BRANCH
INFORMATION TECHNOLOGY BRANCH
Dr. Milne
EXTRAMURAL RESEARCH & RESOURCES BRANCH
Dr. Nadkarni
LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY
Dr. Johns
LABORATORY OF MOLECULAR PHARMACOLOGY
Dr Kohn
LABORATORY OF CHEMICAL PHARMACOLOGY
Dr Cysyk, Actg
LABORATORY OF TUMOR CELL BIOLOGY
Dr. Gallo
LABORATORY OF EXPERIMENTAL THERAPEUTICS AND METABOLISM
Dr. Boyd
BIOLOGICAL RESPONSE MODIFIERS PROGRAM
Dr. Oldham, Assoc. Dir.
Mr Doyle, Admin. Ofcr.
BIOLOGICAL THERAPEUTICS BRANCH
Dr. Herberman
BIOLOGICAL RESOURCES BRANCH
Dr Smalley

Publications

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2. Chabner, B.A.: Bleomycin. In Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. W.B. Saunders Co., Philadelphia, 1982, pp. 377-386.

3. Chabner, B.A.: Clinical pharmacokinetics and drug monitoring. In Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. W.B. Saunders Co., Philadelphia, 1982, pp. 110-108.

4. Chabner, B.A.: Cytosine arabinoside. In Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. W.B. Saunders Co., Philadelphia, 1982, pp. 387-401.

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6. Chabner, B.A.: Principles of Cancer Therapy. In Wyngaarden, J.B. and Smith, L.H., Jr. (Eds.): Cecil Textbook of Medicine. W.B. Saunders Co., Philadelphia, 1982, pp. 1032-1047.

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8. Chabner, B.A.: The Role of Drugs in Cancer Treatment. In Chabner, B.A. (Ed.): Pharmacologic Principles of Cancer Treatment. W.B. Saunders Co., Philadelphia, 1982, pp. 3-14.

9. Chabner, B.A., Rustum, Y.M., and Slocum, H.K.: The role of target determinants in antineoplastic therapy. Cancer Treatment Rep. 65: 27-32, 1981.

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12. Lange, B., and Levine, A.S.: Is it ethical not to conduct a prospectively controlled trial of adjuvant chemotherapy in osteosarcoma? Cancer Treatment Rep. 66: 1982.

13. Levine, A.S.: Adjuvant Chemotherapy in Osteosarcoma: A Dissenting Point of View. In Freeman, A. (Ed.): Controversies in Pediatric Oncology. Masson Monographs in Pediatric Hematology/Oncology. (In Press)

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15. Levine, A.S.: Evolution in the Doctor/Patient Relationship: The Roots of Anxiety. In Freeman, A. (Ed.): Controversies in Pediatric Oncology. Masson Monographs in Pediatric Hematology/Oncology. (In Press)

16. Levine, A.S.: The Doctor-Patient Relationship in Oncology: Implications for Practice, Research, and Policy Planning. In Gross, S., and Garb, S. (Eds.): Humanism and Science in Cancer. Westview Press. (In Press)

17. Levine, A.S.: The Epidemic of Acquired Immune Dysfunction in Homosexual Men and Its Sequelae - Opportunistic Infections, Kaposi's Sarcoma, and Other Malignancies: An Update and Interpretation. Cancer Treatment Rep. 66: 1391-1395, 1982.

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Division of Cancer Treatment Stratification

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

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

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

CT5 Statistics, Data Processing, and Other Clinical Trials Research

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

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

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

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES FOR FY82

	ANNUAL LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	33,932,094	68.28
STAGE I	9,717,956	19.55
1XX ACQUISITION OF MATERIALS		
STAGE IIA	9,533,409	19.18
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	8,692,738	17.49
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	314,221	0.63
2B2 DETAILED EVALUATION OF NEW AGENTS	5,597,167	11.26
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	2,781,350	5.60
STAGE III	5,987,991	12.05
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	3,161,243	6.36
3X2 PROD. & FORM. FOR CLINICAL TRIALS	2,826,748	5.69
PBR PRECLINICAL BASIC RESEARCH	5,510,409	11.09
PBS CELLULAR/SUBCELLULAR STUDIES	3,393,288	6.83
PTS TREATMENT STUDIES	2,117,121	4.26
CTR CLINICAL TRIALS RESEARCH	7,043,958	14.17
CT1 PHASE I CLINICAL TRIALS	894,083	1.80
CT2 PHASE II CLINICAL TRIALS	1,946,675	3.92
CT3 PHASE III CLINICAL TRIALS	897,356	1.81
CT4 PHASE IV CLINICAL TRIALS	665,874	1.34
CT5 OTHER CLINICAL TRIALS RESEARCH	2,639,970	5.31
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	2,124,813	4.28
MGT PROGRAM MANAGEMENT	1,085,249	2.18
TOTAL	49,696,523	100.00

* FY-82 FUNDING REQUIREMENTS AS OF MAY 31, 1982 FOR ABOUT 255 CONTRACTS. NOT INCLUDED ARE ABOUT 32 SCHEDULE A CONTRACTS ($5,370,210) AND 47 SCHEDULE B CONTRACTS ($10,427,132). ALSO NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS (ABOUT $4,025,000).

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y CM4000100	180,000
D.T.P.	ALABAMA, UNIVERSITY OF	N CM0735509	14,597
D.T.P.	ALABAMA, UNIVERSITY OF	N CM0735510	14,624
A.P.	ALABAMA, UNIVERSITY OF	N CP9561600B	3,000
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0734600	75,036
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM0726600	97,500
D.T.P.	BRISTOL LABORATORIES	N CM0729900	297,500
A.P.	CHARLES RIVER BREEDING LABS.	N CM1749800	37,456
A.P.	CHARLES RIVER BREEDING LABS.	N CM5059800	36,816
A.P.	CHARLES RIVER BREEDING LABS.	N CM7714100	114,600
A.P.	CHARLES RIVER BREEDING LABS.	N CM8721200	24,443
A.P.	CHARLES RIVER BREEDING LABS.	N CM9016300	37,856
A.P.	CHARLES RIVER BREEDING LABS.	N CM9722900	24,000
D.T.P.	CHEMICAL ABSTRACTS SERVICE	N CM4372200	610,000
D.T.P.	ENVIRONMENTAL PROTECTION AGENCY	Y CM2010900	37,304
A.P.	HARLAN INDUSTRIES	N CM0736200	136,903
A.P.	HARLAN INDUSTRIES	N CM5059100	29,144
A.P.	HARLAN INDUSTRIES	N CM9724200	14,000
ISDT	HAZLETON LABORATORIES, INC.	N CM2560100	112,216
ISDT	HAZLETON LABORATORIES AMERICA, INC.	N CM9721700	9,673
D.T.P.	IIT RESEARCH INSTITUTE	N CM0735903	20,183
D.T.P.	IIT RESEARCH INSTITUTE	N CM9721300	51,999
D.T.P.	IIT RESEARCH INSTITUTE	N CM9731600	135,000
R.R.P.	INSTITUTE OF CANCER RESEARCH	N CM1750200	133,320

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	1,600
A.P.	KING ANIMAL LABORATORY	N CM1749900	26,832
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM5057700	33,072
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM9724400	8,600
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N CM7716500	175,907
ISDT	LITTON BIONETICS, INC.	N CM0732600	150,000
BRMP	LITTON BIONETICS, INC.	N CM1580800	94,433
D.T.P.	LITTON BIONETICS, INC.	N C07538000D	1,245,120
BRMP	LITTON BIONETICS, INC.	N C07538000K	543,360
ISBR	LITTON BIONETICS, INC.	N C07538000M	1,231,200
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700	49,952
BRMP	MELOY LABORATORIES, INC.	N CM1575700	53,959
D.T.P.	MICHIGAN TECHNOLOGICAL UNIVERSITY	N CM0729300	32,300
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N CM5700900	159,600
A.P.	MICROBIOLOGICAL ASSOCIATES	N CM9724600	26,187
A.P.	MISSOURI, UNIVERSITY OF	N CM8715700	27,321
A.P.	MURPHY BREEDING LABS., INC.	N CM5057900A	33,758
A.P.	NORTHROP SERVICES, INC.	N CM0728600	13,817
A.P.	NORTHWESTERN UNIVERSITY	N CM1736300	12,085
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000	28,381
D.T.P.	POLYSCIENCES, INC.	N CM0730000	270,000
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM0735206	11,978
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM0735207	12,293
A.P.	SASCO, INC.	N CM9016400	22,880

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
A.P.	SIMONSEN LABORATORIES	N CM5057800	37,690
A.P.	SIMONSEN LABORATORIES	N CM7716600	179,272
A.P.	SIMONSEN LABORATORIES	N CM9724700	8,000
D.T.P.	SISA, INC.	N CM0735405	4,347
D.T.P.	SMALL BUSINESS ADMINISTRATION	N CM1740000	42,000
ISDT	SMALL BUSINESS ADMINISTRATION	N CM4371900	100,800
A.P.	SOUTHERN ANIMAL FARMS	N CM5059900	28,392
A.P.	SOUTHERN ANIMAL FARMS	N CM9724500	15,092
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM0726008	5,000
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM0726009	17,124
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	19,954
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N CM0735603	1,996
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N CM0735604	4,553
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM0735104	23,060
R.R.P.	STANFORD RESEARCH INSTITUTE	N CM1748500	248,003
D.T.P.	STARKS C.P., INC.	N CM0735704	6,362
D.T.P.	STARKS C.P., INC.	N CM0735705	2,185
D.T.P.	STARKS C.P., INC.	N CM0735708	6,534
D.T.P.	STARKS C.P., INC.	N CM0735711	13,083
D.T.P.	STARKS C.P., INC.	N CM0735712	15,619
D.T.P.	STARKS C.P., INC.	N CM8720600	522,100
D.T.P.	UPJOHN COMPANY	N CM0738000	382,500
D.T.P.	VSE, CORPORATION	N CM0725100	140,000
D.T.P.	WARNER LAMBERT	N CM0737900	382,500

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
BRMP	WELLCOME FOUNDATION, LTD.	N CM1748900	1,000,000
	TOTAL		9,717,971

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	ALABAMA, UNIVERSITY OF	N CP9561600B	8,250
D.T.P.	ARIZONA, UNIVERSITY OF	N CM1749700	311,242
R.R.P.	ARTHUR D. LITTLE, INC.	N CM0725700	58,230
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0730200	231,722
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0734600	275,130
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM0726600	357,500
D.T.P.	BRISTOL LABORATORIES	N CM0729900	52,500
D.T.P.	CALIFORNIA, UNIVERSITY OF	N CM0742000	271,751
D.T.P.	CANCER THERAPY & RESEARCH FOUND. OF SOUTH TEXAS	N CM0732700	321,922
A.P.	CHARLES RIVER BREEDING LABS.	N CM1749800	103,004
A.P.	CHARLES RIVER BREEDING LABS.	N CM5059800	101,244
A.P.	CHARLES RIVER BREEDING LABS.	N CM7714100	315,150
A.P.	CHARLES RIVER BREEDING LABS.	N CM8721200	67,219
A.P.	CHARLES RIVER BREEDING LABS.	N CM9016300	104,104
A.P.	CHARLES RIVER BREEDING LABS.	N CM9722900	66,000
ISBR	COR BEL LABORATORIES, INC.	N CB1556500B	40,000
D.T.P.	ENVIRONMENTAL PROTECTION AGENCY	Y CM2010900	161,651
R.R.P.	FOX CHASE CANCER CENTER	N CM0733000B	67,895
A.P.	HARLAN INDUSTRIES	N CM0736200	376,483
A.P.	HARLAN INDUSTRIES	N CM5059100	80,146
A.P.	HARLAN INDUSTRIES	N CM9724200	38,500
D.T.P.	IIT RESEARCH INSTITUTE	N CM9721300	168,996
D.T.P.	IIT RESEARCH INSTITUTE	N CM9731600	638,999
D.T.P.	INSTITUT JULES BORDET	N CM0735000	170,629

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
R.R.P.	INSTITUTE OF CANCER RESEARCH	N CM1750200	88,880
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	6,400
A.P.	KING ANIMAL LABORATORY	N CM1749900	73,788
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM5057700	90,948
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM9724400	23,650
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N CM7716500	483,745
D.T.P.	LITTON BIONETICS, INC.	N CO7538000D	311,280
BRMP	LITTON BIONETICS, INC.	N CO7538000K	135,840
ISBR	LITTON BIONETICS, INC.	N CO7538000M	307,800
A.P.	MASON RESEARCH INSTITUTE	N CM8716400	17,999
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700	609,415
D.T.P.	MAYO FOUNDATION	N CM0741900	178,025
D.T.P.	MICHIGAN TECHNOLOGICAL UNIVERSITY	N CM0729300	1,700
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N CM5700900	106,400
A.P.	MICROBIOLOGICAL ASSOCIATES	N CM9724600	72,014
A.P.	MISSOURI, UNIVERSITY OF	N CM8715700	75,133
A.P.	MURPHY BREEDING LABS., INC.	N CM5057900A	92,836
A.P.	NORTHROP SERVICES, INC.	N CM0728600	37,996
A.P.	NORTHWESTERN UNIVERSITY	N CM1736300	33,233
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000	78,049
A.P.	SASCO, INC.	N CM9016400	62,920
A.P.	SIMONSEN LABORATORIES	N CM5057800	103,646
A.P.	SIMONSEN LABORATORIES	N CM7716600	492,997
A.P.	SIMONSEN LABORATORIES	N CM9724700	22,000

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	SOUTHERN ANIMAL FARMS	N CM5059900	78,078
A.P.	SOUTHERN ANIMAL FARMS	N CM9724500	41,502
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	638,533
R.R.P.	STANFORD RESEARCH INSTITUTE	N CM1748500	165,335
D.T.P.	UPJOHN COMPANY	N CM0738000	67,500
D.T.P.	VSE, CORPORATION	N CM0725100	580,000
D.T.P.	WARNER LAMBERT	N CM0737900	67,500
	TOTAL		9,533,409

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II FORMULATION:		
D.T.P.	IOWA, UNIVERSITY OF	N CM0730300	66,360
D.T.P.	KANSAS, UNIVERSITY OF	N CM0730400	95,235
D.T.P.	KENTUCKY, UNIVERSITY OF	N CM0738100	95,626
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N CM6705300	15,000
D.T.P.	YAMANOUCHI PHARMACEUTICAL CO.	N CM9730700	42,000
	TOTAL		314,221

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	ALABAMA, UNIVERSITY OF	N CP9561600B	3,750
D.T.P.	ARIZONA, UNIVERSITY OF	N CM1749700	34,582
R.R.P.	ARTHUR D. LITTLE, INC.	N CM0725700	135,871
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0734600	125,059
D.T.P.	ARTHUR D. LITTLE, INC.	N CM1739700	142,347
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM0726600	162,500
D.T.P.	CALIFORNIA, UNIVERSITY OF	N CM0742000	30,195
D.T.P.	CANCER THERAPY & RESEARCH FOUND. OF SOUTH TEXAS	N CM0732700	35,769
A.P.	CHARLES RIVER BREEDING LABS.	N CM1749800	46,820
A.P.	CHARLES RIVER BREEDING LABS.	N CM5059800	46,020
A.P.	CHARLES RIVER BREEDING LABS.	N CM7714100	143,250
A.P.	CHARLES RIVER BREEDING LABS.	N CM8721200	30,554
A.P.	CHARLES RIVER BREEDING LABS.	N CM9016300	47,320
A.P.	CHARLES RIVER BREEDING LABS.	N CM9722900	30,000
D.T.P.	ENVIRONMENTAL PROTECTION AGENCY	Y CM2010900	49,739
R.R.P.	FOX CHASE CANCER CENTER	N CM0733000B	16,974
A.P.	HARLAN INDUSTRIES	N CM0736200	171,129
A.P.	HARLAN INDUSTRIES	N CM5059100	36,430
A.P.	HARLAN INDUSTRIES	N CM9724200	17,500
ISDT	HAZLETON LABORATORIES, INC.	N CM2560100	56,108
ISDT	HAZLETON LABORATORIES AMERICA, INC.	N CM9721700	4,836
D.T.P.	IIT RESEARCH INSTITUTE	N CM9721300	38,999
D.T.P.	IIT RESEARCH INSTITUTE	N CM9731600	99,000
D.T.P.	INSTITUT JULES BORDET	N CM0735000	42,657

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	KING ANIMAL LABORATORY	N CM1749900	33,540
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM5057700	41,340
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM9724400	10,750
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N CM7716500	219,884
BRMP	LITTON BIONETICS, INC.	N CM1580800	94,433
D.T.P.	LITTON BIONETICS, INC.	N CO7538000D	778,200
BRMP	LITTON BIONETICS, INC.	N CO7538000K	339,600
ISBR	LITTON BIONETICS, INC.	N CO7538000M	769,500
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM0732500	189,099
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700	239,770
D.T.P.	MAYO FOUNDATION	N CM0741900	19,781
BRMP	MELOY LABORATORIES, INC.	N CM1575700	53,959
A.P.	MICROBIOLOGICAL ASSOCIATES	N CM9724600	32,734
A.P.	MISSOURI, UNIVERSITY OF	N CM8715700	34,151
A.P.	MURPHY BREEDING LABS., INC.	N CM5057900A	42,198
A.P.	NORTHROP SERVICES, INC.	N CM0728600	17,271
A.P.	NORTHWESTERN UNIVERSITY	N CM1736300	15,106
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000	35,477
A.P.	SASCO, INC.	N CM9016400	28,600
A.P.	SIMONSEN LABORATORIES	N CM5057800	47,112
A.P.	SIMONSEN LABORATORIES	N CM7716600	224,090
A.P.	SIMONSEN LABORATORIES	N CM9724700	10,000
A.P.	SOUTHERN ANIMAL FARMS	N CM5059900	35,490
A.P.	SOUTHERN ANIMAL FARMS	N CM9724500	18,865

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	518,808
D.T.P.	VSE, CORPORATION	N CM0725100	200,000
	TOTAL		5,597,167

ANALYSIS OF CONTRACTS BY ACTIVITY.

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	AEROJET STRATEGIC PROPULSION CO.	N CM1749000	197,872
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y CM4000100	20,000
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N CM1749200	153,214
D.T.P.	ASH STEVENS, INC.	N CM1748800	283,724
BRMP	IOWA, UNIVERSITY OF	N CM0733400	75,693
ISDT	LITTON BIONETICS, INC.	N CM0572400	418,672
ISDT	LITTON BIONETICS, INC.	N CM0734700	272,069
D.T.P.	LITTON BIONETICS, INC.	N CO7538000D	259,400
BRMP	LITTON BIONETICS, INC.	N CO7538000K	113,200
ISBR	LITTON BIONETICS, INC.	N CO7538000M	256,500
D.T.P.	MIDWEST RESEARCH INSTITUTE	N CM8723400	32,519
D.T.P.	PHARM-ECO	N CM1748700	141,108
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM8718300	116,034
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM9725600	5,714
D.T.P.	STARKS C.P., INC.	N CM1737400	270,825
D.T.P.	WARNER LAMBERT	N CM1749100	164,806
	TOTAL		2,781,350

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PHARMACOLOGY/TOXICOLOGY:		
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM1736500	2,568,756
ISDT	HAZLETON LABORATORIES, INC.	N CM2560100	224,431
A.P.	HAZLETON LABORATORIES, INC.	N CM6012500	36,900
ISDT	HAZLETON LABORATORIES AMERICA, INC.	N CM9721700	19,345
A.P.	MARSHALL RESEARCH ANIMALS, INC.	N CM6012300	33,120
A.P.	MASON RESEARCH INSTITUTE	N CM8716400	173,243
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N CM8716100	25,540
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N CM9726400	40,000
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	39,908
	TOTAL		3,161,243

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PROD. AND FORM. FOR CLINICAL TRIALS:		
D.T.P.	AEROJET STRATEGIC PROPULSION CO.	N CM1749000	197,872
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N CM1749200	153,214
D.T.P.	ASH STEVENS, INC.	N CM1748800	283,724
D.T.P.	BANNER GELATIN PRODUCTS	N CM1740200	162,900
D.T.P.	FLOW LABORATORIES, INC.	N CM1739800	318,381
CTEP	GEORGETOWN UNIVERSITY	N CM9731000	1,250
D.T.P.	IOWA, UNIVERSITY OF	N CM0730300	265,439
BRMP	IOWA, UNIVERSITY OF	N CM0733400	32,440
C.O.P.	LITTON BIONETICS, INC.	N CM6706700	9,900
CTEP	MAYO FOUNDATION	N CM9726800	1,250
D.T.P.	MIDWEST RESEARCH INSTITUTE	N CM8723400	130,074
D.T.P.	PHARM-ECO	N CM1748700	141,108
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N CM6705300	60,000
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM8718300	464,137
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM9725600	1,428
D.T.P.	STARKS C.P., INC.	N CM1737400	270,825
D.T.P.	WARNER LAMBERT	N CM1749100	164,806
D.T.P.	YAMANOUCHI PHARMACEUTICAL CO.	N CM9730700	168,000
	TOTAL		2,826,748

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - BIOLOGICAL STUDIES:		
R.R.P.	BUREAU OF RADIOLOGICAL HEALTH, FDA	Y CO1070000B	50,000
R.R.P.	ENERGY, DEPARTMENT OF-OAK RIDGE NATIONAL LABS	Y CM2011100	144,860
R.R.P.	ENERGY, DEPARTMENT OF-OAK RIDGE NATIONAL LABS	Y CM2011200	125,680
R.R.P.	ENERGY, DEPARTMENT OF-OAK RIDGE NATIONAL LABS	Y CM2011300	156,160
R.R.P.	ENERGY, DEPARTMENT OF	Y CO0032000B	427,500
R.R.P.	ENERGY, DEPT. OF-BROOKHAVEN NAT'L LABS.	Y CO1071100B	224,895
R.R.P.	ENERGY, DEPT. OF-BROOKHAVEN NAT'L LABS.	Y CO1071200B	212,760
CTEP	GEORGETOWN UNIVERSITY	N CM9731000	1,250
C.O.P.	HAZLETON LABORATORIES, INC.	N CM1577000	105,000
ISDT	HAZLETON LABORATORIES, INC.	N CM2560100	168,323
ISDT	HAZLETON LABORATORIES AMERICA, INC.	N CM9721700	14,509
ISDT	LITTON BIONETICS, INC.	N CM0734700	272,069
C.O.P.	LITTON BIONETICS, INC.	N CM1573700	207,749
ISDT	LITTON BIONETICS, INC.	N CM2561600	579,507
C.O.P.	LITTON BIONETICS, INC.	N CM6706700	89,100
BCRP	MARYLAND, UNIVERSITY OF	N CM4374800	125,000
A.P.	MASON RESEARCH INSTITUTE	N CM8716400	33,749
CTEP	MAYO FOUNDATION	N CM9726800	1,250
C.O.P.	MICROBIOLOGICAL ASSOCIATES	N CM0736900	132,906
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CB4387900B	9,074
ISBR	NAVAL MEDICAL RESEARCH INSTITUTE	Y CB0031900B	140,500
ISDT	SMALL BUSINESS ADMINISTRATION	N CM4371900	43,200
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	19,954
BRMP	WASHINGTON, UNIVERSITY OF	N CB8424700B	108,293

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	ACTIVITY CONTRACT	#	DOLLAR LEVEL
	TOTAL		3,393,288

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - TREATMENT STUDIES:		
CTEP	ALBANY MEDICAL COLLEGE	N CB5394000B	59,908
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0730200	189,590
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0734600	25,012
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM0726600	32,500
R.R.P.	ENERGY, DEPARTMENT OF-LAWRENCE BERKELEY LAB	Y CM2011000	66,402
CTEP	HEALTH RESEARCH, INC.	N CB6400700B	4,800
D.T.P.	IIT RESEARCH INSTITUTE	N CM9731600	27,000
C.O.P.	LITTON BIONETICS, INC.	N CM1573700	89,035
C.O.P.	MASON RESEARCH INSTITUTE	N CM6701100	294,113
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700	99,904
R.R.P.	MASSACHUSETTS GENERAL HOSPITAL	N CM2753200	84,969
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CB4387900B	6,049
R.R.P.	NEW MEXICO, UNIVERSITY OF	N CM2753100	49,320
R.R.P.	PENNSYLVANIA, UNIVERSITY OF	N CM2752900	63,725
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	758,258
BRMP	TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER	N CB8424800B	78,243
D.T.P.	VSE, CORPORATION	N CM0725100	80,000
BRMP	WASHINGTON, UNIVERSITY OF	N CB8424700B	108,293
	TOTAL		2,117,121

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT		#	DOLLAR LEVEL
	PROGRAM MANAGEMENT:			
O.D.	CDP ASSOCIATES	N	CM9714300	29,927
CTEP	GEORGETOWN UNIVERSITY	N	CM9731000	1,250
CTEP	INDIANA UNIVERSITY FOUNDATION	N	CM1747500	3,430
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N	CM1734900	1,276
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N	CM2205400	3,200
O.D.	JWK INTERNATIONAL CORP.	N	CM2560200	303,125
BCRP	MARYLAND, UNIVERSITY OF	N	CM4374800	25,000
CTEP	MAYO FOUNDATION	N	CM9726800	1,250
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N	CM1734800	7,142
CTEP	MONTEFIORE HOSPITAL	N	CM1747400	6,132
A.P.	NATIONAL ACADEMY OF SCIENCES	N	CM5385000	29,000
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y	CM0010300	7,640
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N	CM1747300	5,467
CTEP	NORTH CAROLINA, UNIVERSITY OF	N	CM1747100	8,615
CTEP	PAN AMERICAN HEALTH ORGANIZATION	N	CM2739100	172,920
CTEP	PAN AMERICAN HEALTH ORGANIZATION	N	CO6533200C	29,466
CTEP	SOCIAL & SCIENTIFIC SYSTEMS, INC.	N	CM1752100	303,333
CTEP	SOCIAL & SCIENTIFIC SYSTEMS, INC.	N	CM2560600	142,046
CTEP	TENNESSEE UNIVERSITY CENTER FOR HEALTH SCIENCES	N	CM1747200	5,030
	TOTAL			1,085,249

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE I CLINICAL TRIALS:		
CTEP	ARIZONA, UNIVERSITY OF	N CM1750000	18,838
R.R.P.	ARIZONA, UNIVERSITY OF	N CM1752200	71,125
BRMP	CALIFORNIA, UNIVERSITY OF	N CM0744401	24,984
BRMP	GEORGE WASHINTON UNIVERSITY	N CM0744601	10,000
CTEP	GEORGETOWN UNIVERSITY	N CM9720800	30,798
CTEP	GEORGETOWN UNIVERSITY	N CM9731000	750
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	2,400
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER	N CM9727200	17,266
BCRP	MARYLAND, UNIVERSITY OF	N CM4374800	40,000
R.R.P.	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	N CM2752500	94,370
CTEP	MAYO FOUNDATION	N CM9726800	750
CTEP	MAYO FOUNDATION	N CM9727300	45,593
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM9727400	31,315
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CM9727500	23,285
BRMP	NORTHERN CALIFORNIA CANCER PROGRAM	N CM0744301	26,171
BRMP	NORTHERN CALIFORNIA CANCER PROGRAM	N CM0744302	16,229
BRMP	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N CM0743501	10,000
BRMP	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N CM0743502	80,000
R.R.P.	STANFORD UNIVERSITY	N CM1748000	74,817
R.R.P.	TEXAS, UNIVERSITY OF	N CM1752400	84,601
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N CM9727700	44,907
R.R.P.	UTAH, UNIVERSITY OF	N CM1752300	83,137
CTEP	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N CM9727800	22,420
CTEP	WAYNE STATE UNIVERSITY	N CM9727900	22,720

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE I CLINICAL TRIALS:		
CTEP	WISCONSIN, UNIVERSITY OF	N CM97280000	17,607
	TOTAL		894,083

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
CTEP	ALBANY MEDICAL COLLEGE	N CB5394000B	239,633
CTEP	ARIZONA, UNIVERSITY OF	N CM1750000	18,838
BRMP	CALIFORNIA, UNIVERSITY OF	N CM0744401	24,984
BRMP	GEORGE WASHINTON UNIVERSITY	N CM0744601	10,000
CTEP	GEORGETOWN UNIVERSITY	N CM9720800	12,579
CTEP	GEORGETOWN UNIVERSITY	N CM9731000	5,000
CTEP	HEALTH RESEARCH, INC.	N CB6400700B	19,200
CTEP	INDIANA UNIVERSITY FOUNDATION	N CM1747500	10,290
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	2,400
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER	N CM9727200	7,052
BCRP	MARYLAND, UNIVERSITY OF	N CM4374800	105,000
R.R.P.	MASSACHUSETTS GENERAL HOSPITAL	N CM1748100	52,812
R.R.P.	MAYO FOUNDATION	N CM2752800	98,465
CTEP	MAYO FOUNDATION	N CM9726800	5,000
CTEP	MAYO FOUNDATION	N CM9727300	18,623
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM0733700	123,854
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM1734800	21,426
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM9727400	12,790
CTEP	MICHIGAN, UNIVERSITY OF	N CM0740500	134,316
CTEP	MONTEFIORE HOSPITAL	N CM1747400	18,395
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CB4387900B	45,370
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CM9727500	9,511
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y CM0010300	307,510
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N CM1747300	16,402

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
CTEP	NORTH CAROLINA, UNIVERSITY OF	N CM1747100	25,845
BRMP	NORTHERN CALIFORNIA CANCER PROGRAM	N CM0744301	26,171
BRMP	NORTHERN CALIFORNIA CANCER PROGRAM	N CM0744302	16,229
BRMP	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N CM0743501	10,000
BRMP	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N CM0743502	80,000
R.R.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	N CM2748300	58,790
CTEP	TENNESSEE UNIVERSITY CENTER FOR HEALTH SCIENCES	N CM1747200	15,090
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N CM0740600	141,271
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N CM9727700	18,342
CTEP	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N CM9727800	9,158
CTEP	WAYNE STATE UNIVERSITY	N CM0740400	130,935
CTEP	WAYNE STATE UNIVERSITY	N CM9727900	9,280
CTEP	WISCONSIN, UNIVERSITY OF	N CM9728000	7,191
CTEP	YALE UNIVERSITY	N CB7419100B	78,923
	TOTAL		1,946,675

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:		
CTEP	ARIZONA, UNIVERSITY OF	N CM1750000	56,515
CTEP	GEORGETOWN UNIVERSITY	N CM9731000	7,500
CTEP	INDIANA UNIVERSITY FOUNDATION	N CM1747500	20,579
BCRP	MARYLAND, UNIVERSITY OF	N CM4374800	100,000
CTEP	MAYO FOUNDATION	N CM9726800	7,500
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM0733700	101,335
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM1734800	42,852
CTEP	MICHIGAN, UNIVERSITY OF	N CM0740500	109,895
CTEP	MONTEFIORE HOSPITAL	N CM1747400	36,791
CTEP	MUHIMBILI MEDICAL CENTER	N CM0734400	45,000
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N CM1747300	32,804
CTEP	NORTH CAROLINA, UNIVERSITY OF	N CM1747100	51,691
CTEP	TENNESSEE UNIVERSITY CENTER FOR HEALTH SCIENCES	N CM1747200	30,180
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N CM0740600	115,585
CTEP	VETERANS ADMINISTRATION	Y CM7010700	32,000
CTEP	WAYNE STATE UNIVERSITY	N CM0740400	107,129
	TOTAL		897,356

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	ALBANY MEDICAL COLLEGE	N CM5703200	13,200
CTEP	CHICAGO, UNIVERSITY OF	N CM0741100	35,638
CTEP	CINCINNATI, UNIVERSITY OF	N CM8722200	28,879
CTEP	GEORGETOWN UNIVERSITY	N CM6709400	7,052
CTEP	HEALTH RESEARCH, INC.	N CM0741000	11,129
CTEP	HEALTH RESEARCH, INC.	N CM5703400	21,465
BCRP	MARYLAND, UNIVERSITY OF	N CM4374800	95,000
CTEP	MARYLAND, UNIVERSITY OF	N CM8722300	15,364
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM3722400	25,300
CTEP	MIAMI, UNIVERSITY OF	N CM0740900	54,787
CTEP	MIAMI, UNIVERSITY OF	N CM6709300	26,978
CTEP	MICHIGAN, UNIVERSITY OF	N CM8722500	38,333
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CM0740700	29,894
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CM6709600	17,735
CTEP	NORTHERN CALIFORNIA CANCER PROGRAM	N CM8715400	30,001
CTEP	SIDNEY FARBER CANCER INSTITUTE	N CM5703500	12,000
CTEP	SOUTH FLORIDA, UNIVERSITY OF	N CM8722000	61,431
CTEP	TEXAS, UNIVERSITY OF, MEDICAL BRANCH	N CM8722100	26,833
CTEP	VETERANS ADMINISTRATION	Y CM7010700	32,000
CTEP	WAYNE STATE UNIVERSITY	N CM0740800	31,211
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N CM0733900	51,644
	TOTAL		665,874

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS - OTHER RESEARCH:		
R.R.P.	CALIFORNIA, UNIVERSITY OF	N CM9731500	809,458
CTEP	DUKE UNIVERSITY	N CM1747700	25,277
R.R.P.	FOX CHASE CANCER CENTER	N CM9731400	1,236,002
R.R.P.	HEALTH RESEARCH, INC.	N CM9731100	74,932
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N CM1734900	11,483
CTEP	IOWA, UNIVERSITY OF	N CM1747600	25,122
BCRP	MARYLAND, UNIVERSITY OF	N CM4374800	10,000
CTEP	MUHIMBILI MEDICAL CENTER	N CM0734400	5,000
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y CM0010300	32,852
CTEP	PAN AMERICAN HEALTH ORGANIZATION	N CM2739100	93,111
CTEP	PAN AMERICAN HEALTH ORGANIZATION	N CO6533200C	15,867
CTEP	VETERANS ADMINISTRATION	Y CM7010700	16,000
R.R.P.	WASHINGTON, UNIVERSITY OF	N CM9728200	234,866
	TOTAL		2,639,970

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1982
AS OF 05/31/82

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
R.R.P.	ARIZONA, UNIVERSITY OF	N CM1752200	71,125
C.O.P.	CALIFORNIA, UNIVERSITY OF	N CM9723900	265,000
CTEP	CHICAGO, UNIVERSITY OF	N CM0741100	3,960
CTEP	EMMES CORPORATION	N CM8719300	315,000
CTEP	EMORY UNIVERSITY	N CM1580400	32,271
CTEP	EMORY UNIVERSITY	N CM2560300	152,324
CTEP	GEORGETOWN UNIVERSITY	N CM9731000	8,000
CTEP	HEALTH RESEARCH, INC.	N CM0741000	1,237
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N CP0102500B	110,000
R.R.P.	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	N CM2752500	94,370
CTEP	MATHTECH, INC.	N CM9719500	598,972
CTEP	MAYO FOUNDATION	N CM9726800	8,000
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM1573500	74,683
CTEP	MIAMI, UNIVERSITY OF	N CM0740900	6,087
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CM0740700	3,322
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N CM6709600	1,971
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y CM0010300	33,998
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N CM8716100	76,619
R.R.P.	STANFORD UNIVERSITY	N CM1748000	74,817
R.R.P.	TEXAS, UNIVERSITY OF	N CM1752400	84,601
CTEP	TORONTO GENERAL HOSPITAL	N CM1580300	16,113
R.R.P.	UTAH, UNIVERSITY OF	N CM1752300	83,137
CTEP	WAYNE STATE UNIVERSITY	N CM0740800	3,468
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N CM0733900	5,738
	TOTAL		2,124,813

TABLE III

ANALYSIS OF ACTIVITIES BY CONTRACTS
FOR FISCAL YEAR 1982

AS OF 05/31/82

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
AEROJET STRATEGIC PROPULSION CO.	N CM1749000 D.T.P.		395,743
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	197,872
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	197,872
AGRICULTURE, DEPARTMENT OF	Y CM4000100 D.T.P.		200,000
ACQUISITION OF MATERIALS		* 90.00*	180,000
NEW AGENT PROCUREMENT, PLANT PRODUCTS		85.00	170,000
DATA PROCESSING AND SUPPORT		5.00	10,000
PROCUREMENT OF PRECLINICAL MATERIAL PLANT PRODUCTS		* 10.00*	20,000
ALABAMA, UNIVERSITY OF	N CP9561600B A.P.		15,000
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	3,000
BASIC SCREEN ANIMALS		* 55.00*	8,250
VERIFICATION SCREEN ANIMALS		* 25.00*	3,750
ALABAMA, UNIVERSITY OF	N CM0735509 D.T.P.		14,597
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	14,597
ALABAMA, UNIVERSITY OF	N CM0735510 D.T.P.		14,624
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	14,624
ALBANY MEDICAL COLLEGE	N CB5394000B CTEP		299,541
TREATMENT STUDIES		* 20.00*	59,908
COMB. MODAL. THERAPY		20.00	59,908
COMB. MODAL. THERAPY - SURG.		5.00	14,977
COMB. MODAL. THERAPY - IMM.		15.00	44,931
PHASE II CLINICAL TRIALS		* 80.00*	239,633
LUNG IMM.			

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ALBANY MEDICAL COLLEGE		N CM5703200 CTEP		13,200
PHASE IV CLINICAL TRIALS			*100.00*	13,200
COLORECTAL	SURG.		30.00	3,960
COLORECTAL	RAD.		10.00	1,320
COLORECTAL	CHEM.		25.00	3,300
COLORECTAL	IMM.		25.00	3,300
COLORECTAL	PAT. SUPPORT. CARE		10.00	1,320
ALDRICH CHEMICAL COMPANY, INC.		N CM1749200 D.T.P.		306,427
PROCUREMENT OF PRECLINICAL MATERIAL			* 50.00*	153,214
SYNTHETICS				
PROD. AND FORM. FOR CLINICAL TRIALS			* 50.00*	153,214
PRODUCTION, SYNTHETICS				
ARIZONA, UNIVERSITY OF		N CM1750000 CTEP		94,191
PHASE I CLINICAL TRIALS			* 20.00*	18,838
UTERINE CERVIX	CHEM.			
PHASE II CLINICAL TRIALS			* 20.00*	18,838
UTERINE CERVIX	CHEM.			
PHASE III CLINICAL TRIALS			* 60.00*	56,515
UTERINE CERVIX	CHEM.			
ARIZONA, UNIVERSITY OF		N CM1749700 D.T.P.		345,824
BASIC SCREEN			* 90.00*	311,242
PRIMARY SCREENING, IN VITRO				
VERIFICATION SCREEN			* 10.00*	34,582
DETAILED DRUG EVALUATION, IN VITRO				
ARIZONA, UNIVERSITY OF		N CM1752200 R.R.P.		142,249
PHASE I CLINICAL TRIALS			* 50.00*	71,125
NON-SPECIFIC	RAD.			
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 50.00*	71,125
NON-SPECIFIC	HYPERTHERMIA			
ARTHUR D. LITTLE, INC.		N CM1739700 D.T.P.		142,347
VERIFICATION SCREEN			*100.00*	142,347
BIOCHEM TESTS				
ARTHUR D. LITTLE, INC.		N CM0730200 D.T.P.		421,312
BASIC SCREEN			* 55.00*	231,722
IN VIVO ANALOG SCR.				
TREATMENT STUDIES			* 45.00*	189,590
COMB. MODAL. THERAPY			45.00	189,590

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
COMB. MODAL. THERAPY - RAD.		20.00	84,262
COMB. MODAL. THERAPY - CHEM.		25.00	105,328
ARTHUR D. LITTLE, INC.	N CM0734600 D.T.P.		500,237
ACQUISITION OF MATERIALS		* 15.00*	75,036
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 55.00*	275,130
PRIMARY SCREENING, IN VIVO		50.00	250,119
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	25,012
VERIFICATION SCREEN		* 25.00*	125,059
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 5.00*	25,012
CHEMOTHERAPY			
ARTHUR D. LITTLE, INC.	N CM0725700 R.R.P.		194,101
BASIC SCREEN		* 30.00*	58,230
PRIMARY SCREENING, IN VITRO		10.00	19,410
PRIMARY SCREENING, IN VIVO		10.00	19,410
PHYS/CHEM PROPS.		10.00	19,410
VERIFICATION SCREEN		* 70.00*	135,871
DETAILED DRUG EVALUATION, IN VIVO			
ASH STEVENS, INC.	N CM1748800 D.T.P.		567,448
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	283,724
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	283,724
PRODUCTION, SYNTHETICS			
BANNER GELATIN PRODUCTS	N CM1740200 D.T.P.		162,900
PROD. AND FORM. FOR CLINICAL TRIALS		*100.00*	162,900
FORMULATION			
BATTELLE MEMORIAL INSTITUTE	N CM1736500 D.T.P.		2,568,756
PHARMACOLOGY/TOXICOLOGY		*100.00*	2,568,756
PROTOCOL TOXICITY STUDIES		50.00	1,284,378
SPECIAL TOXICITY STUDIES		34.00	873,377
ANIMALS		1.00	25,688
DATA PROCESSING AND SUPPORT		15.00	385,313
BATTELLE MEMORIAL INSTITUTE	N CM0726600 D.T.P.		650,000
ACQUISITION OF MATERIALS		* 15.00*	97,500
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 55.00*	357,500
PRIMARY SCREENING, IN VIVO		50.00	325,000
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	32,500

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
VERIFICATION SCREEN		* 25.00*	162,500
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 5.00*	32,500
CHEMOTHERAPY			
BRISTOL LABORATORIES	N CM0729900 D.T.P.		350,000
ACQUISITION OF MATERIALS		* 85.00*	297,500
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	290,500
DATA PROCESSING AND SUPPORT		2.00	7,000
BASIC SCREEN		* 15.00*	52,500
PRIMARY SCREENING, IN VITRO		10.00	35,000
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	17,500
BUREAU OF RADIOLOGICAL HEALTH,	Y CD1070000B R.R.P.		50,000
CELLULAR/SUBCELLULAR STUDIES		*100.00*	50,000
RADIOBIOLOGY		100.00	50,000
CALIFORNIA, UNIVERSITY OF	N CM9723900 C.O.P.		265,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	265,000
NON-SPECIFIC TRANSFUSION RES.			
CALIFORNIA, UNIVERSITY OF	N CM0742000 D.T.P.		301,945
BASIC SCREEN		* 90.00*	271,751
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	30,195
DETAILED DRUG EVALUATION, IN VITRO			
CALIFORNIA, UNIVERSITY OF	N CM9731500 R.R.P.		809,458
OTHER CLINICAL TRIALS RESEARCH		*100.00*	809,458
NON-SPECIFIC HARDWARE DEVEL.			
CALIFORNIA, UNIVERSITY OF	N CM0744401 BRMP		49,968
PHASE I CLINICAL TRIALS		* 50.00*	24,984
NON-SPECIFIC IMM.			
PHASE II CLINICAL TRIALS		* 50.00*	24,984
NON-SPECIFIC IMM.			
CANCER THERAPY & RESEARCH FOUND. OF	N CM0732700 D.T.P.		357,691
BASIC SCREEN		* 90.00*	321,922
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	35,769
DETAILED DRUG EVALUATION, IN VITRO			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
CDP ASSOCIATES	N CM9714300 O.D.		29,927
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	29,927
CHARLES RIVER BREEDING LABS.	N CM9016300 A.P.		189,280
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	37,856
BASIC SCREEN ANIMALS		* 55.00*	104,104
VERIFICATION SCREEN ANIMALS		* 25.00*	47,320
CHARLES RIVER BREEDING LABS.	N CM5059800 A.P.		184,080
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	36,816
BASIC SCREEN ANIMALS		* 55.00*	101,244
VERIFICATION SCREEN ANIMALS		* 25.00*	46,020
CHARLES RIVER BREEDING LABS.	N CM7714100 A.P.		573,000
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	114,600
BASIC SCREEN ANIMALS		* 55.00*	315,150
VERIFICATION SCREEN ANIMALS		* 25.00*	143,250
CHARLES RIVER BREEDING LABS.	N CM1749800 A.P.		187,280
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	37,456
BASIC SCREEN ANIMALS		* 55.00*	103,004
VERIFICATION SCREEN ANIMALS		* 25.00*	46,820
CHARLES RIVER BREEDING LABS.	N CM8721200 A.P.		122,217
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	24,443
BASIC SCREEN ANIMALS		* 55.00*	67,219
VERIFICATION SCREEN ANIMALS		* 25.00*	30,554

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
CHARLES RIVER BREEDING LABS.	N CM9722900 A.P.		120,000
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	24,000
BASIC SCREEN ANIMALS		* 55.00*	66,000
VERIFICATION SCREEN ANIMALS		* 25.00*	30,000
CHEMICAL ABSTRACTS SERVICE	N CM4372200 D.T.P.		610,000
ACQUISITION OF MATERIALS DATA PROCESSING AND SUPPORT		*100.00*	610,000
CHICAGO, UNIVERSITY OF	N CM0741100 CTEP		39,598
PHASE IV CLINICAL TRIALS		* 90.00*	35,638
STOMACH SURG.		25.00	9,900
STOMACH RAD.		25.00	9,900
STOMACH CHEM.		40.00	15,839
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	3,960
STOMACH MARKERS			
CINCINNATI, UNIVERSITY OF	N CM8722200 CTEP		28,879
PHASE IV CLINICAL TRIALS		*100.00*	28,879
HEAD AND NECK SURG.		30.00	8,664
HEAD AND NECK RAD.		30.00	8,664
HEAD AND NECK CHEM.		20.00	5,776
HEAD AND NECK PAT. SUPPORT. CARE		20.00	5,776
COR BEL LABORATORIES, INC.	N CB1556500B IS3R		40,000
BASIC SCREEN RESPONSE MODIFIERS		*100.00*	40,000
DUKE UNIVERSITY	N CM1747700 CTEP		25,277
OTHER CLINICAL TRIALS RESEARCH		*100.00*	25,277
CNS TUMORS PATHOLOGY			
EMMES CORPORATION	N CM8719300 CTEP		315,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	315,000
LUNG DATA PROC. & SUPP.			
EMORY UNIVERSITY	N CM2560300 CTEP		152,324
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	152,324
NON-SPECIFIC NUTRITION			

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
EMORY UNIVERSITY	N CM1580400 CTEP			32,271
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	32,271	
OTHER NUTRITION				
ENERGY, DEPARTMENT OF	Y C00032000B R.R.P.			427,500
CELLULAR/SUBCELLULAR STUDIES		*100.00*	427,500	
RADIOBIOLOGY		100.00	427,500	
ENERGY, DEPARTMENT OF-LAWRENCE BERKELEY	Y CM2011000 R.R.P.			66,402
TREATMENT STUDIES		*100.00*	66,402	
RADIATION PHYSICS				
ENERGY, DEPARTMENT OF-OAK RIDGE	Y CM2011100 R.R.P.			144,860
CELLULAR/SUBCELLULAR STUDIES		*100.00*	144,860	
RADIOBIOLOGY		100.00	144,860	
ENERGY, DEPARTMENT OF-OAK RIDGE	Y CM2011200 R.R.P.			125,680
CELLULAR/SUBCELLULAR STUDIES		*100.00*	125,680	
RADIOBIOLOGY		100.00	125,680	
ENERGY, DEPARTMENT OF-OAK RIDGE	Y CM2011300 R.R.P.			156,160
CELLULAR/SUBCELLULAR STUDIES		*100.00*	156,160	
RADIOBIOLOGY		100.00	156,160	
ENERGY, DEPT. OF-BROOKHAVEN NAT'L	Y C01071100B R.R.P.			224,895
CELLULAR/SUBCELLULAR STUDIES		*100.00*	224,895	
RADIOBIOLOGY		100.00	224,895	
ENERGY, DEPT. OF-BROOKHAVEN NAT'L	Y C01071200B R.R.P.			212,760
CELLULAR/SUBCELLULAR STUDIES		*100.00*	212,760	
RADIOBIOLOGY		100.00	212,760	
ENVIRONMENTAL PROTECTION AGENCY	Y CM2010900 D.T.P.			248,694
ACQUISITION OF MATERIALS		* 15.00*	37,304	
DATA PROCESSING AND SUPPORT				
BASIC SCREEN		* 65.00*	161,651	
DATA PROCESSING AND SUPPORT				
VERIFICATION SCREEN		* 20.00*	49,739	
DATA PROCESSING AND SUPPORT				

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
FLOW LABORATORIES, INC.	N CM1739800 D.T.P.		318,381
PROD. AND FORM. FOR CLINICAL TRIALS		*100.00*	318,381
DATA PROCESSING AND SUPPORT		30.00	95,514
ST & DIST CLIN DRUGS		70.00	222,867
FOX CHASE CANCER CENTER	N CM0733000B R.R.P.		84,869
BASIC SCREEN		* 80.00*	67,895
PRIMARY SCREENING, IN VIVO			
VERIFICATION SCREEN		* 20.00*	16,974
DETAILED DRUG EVALUATION, IN VIVO			
FOX CHASE CANCER CENTER	N CM9731400 R.R.P.		1,236,002
OTHER CLINICAL TRIALS RESEARCH		*100.00*	1,236,002
NON-SPECIFIC HARDWARE DEVEL.			
GEORGE WASHINTON UNIVERSITY	N CM0744601 BRMP		20,000
PHASE I CLINICAL TRIALS		* 50.00*	10,000
NON-SPECIFIC IMM.			
PHASE II CLINICAL TRIALS		* 50.00*	10,000
NON-SPECIFIC IMM.			
GEORGETOWN UNIVERSITY	N CM6709400 CTEP		7,052
PHASE IV CLINICAL TRIALS		*100.00*	7,052
COLORECTAL SURG.		20.00	1,410
COLORECTAL RAD.		15.00	1,058
COLORECTAL CHEM.		45.00	3,173
COLORECTAL IMM.		20.00	1,410
GEORGETOWN UNIVERSITY	N CM9720800 CTEP		43,377
PHASE I CLINICAL TRIALS		* 71.00*	30,798
NON-SPECIFIC CHEM.			
PHASE II CLINICAL TRIALS		* 29.00*	12,579
LEUK.-ACUTE GRAN. CHEM.		2.00	868
LEUK.-ACUTE LYMPH. CHEM.		2.00	868
GENERAL CHEM.		5.00	2,169
BREAST CHEM.		5.00	2,169
COLORECTAL CHEM.		5.00	2,169
LUNG CHEM.		5.00	2,169
MELANOMA CHEM.		5.00	2,169
GEORGETOWN UNIVERSITY	N CM9731000 CTEP		25,000
PROD. AND FORM. FOR CLINICAL TRIALS		* 5.00*	1,250
FORMULATION			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
CELLULAR/SUBCELLULAR STUDIES		* 5.00*	1,250
NUTRITION		5.00	1,250
PROGRAM MANAGEMENT		* 5.00*	1,250
ADMINISTRATION		2.00	500
COMMUNICATION AND EDUCATION		3.00	750
PHASE I CLINICAL TRIALS		* 3.00*	750
COLORECTAL	CHEM.		
PHASE II CLINICAL TRIALS		* 20.00*	5,000
COLORECTAL	CHEM.	10.00	2,500
PANCREATIC	CHEM.	5.00	1,250
STOMACH	CHEM.	5.00	1,250
PHASE III CLINICAL TRIALS		* 30.00*	7,500
COLORECTAL	CHEM.	10.00	2,500
LUNG	CHEM.	5.00	1,250
STOMACH	RAD.	5.00	1,250
STOMACH	CHEM.	10.00	2,500
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 32.00*	8,000
COLORECTAL	SPEC. PHARM./TOX.	5.00	1,250
COLORECTAL	BLOOD PRODUCTS	5.00	1,250
COLORECTAL	PROTECTED ENVIRON.	5.00	1,250
COLORECTAL	NUTRITION	5.00	1,250
STOMACH	MARKERS	5.00	1,250
COLORECTAL	DATA PROC. & SUPP.	2.00	500
PANCREATIC	DIAG. RADIOLOGY	5.00	1,250
HARLAN INDUSTRIES	N CM5059100 A.P.		145,720
ACQUISITION OF MATERIALS		* 20.00*	29,144
ANIMALS			
BASIC SCREEN		* 55.00*	80,146
ANIMALS			
VERIFICATION SCREEN		* 25.00*	36,430
ANIMALS			
HARLAN INDUSTRIES	N CM0736200 A.P.		684,515
ACQUISITION OF MATERIALS		* 20.00*	136,903
ANIMALS			
BASIC SCREEN		* 55.00*	376,483
ANIMALS			
VERIFICATION SCREEN		* 25.00*	171,129
ANIMALS			
HARLAN INDUSTRIES	N CM9724200 A.P.		70,000
ACQUISITION OF MATERIALS		* 20.00*	14,000
ANIMALS			
BASIC SCREEN		* 55.00*	38,500
ANIMALS			
VERIFICATION SCREEN		* 25.00*	17,500
ANIMALS			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
HAZLETON LABORATORIES	N CM9721700 ISDT		48,363
ACQUISITION OF MATERIALS		* 20.00*	9,673
ANIMALS			
VERIFICATION SCREEN		* 10.00*	4,836
ANIMALS			
PHARMACOLOGY/TOXICOLOGY		* 40.00*	19,345
PHARMACOLOGY		10.00	4,836
SPECIAL TOXICITY STUDIES		30.00	14,509
CELLULAR/SUBCELLULAR STUDIES		* 30.00*	14,509
MARKERS		30.00	14,509
HAZLETON LABORATORIES, INC.	N CM6012500 A.P.		36,900
PHARMACOLOGY/TOXICOLOGY		*100.00*	36,900
ANIMALS			
HAZLETON LABORATORIES, INC.	N CM1577000 C.O.P.		105,000
CELLULAR/SUBCELLULAR STUDIES		*100.00*	105,000
BLOOD PRODUCTS		50.00	52,500
IMMUNOBIOLOGY		50.00	52,500
HAZLETON LABORATORIES, INC.	N CM2560100 ISDT		561,078
ACQUISITION OF MATERIALS		* 20.00*	112,216
ANIMALS			
VERIFICATION SCREEN		* 10.00*	56,108
ANIMALS			
PHARMACOLOGY/TOXICOLOGY		* 40.00*	224,431
PHARMACOLOGY		10.00	56,108
SPECIAL TOXICITY STUDIES		30.00	168,323
CELLULAR/SUBCELLULAR STUDIES		* 30.00*	168,323
MARKERS		30.00	168,323
HEALTH RESEARCH, INC.	N CB6400700B CTEP		24,000
TREATMENT STUDIES		* 20.00*	4,800
COMB. MODAL. THERAPY		20.00	4,800
COMB. MODAL. THERAPY - SURG.		5.00	1,200
COMB. MODAL. THERAPY - IMM.		15.00	3,600
PHASE II CLINICAL TRIALS		* 80.00*	19,200
LUNG IMM.			
HEALTH RESEARCH, INC.	N CM9731100 R.R.P.		74,932
OTHER CLINICAL TRIALS RESEARCH		*100.00*	74,932
NON-SPECIFIC PHOTORADIATION THE			

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
HEALTH RESEARCH, INC.		N CM0741000 CTEP		12,365
PHASE IV CLINICAL TRIALS			* 90.00*	11,129
STOMACH	SURG.		25.00	3,091
STOMACH	RAD.		25.00	3,091
STOMACH	CHEM.		40.00	4,946
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	1,237
STOMACH	MARKERS			
HEALTH RESEARCH, INC.		N CM5703400 CTEP		21,465
PHASE IV CLINICAL TRIALS			*100.00*	21,465
COLORECTAL	SURG.		20.00	4,293
COLORECTAL	RAD.		15.00	3,220
COLORECTAL	CHEM.		45.00	9,659
COLORECTAL	IMM.		20.00	4,293
IIT RESEARCH INSTITUTE		N CM9731600 D.T.P.		899,998
ACQUISITION OF MATERIALS			* 15.00*	135,000
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN			* 71.00*	638,999
PRIMARY SCREENING, IN VIVO			64.00	575,999
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			7.00	63,000
VERIFICATION SCREEN			* 11.00*	99,000
DETAILED DRUG EVALUATION, IN VIVO				
TREATMENT STUDIES			* 3.00*	27,000
CHEMOTHERAPY				
IIT RESEARCH INSTITUTE		N CM9721300 D.T.P.		259,994
ACQUISITION OF MATERIALS			* 20.00*	51,999
BIOASSAY OF NATURAL PRODUCTS				
BASIC SCREEN			* 65.00*	168,996
PRIMARY SCREENING, IN VITRO			5.00	13,000
PRIMARY SCREENING, IN VIVO			55.00	142,997
DATA PROCESSING AND SUPPORT			5.00	13,000
VERIFICATION SCREEN			* 15.00*	38,999
DETAILED DRUG EVALUATION, IN VIVO				
IIT RESEARCH INSTITUTE		N CM0735903 D.T.P.		20,183
ACQUISITION OF MATERIALS			*100.00*	20,183
NEW AGENT PROCUREMENT, SYNTHETICS				
INDIANA UNIVERSITY FOUNDATION		N CM1747500 CTEP		34,299
PROGRAM MANAGEMENT			* 10.00*	3,430
ADMINISTRATION				
PHASE II CLINICAL TRIALS			* 30.00*	10,290

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
CNS TUMORS CHEM.			
PHASE III CLINICAL TRIALS		* 60.00*	20,579
CNS TUMORS RAD.		30.00	10,290
CNS TUMORS CHEM.		30.00	10,290
INFORMATION MANAGEMENT SERVICES,	N CP0102500B CTEP		110,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	110,000
LUNG DATA PROC. & SUPP.			
INFORMATION MANAGEMENT SERVICES,	N CM1734900 CTEP		12,759
PROGRAM MANAGEMENT		* 10.00*	1,276
ADMINISTRATION			
OTHER CLINICAL TRIALS RESEARCH		* 90.00*	11,483
CNS TUMORS DATA PROC. & SUPP.			
INSTITUT JULES BORDET	N CM0735000 D.T.P.		213,286
BASIC SCREEN		* 80.00*	170,629
PRIMARY SCREENING, IN VIVO			
VERIFICATION SCREEN		* 20.00*	42,657
DETAILED DRUG EVALUATION, IN VIVO			
INSTITUTE OF CANCER RESEARCH	N CM1750200 R.R.P.		222,200
ACQUISITION OF MATERIALS		* 60.00*	133,320
RAD. MODIFIERS			
BASIC SCREEN		* 40.00*	88,880
PRIMARY SCREENING, IN VITRO		20.00	44,440
PRIMARY SCREENING, IN VIVO		20.00	44,440
IOWA, UNIVERSITY OF	N CM0733400 BRMP		108,133
PROCUREMENT OF PRECLINICAL MATERIAL		* 70.00*	75,693
ANALYTICAL AND QUALITY CONTROL			
PROD. AND FORM. FOR CLINICAL TRIALS		* 30.00*	32,440
ANALYTICAL AND QUALITY CONTROL			
IOWA, UNIVERSITY OF	N CM1747600 CTEP		25,122
OTHER CLINICAL TRIALS RESEARCH		*100.00*	25,122
CNS TUMORS PATHOLOGY			
IOWA, UNIVERSITY OF	N CM0730300 D.T.P.		331,799
FORMULATION		* 20.00*	66,360
DEVEL. OF EXP. FORMULATIONS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	265,439
FORMULATION		70.00	232,259
ANALYTICAL AND QUALITY CONTROL		10.00	33,180

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400 O.D.		16,000
ACQUISITION OF MATERIALS		* 10.00*	1,600
NEW AGENT PROCUREMENT, SYNTHETICS		5.00	800
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		5.00	800
BASIC SCREEN		* 40.00*	6,400
PRIMARY SCREENING, IN VITRO		2.00	320
PRIMARY SCREENING, IN VIVO		18.00	2,880
DRUG EVALUATION		20.00	3,200
PROGRAM MANAGEMENT		* 20.00*	3,200
COMMUNICATION AND EDUCATION		10.00	1,600
RESOURCE DEVELOPMENT		10.00	1,600
PHASE I CLINICAL TRIALS		* 15.00*	2,400
LEUK.-ACUTE GRAN. CHEM.		5.00	800
LEUK.-ACUTE LYMPH. CHEM.		5.00	800
NON-SPECIFIC CHEM.		5.00	800
PHASE II CLINICAL TRIALS		* 15.00*	2,400
LEUK.-ACUTE GRAN. CHEM.		5.00	800
LEUK.-ACUTE LYMPH. CHEM.		5.00	800
NON-SPECIFIC CHEM.		5.00	800
JWK INTERNATIONAL CORP.	N CM2560200 O.D.		303,125
PROGRAM MANAGEMENT		*100.00*	303,125
COMMUNICATION AND EDUCATION			
KANSAS, UNIVERSITY OF	N CM0730400 D.T.P.		95,235
FORMULATION		*100.00*	95,235
DEVEL. OF EXP. FORMULATIONS			
KANSAS, UNIVERSITY OF-MEDICAL CENTER	N CM9727200 CTEP		24,318
PHASE I CLINICAL TRIALS		* 71.00*	17,266
NON-SPECIFIC CHEM.			
PHASE II CLINICAL TRIALS		* 29.00*	7,052
LEUK.-ACUTE GRAN. CHEM.		2.00	486
LEUK.-ACUTE LYMPH. CHEM.		2.00	486
GENERAL CHEM.		5.00	1,216
BREAST CHEM.		5.00	1,216
COLORECTAL CHEM.		5.00	1,216
LUNG CHEM.		5.00	1,216
MELANOMA CHEM.		5.00	1,216
KENTUCKY, UNIVERSITY OF	N CM0738100 D.T.P.		95,626
FORMULATION		*100.00*	95,626
DEVEL. OF EXP. FORMULATIONS			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
KING ANIMAL LABORATORY	N CM1749900 A.P.		134,160
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	26,832
BASIC SCREEN ANIMALS		* 55.00*	73,788
VERIFICATION SCREEN ANIMALS		* 25.00*	33,540
LABORATORY SUPPLY COMPANY, INC.	N CM9724400 A.P.		43,000
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	8,600
BASIC SCREEN ANIMALS		* 55.00*	23,650
VERIFICATION SCREEN ANIMALS		* 25.00*	10,750
LABORATORY SUPPLY COMPANY, INC.	N CM5057700 A.P.		165,360
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	33,072
BASIC SCREEN ANIMALS		* 55.00*	90,948
VERIFICATION SCREEN ANIMALS		* 25.00*	41,340
LEO GOODWIN INST. FOR CANCER RESEARCH	N CM7716500 A.P.		879,536
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	175,907
BASIC SCREEN ANIMALS		* 55.00*	483,745
VERIFICATION SCREEN ANIMALS		* 25.00*	219,884
LITTON BIONETICS, INC.	N CM1580800 BRMP		188,865
ACQUISITION OF MATERIALS COLL,STOR,DISTR -BRM		* 50.00*	94,433
VERIFICATION SCREEN RESPONSE MODIFIERS		* 50.00*	94,433
LITTON BIONETICS, INC.	N CO7538000K BRMP		1,132,000
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		* 48.00*	543,360
BASIC SCREEN		* 12.00*	135,840
PRIMARY SCREENING, IN VITRO		3.00	33,960
ANIMALS		9.00	101,880

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
VERIFICATION SCREEN		* 30.00*	339,600
ANIMALS			
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	113,200
FERMENTATION/ANTIBIOTICS			
LITTON BIONETICS, INC.	N CM6706700 C.O.P.		99,000
PROD. AND FORM. FOR CLINICAL TRIALS		* 10.00*	9,900
DATA PROCESSING AND SUPPORT			
CELLULAR/SUBCELLULAR STUDIES		* 90.00*	89,100
IMMUNOLOGY		90.00	89,100
LITTON BIONETICS, INC.	N CM1573700 C.O.P.		296,784
CELLULAR/SUBCELLULAR STUDIES		* 70.00*	207,749
BIOCHEMISTRY		20.00	59,357
CELL BIOLOGY		20.00	59,357
CELL KINETICS		10.00	29,678
MARKERS		10.00	29,678
CARCINOGENESIS		10.00	29,678
TREATMENT STUDIES		* 30.00*	89,035
COMB. MODAL. THERAPY		30.00	89,035
COMB. MODAL. THERAPY - SURG.		10.00	29,678
COMB. MODAL. THERAPY - CHEM.		10.00	29,678
COMB. MODAL. THERAPY - IMM.		10.00	29,678
LITTON BIONETICS, INC.	N C07538000D D.T.P.		2,594,000
ACQUISITION OF MATERIALS		* 48.00*	1,245,120
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			
BASIC SCREEN		* 12.00*	311,280
PRIMARY SCREENING, IN VITRO		3.00	77,320
ANIMALS		9.00	233,460
VERIFICATION SCREEN		* 30.00*	778,200
ANIMALS			
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	259,400
FERMENTATION/ANTIBIOTICS			
LITTON BIONETICS, INC.	N C07538000M ISBR		2,565,000
ACQUISITION OF MATERIALS		* 48.00*	1,231,200
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			
BASIC SCREEN		* 12.00*	307,800
PRIMARY SCREENING, IN VITRO		3.00	76,950
ANIMALS		9.00	230,850
VERIFICATION SCREEN		* 30.00*	769,500
ANIMALS			
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	256,500
FERMENTATION/ANTIBIOTICS			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
LITTON BIONETICS, INC.	N CM0734700 ISDT		544,137
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	272,069
ANTIBODIES/ANTIGENS			
CELLULAR/SUBCELLULAR STUDIES		* 50.00*	272,069
MOLECULAR BIOLOGY		50.00	272,069
LITTON BIONETICS, INC.	N CM2561600 ISDT		579,507
CELLULAR/SUBCELLULAR STUDIES		*100.00*	579,507
BIOCHEMISTRY		30.00	173,852
CELL BIOLOGY		30.00	173,852
IMMUNOBIOLOGY		10.00	57,951
MOLECULAR BIOLOGY		30.00	173,852
LITTON BIONETICS, INC.	N CM0732600 ISDT		150,000
ACQUISITION OF MATERIALS		*100.00*	150,000
ANIMAL VIRUSES			
LITTON BIONETICS, INC.	N CM0572400 ISDT		418,672
PROCUREMENT OF PRECLINICAL MATERIAL		*100.00*	418,672
ANIMAL FACILITIES			
MARSHALL RESEARCH ANIMALS, INC.	N CM6012300 A.P.		33,120
PHARMACOLOGY/TOXICOLOGY		*100.00*	33,120
ANIMALS			
MARYLAND, UNIVERSITY OF	N CM4374800 BCRP		500,000
CELLULAR/SUBCELLULAR STUDIES		* 25.00*	125,000
BIOCHEMISTRY		11.00	55,000
CELL BIOLOGY		1.00	5,000
CELL KINETICS		1.00	5,000
MOLECULAR BIOLOGY		11.00	55,000
DATA PROCESSING AND SUPPORT		1.00	5,000
PROGRAM MANAGEMENT		* 5.00*	25,000
ADMINISTRATION			
PHASE I CLINICAL TRIALS		* 8.00*	40,000
NON-SPECIFIC CHEM.		7.00	35,000
NON-SPECIFIC PHARM./TOX.		1.00	5,000
PHASE II CLINICAL TRIALS		* 21.00*	105,000
LEUK.-ACUTE GRAN. CHEM.		2.00	10,000
LEUK.-ACUTE GRAN. PAT. SUPPORT. CARE		1.00	5,000
LEUK.-ACUTE LYMPH. CHEM.		1.00	5,000
LEUK.-CHRON. GRAN. CHEM.		1.00	5,000
LEUK.-CHRON. GRAN. PAT. SUPPORT. CARE		1.00	5,000
LYMPHOMA-HODGKIN'S CHEM.		1.00	5,000

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
LYMPHOMA-LYMPHOCYT	CHEM.	1.00	5,000
BREAST	CHEM.	2.00	10,000
BREAST	PHARM./TOX.	1.00	5,000
BREAST	PAT. SUPPORT. CARE	1.00	5,000
COLORECTAL	CHEM.	1.00	5,000
KIDNEY	CHEM.	1.00	5,000
LUNG	CHEM.	1.00	5,000
LUNG	PHARM./TOX.	1.00	5,000
OVARIAN	CHEM.	1.00	5,000
SARCOMAS (GEN.)	CHEM.	1.00	5,000
SARCOMAS (GEN.)	PHARM./TOX.	1.00	5,000
NON-SPECIFIC	CHEM.	2.00	10,000
PHASE III CLINICAL TRIALS		* 20.00*	100,000
LEUK.-ACUTE GRAN.	CHEM.	3.00	15,000
LEUK.-ACUTE GRAN.	PAT. SUPPORT. CARE	1.00	5,000
LEUK.-ACUTE LYMPH.	CHEM.	1.00	5,000
LEUK.-ACUTE LYMPH.	PAT. SUPPORT. CARE	1.00	5,000
LYMPHOMA-HODGKIN'S	CHEM.	2.00	10,000
LYMPHOMA-HODGKIN'S	PAT. SUPPORT. CARE	1.00	5,000
LYMPHOMA-LYMPHOCYT	CHEM.	1.00	5,000
LYMPHOMA-MYCO.FUNG	CHEM.	1.00	5,000
MYELOMA	CHEM.	1.00	5,000
BREAST	CHEM.	1.00	5,000
COLORECTAL	CHEM.	1.00	5,000
LUNG	CHEM.	2.00	10,000
LUNG	PAT. SUPPORT. CARE	1.00	5,000
NON-SPECIFIC	CHEM.	2.00	10,000
NON-SPECIFIC	PAT. SUPPORT. CARE	1.00	5,000
PHASE IV CLINICAL TRIALS		* 19.00*	95,000
LEUK.-ACUTE GRAN.	CHEM.	4.00	20,000
LEUK.-ACUTE GRAN.	PAT. SUPPORT. CARE	1.00	5,000
LEUK.-ACUTE LYMPH.	CHEM.	1.00	5,000
LEUK.-CHRON LYMPH.	CHEM.	1.00	5,000
LYMPHOMA-HODGKIN'S	SURG.	2.00	10,000
LYMPHOMA-HODGKIN'S	RAD.	2.00	10,000
LYMPHOMA-HODGKIN'S	CHEM.	3.00	15,000
LYMPHOMA-HODGKIN'S	PAT. SUPPORT. CARE	2.00	10,000
LYMPHOMA-LYMPHOCYT	CHEM.	1.00	5,000
LYMPHOMA-LYMPHOCYT	PAT. SUPPORT. CARE	1.00	5,000
NON-SPECIFIC	CHEM.	1.00	5,000
OTHER CLINICAL TRIALS RESEARCH		* 2.00*	10,000
NON-SPECIFIC	DATA PROC. & SUPP.		
MARYLAND, UNIVERSITY OF	N CM8722300 CTEP		15,364
PHASE IV CLINICAL TRIALS		*100.00*	15,364
HEAD AND NECK	SURG.	30.00	4,609
HEAD AND NECK	RAD.	30.00	4,609
HEAD AND NECK	CHEM.	20.00	3,073
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	3,073

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MASON RESEARCH INSTITUTE	N CM8716400 A.P.		224,991
BASIC SCREEN		* 8.00*	17,999
TUMOR BANK			
PHARMACOLOGY/TOXICOLOGY		* 77.00*	173,243
PHARMACOLOGY			
CELLULAR/SUBCELLULAR STUDIES		* 15.00*	33,749
BIOCHEMISTRY		15.00	33,749
MASON RESEARCH INSTITUTE	N CM6701100 C.O.P.		294,113
TREATMENT STUDIES		*100.00*	294,113
DATA PROCESSING AND SUPPORT			
MASON RESEARCH INSTITUTE/EG&G	N CM9731700 D.T.P.		999,041
ACQUISITION OF MATERIALS		* 5.00*	49,952
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 61.00*	609,415
PRIMARY SCREENING, IN VIVO		50.00	499,521
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		11.00	109,895
VERIFICATION SCREEN		* 24.00*	239,770
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 10.00*	99,904
COMB. MODAL. THERAPY		10.00	99,904
COMB. MODAL. THERAPY - SURG.		8.00	79,923
COMB. MODAL. THERAPY - CHEM.		2.00	19,981
MASON RESEARCH INSTITUTE/EG&G	N CM0732500 D.T.P.		189,099
VERIFICATION SCREEN		*100.00*	189,099
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			
MASSACHUSETTS GENERAL HOSPITAL	N CM2753200 R.R.P.		84,969
TREATMENT STUDIES		*100.00*	84,969
RADIATION PHYSICS			
MASSACHUSETTS GENERAL HOSPITAL	N CM1748100 R.R.P.		52,812
PHASE II CLINICAL TRIALS		*100.00*	52,812
COLORECTAL RAD.		30.00	15,844
PANCREATIC RAD.		30.00	15,844
STOMACH RAD.		30.00	15,844
NON-SPEC SOL. TUM. RAD.		10.00	5,281
MASSACHUSETTS INSTITUTE OF	N CM2752500 R.R.P.		188,739
PHASE I CLINICAL TRIALS		* 50.00*	94,370
NON-SPECIFIC RAD.			

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 50.00*	94,370	
NON-SPECIFIC	HYPERTHERMIA			
MATHTECH, INC.	N CM9719500 CTEP			598,972
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	598,972	
NON-SPECIFIC	DATA PROC. & SUPP.			
MAYO FOUNDATION	N CM9727300 CTEP			64,216
PHASE I CLINICAL TRIALS		* 71.00*	45,593	
NON-SPECIFIC	CHEM.			
PHASE II CLINICAL TRIALS		* 29.00*	18,623	
LEUK.-ACUTE GRAN.	CHEM.	2.00	1,284	
LEUK.-ACUTE LYMPH.	CHEM.	2.00	1,284	
GENERAL	CHEM.	5.00	3,211	
BREAST	CHEM.	5.00	3,211	
COLORECTAL	CHEM.	5.00	3,211	
LUNG	CHEM.	5.00	3,211	
MELANOMA	CHEM.	5.00	3,211	
MAYO FOUNDATION	N CM9726800 CTEP			25,000
PROD. AND FORM. FOR CLINICAL TRIALS		* 5.00*	1,250	
FORMULATION				
CELLULAR/SUBCELLULAR STUDIES		* 5.00*	1,250	
NUTRITION		5.00	1,250	
PROGRAM MANAGEMENT		* 5.00*	1,250	
ADMINISTRATION		2.00	500	
COMMUNICATION AND EDUCATION		3.00	750	
PHASE I CLINICAL TRIALS		* 3.00*	750	
COLORECTAL	CHEM.			
PHASE II CLINICAL TRIALS		* 20.00*	5,000	
COLORECTAL	CHEM.	10.00	2,500	
PANCREATIC	CHEM.	5.00	1,250	
STOMACH	CHEM.	5.00	1,250	
PHASE III CLINICAL TRIALS		* 30.00*	7,500	
COLORECTAL	CHEM.	10.00	2,500	
LUNG	CHEM.	5.00	1,250	
STOMACH	RAD.	5.00	1,250	
STOMACH	CHEM.	10.00	2,500	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 32.00*	8,000	
COLORECTAL	SPEC. PHARM./TOX.	5.00	1,250	
COLORECTAL	BLOOD PRODUCTS	5.00	1,250	
COLORECTAL	PROTECTED ENVIRON.	5.00	1,250	
COLORECTAL	NUTRITION	5.00	1,250	
STOMACH	MARKERS	5.00	1,250	
COLORECTAL	DATA PROC. & SUPP.	2.00	500	
PANCREATIC	DIAG. RADIOLOGY	5.00	1,250	

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MAYO FOUNDATION		N CM0741900 D.T.P.		197,805
BASIC SCREEN			* 90.00*	178,025
PRIMARY SCREENING, IN VITRO				
VERIFICATION SCREEN			* 10.00*	19,781
DETAILED DRUG EVALUATION, IN VITRO				
MAYO FOUNDATION		N CM2752800 R.R.P.		98,465
PHASE II CLINICAL TRIALS			*100.00*	98,465
COLORECTAL	RAD.		30.00	29,540
PANCREATIC	RAD.		30.00	29,540
STOMACH	RAD.		30.00	29,540
NON-SPEC SOL. TUM.	RAD.		10.00	9,847
MELOY LABORATORIES, INC.		N CM1575700 BRMP		107,918
ACQUISITION OF MATERIALS			* 50.00*	53,959
COLL,STOR,DISTR -BRM				
VERIFICATION SCREEN			* 50.00*	53,959
RESPONSE MODIFIERS				
MEMORIAL HOSP. FOR CANCER & ALLIED		N CM1734800 CTEP		71,420
PROGRAM MANAGEMENT			* 10.00*	7,142
ADMINISTRATION				
PHASE II CLINICAL TRIALS			* 30.00*	21,426
CNS TUMORS	CHEM.			
PHASE III CLINICAL TRIALS			* 60.00*	42,852
CNS TUMORS	RAD.		30.00	21,426
CNS TUMORS	CHEM.		30.00	21,426
MEMORIAL HOSP. FOR CANCER & ALLIED		N CM1573500 CTEP		74,683
CLINICAL TRIALS SUPPORTIVE RESEARCH			*100.00*	74,683
OTHER	NUTRITION			
MEMORIAL HOSP. FOR CANCER & ALLIED		N CM0733700 CTEP		225,189
PHASE II CLINICAL TRIALS			* 55.00*	123,854
BREAST	CHEM.		15.00	33,778
COLORECTAL	CHEM.		15.00	33,778
HEAD AND NECK	CHEM.		5.00	11,259
LUNG	CHEM.		5.00	11,259
MELANOMA	CHEM.		10.00	22,519
SARCOMAS (GEN.)	CHEM.		5.00	11,259
PHASE III CLINICAL TRIALS			* 45.00*	101,335
BREAST	CHEM.		10.00	22,519
COLORECTAL	CHEM.		5.00	11,259
HEAD AND NECK	CHEM.		5.00	11,259

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
LUNG	CHEM.		5.00	11,259
MELANOMA	CHEM.		15.00	33,778
SARCOMAS (GEN.)	CHEM.		5.00	11,259
MEMORIAL HOSP. FOR CANCER & ALLIED		N CM8722400 CTEP		25,300
PHASE IV CLINICAL TRIALS			*100.00*	25,300
HEAD AND NECK	SURG.		30.00	7,590
HEAD AND NECK	RAD.		30.00	7,590
HEAD AND NECK	CHEM.		20.00	5,060
HEAD AND NECK	PAT. SUPPORT. CARE		20.00	5,060
MEMORIAL HOSP. FOR CANCER & ALLIED		N CM9727400 CTEP		44,105
PHASE I CLINICAL TRIALS			* 71.00*	31,315
NON-SPECIFIC	CHEM.			
PHASE II CLINICAL TRIALS			* 29.00*	12,790
LEUK.-ACUTE GRAN.	CHEM.		2.00	882
LEUK.-ACUTE LYMPH.	CHEM.		2.00	882
GENERAL	CHEM.		5.00	2,205
BREAST	CHEM.		5.00	2,205
COLORECTAL	CHEM.		5.00	2,205
LUNG	CHEM.		5.00	2,205
MELANOMA	CHEM.		5.00	2,205
MIAMI, UNIVERSITY OF		N CM6709300 CTEP		26,978
PHASE IV CLINICAL TRIALS			*100.00*	26,978
COLORECTAL	SURG.		20.00	5,396
COLORECTAL	RAD.		15.00	4,047
COLORECTAL	CHEM.		45.00	12,140
COLORECTAL	IMM.		20.00	5,396
MIAMI, UNIVERSITY OF		N CM0740900 CTEP		60,874
PHASE IV CLINICAL TRIALS			* 90.00*	54,787
STOMACH	SURG.		25.00	15,219
STOMACH	RAD.		25.00	15,219
STOMACH	CHEM.		40.00	24,350
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	6,087
STOMACH	MARKERS			
MICHIGAN TECHNOLOGICAL		N CM0729300 D.T.P.		34,000
ACQUISITION OF MATERIALS			* 95.00*	32,300
NEW AGENT PROCUREMENT, SYNTHETICS				
BASIC SCREEN			* 5.00*	1,700
PRIMARY SCREENING, IN VITRO				

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MICHIGAN, UNIVERSITY OF	N CM0740500 CTEP		244,210
PHASE II CLINICAL TRIALS		* 55.00*	134,316
BREAST	CHEM.	15.00	36,632
COLORECTAL	CHEM.	15.00	36,632
HEAD AND NECK	CHEM.	5.00	12,211
LUNG	CHEM.	5.00	12,211
MELANOMA	CHEM.	10.00	24,421
SARCOMAS (GEN.)	CHEM.	5.00	12,211
PHASE III CLINICAL TRIALS		* 45.00*	109,895
BREAST	CHEM.	10.00	24,421
COLORECTAL	CHEM.	5.00	12,211
HEAD AND NECK	CHEM.	5.00	12,211
LUNG	CHEM.	5.00	12,211
MELANOMA	CHEM.	15.00	36,632
SARCOMAS (GEN.)	CHEM.	5.00	12,211
MICHIGAN, UNIVERSITY OF	N CM8722500 CTEP		38,333
PHASE IV CLINICAL TRIALS		*100.00*	38,333
HEAD AND NECK	SURG.	30.00	11,500
HEAD AND NECK	RAD.	30.00	11,500
HEAD AND NECK	CHEM.	20.00	7,667
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	7,667
MICROBIAL CHEMISTRY RESEARCH FDN.	N CM5700900 D.T.P.		266,000
ACQUISITION OF MATERIALS		* 60.00*	159,600
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			
BASIC SCREEN		* 40.00*	106,400
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			
MICROBIOLOGICAL ASSOCIATES	N CM9724600 A.P.		130,934
ACQUISITION OF MATERIALS		* 20.00*	26,187
ANIMALS			
BASIC SCREEN		* 55.00*	72,014
ANIMALS			
VERIFICATION SCREEN		* 25.00*	32,734
ANIMALS			
MICROBIOLOGICAL ASSOCIATES	N CM0736900 C.O.P.		132,906
CELLULAR/SUBCELLULAR STUDIES		*100.00*	132,906
IMMUNOBIOLOGY		50.00	66,453
SERUM STORAGE		50.00	66,453
MIDWEST RESEARCH INSTITUTE	N CM8723400 D.T.P.		162,593
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	32,519

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ANALYTICAL AND QUALITY CONTROL				
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	130,074	
ANALYTICAL AND QUALITY CONTROL				
MISSOURI, UNIVERSITY OF	N CM8715700 A.P.			136,605
ACQUISITION OF MATERIALS		* 20.00*	27,321	
ANIMALS				
BASIC SCREEN		* 55.00*	75,133	
ANIMALS				
VERIFICATION SCREEN		* 25.00*	34,151	
ANIMALS				
MONTEFIORE HOSPITAL	N CM1747400 CTEP			61,318
PROGRAM MANAGEMENT		* 10.00*	6,132	
ADMINISTRATION				
PHASE II CLINICAL TRIALS		* 30.00*	18,395	
CNS TUMORS	CHEM.			
PHASE III CLINICAL TRIALS		* 60.00*	36,791	
CNS TUMORS	RAD.	30.00	18,395	
CNS TUMORS	CHEM.	30.00	18,395	
MOUNT SINAI SCHOOL OF MEDICINE	N CM0740700 CTEP			33,216
PHASE IV CLINICAL TRIALS		* 90.00*	29,894	
STOMACH	SURG.	25.00	8,304	
STOMACH	RAD.	25.00	8,304	
STOMACH	CHEM.	40.00	13,286	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	3,322	
STOMACH	MARKERS			
MOUNT SINAI SCHOOL OF MEDICINE	N CM6709600 CTEP			19,706
PHASE IV CLINICAL TRIALS		* 90.00*	17,735	
COLORECTAL	SURG.	18.00	3,547	
COLORECTAL	RAD.	18.00	3,547	
COLORECTAL	CHEM.	36.00	7,094	
COLORECTAL	IMM.	18.00	3,547	
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	1,971	
COLORECTAL	MARKERS			
MOUNT SINAI SCHOOL OF MEDICINE	N CB4337900B CTEP			60,493
CELLULAR/SUBCELLULAR STUDIES		* 15.00*	9,074	
IMMUNOBIOLOGY		15.00	9,074	
TREATMENT STUDIES		* 10.00*	6,049	
IMMUNOTHERAPY				
PHASE II CLINICAL TRIALS		* 75.00*	45,370	
LEUK.-ACUTE GRAN.	CHEM.	25.00	15,123	
LEUK.-ACUTE GRAN.	IMM.	50.00	30,247	

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MOUNT SINAI SCHOOL OF MEDICINE	N CM9727500 CTEP		32,796
PHASE I CLINICAL TRIALS		* 71.00*	23,285
NON-SPECIFIC CHEM.			
PHASE II CLINICAL TRIALS		* 29.00*	9,511
LEUK.-ACUTE GRAN. CHEM.		2.00	656
LEUK.-ACUTE LYMPH. CHEM.		2.00	656
GENERAL CHEM.		5.00	1,640
BREAST CHEM.		5.00	1,640
COLORECTAL CHEM.		5.00	1,640
LUNG CHEM.		5.00	1,640
MELANOMA CHEM.		5.00	1,640
MUHIMBILI MEDICAL CENTER	N CM0734400 CTEP		50,000
PHASE III CLINICAL TRIALS		* 90.00*	45,000
SKIN CHEM.			
OTHER CLINICAL TRIALS RESEARCH		* 10.00*	5,000
SKIN PROTOCOL-REL. STAT		5.00	2,500
SKIN DATA PROC. & SUPP.		5.00	2,500
MURPHY BREEDING LABS., INC.	N CM5057900A A.P.		168,792
ACQUISITION OF MATERIALS		* 20.00*	33,758
ANIMALS			
BASIC SCREEN		* 55.00*	92,836
ANIMALS			
VERIFICATION SCREEN		* 25.00*	42,198
ANIMALS			
NATIONAL ACADEMY OF SCIENCES	N CM5385000 A.P.		29,000
PROGRAM MANAGEMENT		*100.00*	29,000
COMMUNICATION AND EDUCATION			
NATIONAL NAVAL MEDICAL CENTER	Y CM0010300 C.O.P.		382,000
PROGRAM MANAGEMENT		* 2.00*	7,640
COMMUNICATION AND EDUCATION		1.00	3,820
RESOURCE DEVELOPMENT		1.00	3,820
PHASE II CLINICAL TRIALS		* 80.50*	307,510
LEUK.-ACUTE GRAN. CHEM.		1.00	3,820
LEUK.-ACUTE GRAN. PAT. SUPPORT. CARE		1.00	3,820
LEUK.-ACUTE LYMPH. CHEM.		1.50	5,730
LEUK.-ACUTE LYMPH. IMM.		0.30	1,146
LEUK.-ACUTE LYMPH. PAT. SUPPORT. CARE		1.50	5,730
LEUK.-CHRON. GRAN. CHEM.		0.50	1,910
LEUK.-CHRON. GRAN. PAT. SUPPORT. CARE		0.20	764
LEUK.-CHRON LYMPH. CHEM.		0.50	1,910
LEUK.-CHRON LYMPH. PAT. SUPPORT. CARE		0.50	1,910

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
LYMPHOMA-HISTIOCYT	CHEM.	0.50	1,910
LYMPHOMA-HISTIOCYT	PAT. SUPPORT. CARE	0.50	1,910
LYMPHOMA-HODGKIN'S	RAD.	1.00	3,820
LYMPHOMA-HODGKIN'S	CHEM.	1.00	3,820
LYMPHOMA-HODGKIN'S	PHARM./TOX.	0.50	1,910
LYMPHOMA-HODGKIN'S	PAT. SUPPORT. CARE	1.00	3,820
LYMPHOMA-LYMPHOCYT	CHEM.	0.50	1,910
LYMPHOMA-LYMPHOCYT	PAT. SUPPORT. CARE	0.50	1,910
NON-HODGKIN LYMPH.	RAD.	1.00	3,820
NON-HODGKIN LYMPH.	CHEM.	1.00	3,820
NON-HODGKIN LYMPH.	PHARM./TOX.	0.50	1,910
NON-HODGKIN LYMPH.	PAT. SUPPORT. CARE	1.00	3,820
MYELOMA	CHEM.	2.00	7,640
MYELOMA	PAT. SUPPORT. CARE	2.00	7,640
BLADDER	CHEM.	0.50	1,910
BLADDER	PAT. SUPPORT. CARE	0.50	1,910
BREAST	SURG.	3.00	11,460
BREAST	RAD.	3.00	11,460
BREAST	CHEM.	6.00	22,920
BREAST	PHARM./TOX.	1.00	3,820
BREAST	PAT. SUPPORT. CARE	3.00	11,460
COLORECTAL	SURG.	2.00	7,640
COLORECTAL	RAD.	1.00	3,820
COLORECTAL	CHEM.	2.00	7,640
COLORECTAL	PAT. SUPPORT. CARE	4.00	15,280
ESOPHAGEAL	CHEM.	0.50	1,910
ESOPHAGEAL	PAT. SUPPORT. CARE	0.50	1,910
HEAD AND NECK	SURG.	1.00	3,820
HEAD AND NECK	RAD.	0.50	1,910
HEAD AND NECK	CHEM.	1.50	5,730
HEAD AND NECK	PAT. SUPPORT. CARE	0.50	1,910
KIDNEY	CHEM.	1.00	3,820
KIDNEY	PAT. SUPPORT. CARE	1.00	3,820
LUNG	SURG.	3.00	11,460
LUNG	RAD.	3.00	11,460
LUNG	CHEM.	5.00	19,100
LUNG	IMM.	3.00	11,460
LUNG	PAT. SUPPORT. CARE	4.00	15,280
MELANOMA	SURG.	0.50	1,910
MELANOMA	CHEM.	0.50	1,910
MELANOMA	IMM.	0.50	1,910
MELANOMA	PAT. SUPPORT. CARE	0.50	1,910
PANCREATIC	CHEM.	0.50	1,910
PANCREATIC	PAT. SUPPORT. CARE	0.50	1,910
PROSTATIC	CHEM.	0.50	1,910
PROSTATIC	PAT. SUPPORT. CARE	0.50	1,910
SARCOMAS (GEN.)	CHEM.	0.50	1,910
SARCOMAS (GEN.)	PAT. SUPPORT. CARE	0.50	1,910
STOMACH	SURG.	0.50	1,910
STOMACH	CHEM.	0.50	1,910
STOMACH	PAT. SUPPORT. CARE	0.50	1,910

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
TESTICULAR	SURG.	0.50	1,910
TESTICULAR	CHEM.	2.00	7,640
TESTICULAR	PAT. SUPPORT. CARE	1.00	3,820
OTHER CLINICAL TRIALS RESEARCH		* 8.60*	32,852
LEUK.-ACUTE GRAN.	PROTOCOL-REL. STAT	0.30	1,146
LEUK.-ACUTE LYMPH.	PROTOCOL-REL. STAT	0.20	764
LEUK.-CHRON. GRAN.	PROTOCOL-REL. STAT	0.10	382
LYMPHOMA-HODGKIN'S	PROTOCOL-REL. STAT	0.50	1,910
NON-HODGKIN LYMPH.	PROTOCOL-REL. STAT	0.50	1,910
BREAST	PROTOCOL-REL. STAT	1.00	3,820
COLORECTAL	PROTOCOL-REL. STAT	0.50	1,910
HEAD AND NECK	PROTOCOL-REL. STAT	0.50	1,910
LUNG	PROTOCOL-REL. STAT	0.50	1,910
TESTICULAR	PROTOCOL-REL. STAT	0.20	764
LEUK.-ACUTE GRAN.	DATA PROC. & SUPP.	0.50	1,910
LYMPHOMA-HODGKIN'S	DATA PROC. & SUPP.	0.50	1,910
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	0.50	1,910
BREAST	DATA PROC. & SUPP.	1.00	3,820
COLORECTAL	DATA PROC. & SUPP.	0.50	1,910
HEAD AND NECK	DATA PROC. & SUPP.	0.50	1,910
LUNG	DATA PROC. & SUPP.	0.50	1,910
TESTICULAR	DATA PROC. & SUPP.	0.30	1,146
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	0.50	1,910
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 8.90*	33,998
LEUK.-ACUTE GRAN.	CELL KINETICS	0.20	764
LEUK.-ACUTE LYMPH.	CELL KINETICS	0.20	764
LEUK.-ACUTE LYMPH.	MARKERS	0.30	1,146
LEUK.-CHRON. GRAN.	CELL KINETICS	0.10	382
LEUK.-CHRON. GRAN.	MARKERS	0.10	382
LEUK.-CHRON LYMPH.	CELL KINETICS	0.50	1,910
LEUK.-CHRON LYMPH.	MARKERS	0.50	1,910
LYMPHOMA-HODGKIN'S	BLOOD PRODUCTS	0.50	1,910
NON-HODGKIN LYMPH.	CELL KINETICS	1.00	3,820
NON-HODGKIN LYMPH.	MARKERS	1.00	3,820
NON-HODGKIN LYMPH.	BLOOD PRODUCTS	0.50	1,910
MYELOMA	TRANSFUSION RES.	1.00	3,820
POLYCYTHERMIA VERA	CELL KINETICS	0.50	1,910
POLYCYTHERMIA VERA	TRANSFUSION RES.	0.50	1,910
HEAD AND NECK	NUTRITION	0.50	1,910
LUNG	SPEC. PHARM./TOX.	0.50	1,910
LUNG	MARKERS	0.50	1,910
STOMACH	NUTRITION	0.50	1,910
NAVAL MEDICAL RESEARCH INSTITUTE	Y CB0031900B ISBR		140,500
CELLULAR/SUBCELLULAR STUDIES		*100.00*	140,500
IMMUNOBIOLOGY		100.00	140,500
NEW MEXICO, UNIVERSITY OF	N CM2753100 R.R.P.		49,320
TREATMENT STUDIES		*100.00*	49,320

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
TESTICULAR	SURG.	0.50	1,910
TESTICULAR	CHEM.	2.00	7,640
TESTICULAR	PAT. SUPPORT. CARE	1.00	3,820
OTHER CLINICAL TRIALS RESEARCH		* 8.60*	32,852
LEUK.-ACUTE GRAN.	PROTOCOL-REL. STAT	0.30	1,146
LEUK.-ACUTE LYMPH.	PROTOCOL-REL. STAT	0.20	764
LEUK.-CHRON. GRAN.	PROTOCOL-REL. STAT	0.10	382
LYMPHOMA-HODGKIN'S	PROTOCOL-REL. STAT	0.50	1,910
NON-HODGKIN LYMPH.	PROTOCOL-REL. STAT	0.50	1,910
BREAST	PROTOCOL-REL. STAT	1.00	3,820
COLORECTAL	PROTOCOL-REL. STAT	0.50	1,910
HEAD AND NECK	PROTOCOL-REL. STAT	0.50	1,910
LUNG	PROTOCOL-REL. STAT	0.50	1,910
TESTICULAR	PROTOCOL-REL. STAT	0.20	764
LEUK.-ACUTE GRAN.	DATA PROC. & SUPP.	0.50	1,910
LYMPHOMA-HODGKIN'S	DATA PROC. & SUPP.	0.50	1,910
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	0.50	1,910
BREAST	DATA PROC. & SUPP.	1.00	3,820
COLORECTAL	DATA PROC. & SUPP.	0.50	1,910
HEAD AND NECK	DATA PROC. & SUPP.	0.50	1,910
LUNG	DATA PROC. & SUPP.	0.50	1,910
TESTICULAR	DATA PROC. & SUPP.	0.30	1,146
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	0.50	1,910
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 8.90*	33,998
LEUK.-ACUTE GRAN.	CELL KINETICS	0.20	764
LEUK.-ACUTE LYMPH.	CELL KINETICS	0.20	764
LEUK.-ACUTE LYMPH.	MARKERS	0.30	1,146
LEUK.-CHRON. GRAN.	CELL KINETICS	0.10	382
LEUK.-CHRON. GRAN.	MARKERS	0.10	382
LEUK.-CHRON LYMPH.	CELL KINETICS	0.50	1,910
LEUK.-CHRON LYMPH.	MARKERS	0.50	1,910
LYMPHOMA-HODGKIN'S	BLOOD PRODUCTS	0.50	1,910
NON-HODGKIN LYMPH.	CELL KINETICS	1.00	3,820
NON-HODGKIN LYMPH.	MARKERS	1.00	3,820
NON-HODGKIN LYMPH.	BLOOD PRODUCTS	0.50	1,910
MYELOMA	TRANSFUSION RES.	1.00	3,820
POLYCYTHERMIA VERA	CELL KINETICS	0.50	1,910
POLYCYTHERMIA VERA	TRANSFUSION RES.	0.50	1,910
HEAD AND NECK	NUTRITION	0.50	1,910
LUNG	SPEC. PHARM./TOX.	0.50	1,910
LUNG	MARKERS	0.50	1,910
STOMACH	NUTRITION	0.50	1,910
NAVAL MEDICAL RESEARCH INSTITUTE	Y CB0031900B ISBR		140,500
CELLULAR/SUBCELLULAR STUDIES		*100.00*	140,500
IMMUNOBIOLOGY		100.00	140,500
NEW MEXICO, UNIVERSITY OF	N CM2753100 R.R.P.		49,320
TREATMENT STUDIES		*100.00*	49,320

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
RADIATION PHYSICS			
NEW YORK UNIVERSITY MEDICAL CENTER	N CM1747300 CTEP		54,674
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	5,467
PHASE II CLINICAL TRIALS		* 30.00*	16,402
CNS TUMORS CHEM.			
PHASE III CLINICAL TRIALS		* 60.00*	32,804
CNS TUMORS RAD.		30.00	16,402
CNS TUMORS CHEM.		30.00	16,402
NORTH CAROLINA, UNIVERSITY OF	N CM1747100 CTEP		86,151
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	8,615
PHASE II CLINICAL TRIALS		* 30.00*	25,845
CNS TUMORS CHEM.			
PHASE III CLINICAL TRIALS		* 60.00*	51,691
CNS TUMORS RAD.		30.00	25,845
CNS TUMORS CHEM.		30.00	25,845
NORTHERN CALIFORNIA CANCER PROGRAM	N CM0744302 BRMP		32,458
PHASE I CLINICAL TRIALS		* 50.00*	16,229
NON-SPECIFIC IMM.			
PHASE II CLINICAL TRIALS		* 50.00*	16,229
NON-SPECIFIC IMM.			
NORTHERN CALIFORNIA CANCER PROGRAM	N CM0744301 BRMP		52,342
PHASE I CLINICAL TRIALS		* 50.00*	26,171
NON-SPECIFIC IMM.			
PHASE II CLINICAL TRIALS		* 50.00*	26,171
NON-SPECIFIC IMM.			
NORTHERN CALIFORNIA CANCER PROGRAM	N CM8715400 CTEP		30,001
PHASE IV CLINICAL TRIALS		*100.00*	30,001
HEAD AND NECK SURG.		30.00	9,000
HEAD AND NECK RAD.		30.00	9,000
HEAD AND NECK CHEM.		20.00	6,000
HEAD AND NECK PAT. SUPPORT. CARE		20.00	6,000
NORTHROP SERVICES, INC.	N CM0728600 A.P.		69,084
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	13,817
BASIC SCREEN		* 55.00*	37,996
ANIMALS			
VERIFICATION SCREEN		* 25.00*	17,271

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS			
NORTHWESTERN UNIVERSITY	N CM1736300 A.P.		60,423
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	12,085
BASIC SCREEN ANIMALS		* 55.00*	33,233
VERIFICATION SCREEN ANIMALS		* 25.00*	15,106
OHIO STATE UNIVERSITY RESEARCH	N CM8716100 D.T.P.		102,158
PHARMACOLOGY/TOXICOLOGY ANAL. METH.		* 25.00*	25,540
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC SPEC. PHARM./TOX.		* 75.00*	76,619
OHIO STATE UNIVERSITY RESEARCH	N CM9726400 D.T.P.		40,000
PHARMACOLOGY/TOXICOLOGY PHARMACOLOGY		*100.00*	40,000
PAN AMERICAN HEALTH ORGANIZATION	N CM2739100 CTEP		266,031
PROGRAM MANAGEMENT		* 65.00*	172,920
ADMINISTRATION		40.00	106,412
COMMUNICATION AND EDUCATION		15.00	39,905
DATA PROCESSING AND SUPPORT		10.00	26,603
OTHER CLINICAL TRIALS RESEARCH		* 35.00*	93,111
LYMPHOMA-HISTIOCYT DATA PROC. & SUPP.		2.00	5,321
LYMPHOMA-HODGKIN'S DATA PROC. & SUPP.		2.00	5,321
NON-HODGKIN LYMPH. DATA PROC. & SUPP.		2.00	5,321
MYELOMA DATA PROC. & SUPP.		2.00	5,321
BREAST DATA PROC. & SUPP.		3.00	7,981
CNS TUMORS DATA PROC. & SUPP.		2.00	5,321
ESOPHAGEAL DATA PROC. & SUPP.		1.00	2,660
HEAD AND NECK DATA PROC. & SUPP.		3.00	7,981
LUNG DATA PROC. & SUPP.		1.00	2,660
MELANOMA DATA PROC. & SUPP.		1.00	2,660
OVARIAN DATA PROC. & SUPP.		0.50	1,330
SARCOMAS (GEN.) DATA PROC. & SUPP.		6.50	17,292
STOMACH DATA PROC. & SUPP.		1.00	2,660
UTERINE CERVIX DATA PROC. & SUPP.		7.50	19,952
OTHER DATA PROC. & SUPP.		0.50	1,330
NON-HODGKIN LYMPH. DATA PROC. & SUPP.		2.00	5,321
PAN AMERICAN HEALTH ORGANIZATION	N CO6533200C CTEP		45,333
PROGRAM MANAGEMENT		* 65.00*	29,466
ADMINISTRATION		40.00	18,133

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
COMMUNICATION AND EDUCATION		15.00	6,800
DATA PROCESSING AND SUPPORT		10.00	4,533
OTHER CLINICAL TRIALS RESEARCH		* 35.00*	15,867
LYMPHOMA-HISTIOCYT	DATA PROC. & SUPP.	2.00	907
LYMPHOMA-HODGKIN'S	DATA PROC. & SUPP.	2.00	907
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	2.00	907
MYELOMA	DATA PROC. & SUPP.	2.00	907
BREAST	DATA PROC. & SUPP.	3.00	1,360
CNS TUMORS	DATA PROC. & SUPP.	2.00	907
ESOPHAGEAL	DATA PROC. & SUPP.	1.00	453
HEAD AND NECK	DATA PROC. & SUPP.	3.00	1,360
LUNG	DATA PROC. & SUPP.	1.00	453
MELANOMA	DATA PROC. & SUPP.	1.00	453
OVARIAN	DATA PROC. & SUPP.	0.50	227
SARCOMAS (GEN.)	DATA PROC. & SUPP.	6.50	2,947
STOMACH	DATA PROC. & SUPP.	1.00	453
UTERINE CERVIX	DATA PROC. & SUPP.	7.50	3,400
OTHER	DATA PROC. & SUPP.	0.50	227
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	2.00	907
PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000 A.P.		141,907
ACQUISITION OF MATERIALS		* 20.00*	28,381
ANIMALS			
BASIC SCREEN		* 55.00*	78,049
ANIMALS			
VERIFICATION SCREEN		* 25.00*	35,477
ANIMALS			
PENNSYLVANIA, UNIVERSITY OF	N CM2752900 R.R.P.		63,725
TREATMENT STUDIES		*100.00*	63,725
RADIATION PHYSICS			
PHARM-ECO	N CM1748700 D.T.P.		282,216
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	141,108
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	141,108
PRODUCTION, SYNTHETICS			
PHILIPS ROXANE LABORATORIES, INC.	N CM6705300 D.T.P.		75,000
FORMULATION		* 20.00*	15,000
DEVEL. OF EXP. FORMULATIONS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	60,000
FORMULATION		70.00	52,500
ANALYTICAL AND QUALITY CONTROL		10.00	7,500

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
POLYSCIENCES, INC.	N CM0730000 D.T.P.		270,000
ACQUISITION OF MATERIALS		*100.00*	270,000
NEW AGENT PROCUREMENT, PLANT PRODUCTS		79.00	213,300
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		10.00	27,000
BIOASSAY OF NATURAL PRODUCTS		11.00	29,700
RESEARCH TRIANGLE INSTITUTE	N CM0735206 D.T.P.		11,978
ACQUISITION OF MATERIALS		*100.00*	11,978
NEW AGENT PROCUREMENT, SYNTHETICS			
RESEARCH TRIANGLE INSTITUTE	N CM0735207 D.T.P.		12,293
ACQUISITION OF MATERIALS		*100.00*	12,293
NEW AGENT PROCUREMENT, SYNTHETICS			
SASCO, INC.	N CM9016400 A.P.		114,400
ACQUISITION OF MATERIALS		* 20.00*	22,880
ANIMALS			
BASIC SCREEN		* 55.00*	62,920
ANIMALS			
VERIFICATION SCREEN		* 25.00*	28,600
ANIMALS			
SIDNEY FARBER CANCER INSTITUTE	N CM5703500 CTEP		12,000
PHASE IV CLINICAL TRIALS		*100.00*	12,000
COLORECTAL RAD.		3.00	360
COLORECTAL CHEM.		35.00	4,200
COLORECTAL IMM.		22.00	2,640
COLORECTAL PAT. SUPPORT. CARE		40.00	4,800
SIMONSEN LABORATORIES	N CM9724700 A.P.		40,000
ACQUISITION OF MATERIALS		* 20.00*	8,000
ANIMALS			
BASIC SCREEN		* 55.00*	22,000
ANIMALS			
VERIFICATION SCREEN		* 25.00*	10,000
ANIMALS			
SIMONSEN LABORATORIES	N CM7716600 A.P.		896,358
ACQUISITION OF MATERIALS		* 20.00*	179,272
ANIMALS			
BASIC SCREEN		* 55.00*	492,997
ANIMALS			
VERIFICATION SCREEN		* 25.00*	224,090

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS			
SIMONSEN LABORATORIES	N CM5057800 A.P.		188,448
ACQUISITION OF MATERIALS		* 20.00*	37,690
ANIMALS			
BASIC SCREEN		* 55.00*	103,646
ANIMALS			
VERIFICATION SCREEN		* 25.00*	47,112
ANIMALS			
SISA, INC.	N CM0735405 D.T.P.		4,347
ACQUISITION OF MATERIALS		*100.00*	4,347
NEW AGENT PROCUREMENT, SYNTHETICS			
SLOAN-KETTERING INSTITUTE FOR CANCER	N CM0743501 BRMP		20,000
PHASE I CLINICAL TRIALS		* 50.00*	10,000
NON-SPECIFIC IMM.			
PHASE II CLINICAL TRIALS		* 50.00*	10,000
NON-SPECIFIC IMM.			
SLOAN-KETTERING INSTITUTE FOR CANCER	N CM0743502 BRMP		160,000
PHASE I CLINICAL TRIALS		* 50.00*	80,000
NON-SPECIFIC IMM.			
PHASE II CLINICAL TRIALS		* 50.00*	80,000
NON-SPECIFIC IMM.			
SMALL BUSINESS ADMINISTRATION	N CM1740000 D.T.P.		42,000
ACQUISITION OF MATERIALS		*100.00*	42,000
DATA PROCESSING AND SUPPORT			
SMALL BUSINESS ADMINISTRATION	N CM4371900 ISDT		144,000
ACQUISITION OF MATERIALS		* 70.00*	100,800
ANIMAL CELLS			
CELLULAR/SUBCELLULAR STUDIES		* 30.00*	43,200
MOLECULAR BIOLOGY		10.00	14,400
RADIOIMMUNE ASSAY		20.00	28,800
SOCIAL & SCIENTIFIC SYSTEMS, INC.	N CM2560600 CTEP		142,046
PROGRAM MANAGEMENT		*100.00*	142,046
COMMUNICATION AND EDUCATION			
SOCIAL & SCIENTIFIC SYSTEMS, INC.	N CM1752100 CTEP		303,333
PROGRAM MANAGEMENT		*100.00*	303,333

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
DATA PROCESSING AND SUPPORT			
SOUTH FLORIDA, UNIVERSITY OF	N CM8722000 CTEP		61,431
PHASE IV CLINICAL TRIALS		*100.00*	61,431
HEAD AND NECK SURG.		30.00	18,429
HEAD AND NECK RAD.		30.00	18,429
HEAD AND NECK CHEM.		20.00	12,286
HEAD AND NECK PAT. SUPPORT. CARE		20.00	12,286
SOUTHERN ANIMAL FARMS	N CM9724500 A.P.		75,459
ACQUISITION OF MATERIALS		* 20.00*	15,092
ANIMALS			
BASIC SCREEN		* 55.00*	41,502
ANIMALS			
VERIFICATION SCREEN		* 25.00*	18,865
ANIMALS			
SOUTHERN ANIMAL FARMS	N CM5059900 A.P.		141,960
ACQUISITION OF MATERIALS		* 20.00*	28,392
ANIMALS			
BASIC SCREEN		* 55.00*	78,078
ANIMALS			
VERIFICATION SCREEN		* 25.00*	35,490
ANIMALS			
SOUTHERN CALIFORNIA, UNIVERSITY OF	N CM2748300 R.R.P.		58,790
PHASE II CLINICAL TRIALS		*100.00*	58,790
LUNG RAD.			
SOUTHERN RESEARCH INSTITUTE	N CM0726008 D.T.P.		5,000
ACQUISITION OF MATERIALS		*100.00*	5,000
NEW AGENT PROCUREMENT, SYNTHETICS			
SOUTHERN RESEARCH INSTITUTE	N CM9730900 D.T.P.		1,995,415
ACQUISITION OF MATERIALS		* 1.00*	19,954
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 32.00*	638,533
PRIMARY SCREENING, IN VITRO		4.00	79,817
PRIMARY SCREENING, IN VIVO		16.00	319,266
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		10.00	199,542
ANALOG SCREENING		2.00	39,908
VERIFICATION SCREEN		* 26.00*	518,808
DETAILED DRUG EVALUATION, IN VIVO		14.00	279,358
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		10.00	199,542
ANTIVIRAL ACTIVITY		2.00	39,908

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PHARMACOLOGY/TOXICOLOGY		* 2.00*	39,908
SPECIAL TOXICITY STUDIES		1.00	19,954
BIOASSAY-DRUG METAB.		1.00	19,954
CELLULAR/SUBCELLULAR STUDIES		* 1.00*	19,954
CELL KINETICS		1.00	19,954
TREATMENT STUDIES		* 38.00*	758,258
COMB. MODAL. THERAPY		38.00	758,258
COMB. MODAL. THERAPY - SURG.		17.00	339,221
COMB. MODAL. THERAPY - CHEM.		21.00	419,037
SOUTHERN RESEARCH INSTITUTE	N CM0726009 D.T.P.		17,124
ACQUISITION OF MATERIALS		*100.00*	17,124
NEW AGENT PROCUREMENT, SYNTHETICS			
SOUTHWEST FOUNDATION FOR RESEARCH &	N CM0735604 D.T.P.		4,553
ACQUISITION OF MATERIALS		*100.00*	4,553
NEW AGENT PROCUREMENT, SYNTHETICS			
SOUTHWEST FOUNDATION FOR RESEARCH &	N CM0735603 D.T.P.		1,996
ACQUISITION OF MATERIALS		*100.00*	1,996
NEW AGENT PROCUREMENT, SYNTHETICS			
STANFORD RESEARCH INSTITUTE	N CM8718300 D.T.P.		580,171
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	116,034
ANALYTICAL AND QUALITY CONTROL			
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	464,137
ANALYTICAL AND QUALITY CONTROL			
STANFORD RESEARCH INSTITUTE	N CM9725600 D.T.P.		7,142
PROCUREMENT OF PRECLINICAL MATERIAL		* 80.00*	5,714
RADIOLABELED MATERIALS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 20.00*	1,428
RADIOLABEL			
STANFORD RESEARCH INSTITUTE	N CM0735104 D.T.P.		23,060
ACQUISITION OF MATERIALS		*100.00*	23,060
NEW AGENT PROCUREMENT, SYNTHETICS			
STANFORD RESEARCH INSTITUTE	N CM1748500 R.R.P.		413,338
ACQUISITION OF MATERIALS		* 60.00*	248,003
RAD. MODIFIERS			
BASIC SCREEN		* 40.00*	165,335
PRIMARY SCREENING, IN VITRO		20.00	82,668
PRIMARY SCREENING, IN VIVO		20.00	82,668

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
STANFORD UNIVERSITY	N CM1748000 R.R.P.		149,634
PHASE I CLINICAL TRIALS NON-SPECIFIC RAD.		* 50.00*	74,817
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC HYPERTHERMIA		* 50.00*	74,817
STARKS C.P., INC.	N CM0735704 D.T.P.		6,362
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	6,362
STARKS C.P., INC.	N CM0735705 D.T.P.		2,185
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	2,185
STARKS C.P., INC.	N CM0735711 D.T.P.		13,083
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	13,083
STARKS C.P., INC.	N CM0735712 D.T.P.		15,619
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	15,619
STARKS C.P., INC.	N CM1737400 D.T.P.		541,650
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	270,825
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	270,825
STARKS C.P., INC.	N CM0735708 D.T.P.		6,534
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	6,534
STARKS C.P., INC.	N CM8720600 D.T.P.		522,100
ACQUISITION OF MATERIALS		*100.00*	522,100
NEW AGENT PROCUREMENT, SYNTHETICS		70.00	365,470
DATA PROCESSING AND SUPPORT		30.00	156,630
TENNESSEE UNIVERSITY CENTER FOR HEALTH	N CM1747200 CTEP		50,300
PROGRAM MANAGEMENT ADMINISTRATION		* 10.00*	5,030
PHASE II CLINICAL TRIALS		* 30.00*	15,090

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CNS TUMORS	CHEM.				
PHASE III CLINICAL TRIALS			* 60.00*	30,180	
CNS TUMORS	RAD.		30.00	15,090	
CNS TUMORS	CHEM.		30.00	15,090	
TEXAS, UNIVERSITY OF, HEALTH SCIENCE		N CB8424800B BRMP			78,243
TREATMENT STUDIES			*100.00*	78,243	
SURGERY			50.00	39,122	
IMMUNOTHERAPY			50.00	39,122	
TEXAS, UNIVERSITY OF, MEDICAL BRANCH		N CM8722100 CTEP			26,833
PHASE IV CLINICAL TRIALS			*100.00*	26,833	
HEAD AND NECK	SURG.		30.00	8,050	
HEAD AND NECK	RAD.		30.00	8,050	
HEAD AND NECK	CHEM.		20.00	5,367	
HEAD AND NECK	PAT. SUPPORT. CARE		20.00	5,367	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N CM0740600 CTEP			256,856
PHASE II CLINICAL TRIALS			* 55.00*	141,271	
BREAST	CHEM.		15.00	38,528	
COLORECTAL	CHEM.		15.00	38,528	
HEAD AND NECK	CHEM.		5.00	12,843	
LUNG	CHEM.		5.00	12,843	
MELANOMA	CHEM.		10.00	25,686	
SARCOMAS (GEN.)	CHEM.		5.00	12,843	
PHASE III CLINICAL TRIALS			* 45.00*	115,585	
BREAST	CHEM.		10.00	25,686	
COLORECTAL	CHEM.		5.00	12,843	
HEAD AND NECK	CHEM.		5.00	12,843	
LUNG	CHEM.		5.00	12,843	
MELANOMA	CHEM.		15.00	38,528	
SARCOMAS (GEN.)	CHEM.		5.00	12,843	
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N CM9727700 CTEP			63,249
PHASE I CLINICAL TRIALS			* 71.00*	44,907	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	18,342	
LEUK.-ACUTE GRAN.	CHEM.		2.00	1,265	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	1,265	
GENERAL	CHEM.		5.00	3,162	
BREAST	CHEM.		5.00	3,162	
COLORECTAL	CHEM.		5.00	3,162	
LUNG	CHEM.		5.00	3,162	
MELANOMA	CHEM.		5.00	3,162	

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
TEXAS, UNIVERSITY OF	N CM1752400 R.R.P.		169,201
PHASE I CLINICAL TRIALS		* 50.00*	84,601
NON-SPECIFIC RAD.			
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 50.00*	84,601
NON-SPECIFIC HYPERTHERMIA			
TORONTO GENERAL HOSPITAL	N CM1580300 CTEP		16,113
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	16,113
OTHER NUTRITION			
UPJOHN COMPANY	N CM0738000 D.T.P.		450,000
ACQUISITION OF MATERIALS		* 85.00*	382,500
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	373,500
DATA PROCESSING AND SUPPORT		2.00	9,000
BASIC SCREEN		* 15.00*	67,500
PRIMARY SCREENING, IN VITRO		10.00	45,000
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	22,500
UTAH, UNIVERSITY OF	N CM1752300 R.R.P.		166,274
PHASE I CLINICAL TRIALS		* 50.00*	83,137
NON-SPECIFIC RAD.			
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 50.00*	83,137
NON-SPECIFIC HYPERTHERMIA			
VERMONT, UNIVERSITY OF, COLLEGE OF	N CM9727800 CTEP		31,578
PHASE I CLINICAL TRIALS		* 71.00*	22,420
NON-SPECIFIC CHEM.			
PHASE II CLINICAL TRIALS		* 29.00*	9,158
LEUK.-ACUTE GRAN. CHEM.		2.00	632
LEUK.-ACUTE LYMPH. CHEM.		2.00	632
GENERAL CHEM.		5.00	1,579
BREAST CHEM.		5.00	1,579
COLORECTAL CHEM.		5.00	1,579
LUNG CHEM.		5.00	1,579
MELANOMA CHEM.		5.00	1,579
VETERANS ADMINISTRATION	Y CM7010700 CTEP		80,000
PHASE III CLINICAL TRIALS		* 40.00*	32,000
HEAD AND NECK CHEM.		5.00	4,000
HEAD AND NECK IMM.		5.00	4,000
LUNG RAD.		10.00	8,000
LUNG CHEM.		20.00	16,000
PHASE IV CLINICAL TRIALS		* 40.00*	32,000
COLORECTAL SURG.		5.00	4,000

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
COLORECTAL	RAD.	5.00	4,000
COLORECTAL	CHEM.	5.00	4,000
ESOPHAGEAL	RAD.	2.50	2,000
ESOPHAGEAL	CHEM.	2.50	2,000
LUNG	SURG.	5.00	4,000
LUNG	CHEM.	5.00	4,000
LUNG	IMM.	5.00	4,000
PANCREATIC	CHEM.	2.50	2,000
STOMACH	CHEM.	2.50	2,000
OTHER CLINICAL TRIALS RESEARCH		* 20.00*	16,000
COLORECTAL	DATA PROC. & SUPP.	2.50	2,000
ESOPHAGEAL	DATA PROC. & SUPP.	2.50	2,000
HEAD AND NECK	DATA PROC. & SUPP.	2.50	2,000
LUNG	DATA PROC. & SUPP.	5.00	4,000
PANCREATIC	DATA PROC. & SUPP.	2.50	2,000
STOMACH	DATA PROC. & SUPP.	5.00	4,000
VSE, CORPORATION	N CM0725100 D.T.P.		1,000,000
ACQUISITION OF MATERIALS		* 14.00*	140,000
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 58.00*	580,000
PRIMARY SCREENING, IN VITRO		5.00	50,000
PRIMARY SCREENING, IN VIVO		53.00	530,000
VERIFICATION SCREEN		* 20.00*	200,000
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 8.00*	80,000
CHEMOTHERAPY			
WARNER LAMBERT	N CM1749100 D.T.P.		329,611
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	164,806
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	164,806
PRODUCTION, SYNTHETICS			
WARNER LAMBERT	N CM0737900 D.T.P.		450,000
ACQUISITION OF MATERIALS		* 85.00*	382,500
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	373,500
DATA PROCESSING AND SUPPORT		2.00	9,000
BASIC SCREEN		* 15.00*	67,500
PRIMARY SCREENING, IN VITRO		10.00	45,000
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	22,500
WASHINGTON, UNIVERSITY OF	N CB8424700B BRMP		216,586
CELLULAR/SUBCELLULAR STUDIES		* 50.00*	108,293
CELL BIOLOGY		50.00	108,293
TREATMENT STUDIES		* 50.00*	108,293
COMB. MODAL. THERAPY		50.00	108,293

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
COMB. MODAL. THERAPY - CHEM.			10.00	21,659	
COMB. MODAL. THERAPY - IMM.			40.00	86,634	
WASHINGTON, UNIVERSITY OF		N CM9728200 R.R.P.			284,866
OTHER CLINICAL TRIALS RESEARCH			*100.00*	284,866	
NON-SPECIFIC	HARDWARE DEVEL.				
WAYNE STATE UNIVERSITY		N CM0740400 CTEP			238,064
PHASE II CLINICAL TRIALS			* 55.00*	130,935	
BREAST	CHEM.		15.00	35,710	
COLORECTAL	CHEM.		15.00	35,710	
HEAD AND NECK	CHEM.		5.00	11,903	
LUNG	CHEM.		5.00	11,903	
MELANOMA	CHEM.		10.00	23,806	
SARCOMAS (GEN.)	CHEM.		5.00	11,903	
PHASE III CLINICAL TRIALS			* 45.00*	107,129	
BREAST	CHEM.		10.00	23,806	
COLORECTAL	CHEM.		5.00	11,903	
HEAD AND NECK	CHEM.		5.00	11,903	
LUNG	CHEM.		5.00	11,903	
MELANOMA	CHEM.		15.00	35,710	
SARCOMAS (GEN.)	CHEM.		5.00	11,903	
WAYNE STATE UNIVERSITY		N CM0740800 CTEP			34,679
PHASE IV CLINICAL TRIALS			* 90.00*	31,211	
STOMACH	SURG.		25.00	8,670	
STOMACH	RAD.		25.00	8,670	
STOMACH	CHEM.		40.00	13,872	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	3,468	
STOMACH	MARKERS				
WAYNE STATE UNIVERSITY		N CM9727900 CTEP			32,000
PHASE I CLINICAL TRIALS			* 71.00*	22,720	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	9,280	
LEUK.-ACUTE GRAN.	CHEM.		2.00	640	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	640	
GENERAL	CHEM.		5.00	1,600	
BREAST	CHEM.		5.00	1,600	
COLORECTAL	CHEM.		5.00	1,600	
LUNG	CHEM.		5.00	1,600	
MELANOMA	CHEM.		5.00	1,600	
WELLCOME FOUNDATION, LTD.		N CM1748900 BRMP			1,000,000
ACQUISITION OF MATERIALS			*100.00*	1,000,000	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			30.00	300,000	

NAME		# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
ANIMALS			70.00	700,000	
WISCONSIN, UNIVERSITY OF		N CM9728000 CTEP			24,798
PHASE I CLINICAL TRIALS			* 71.00*	17,607	
NON-SPECIFIC	CHEM.				
PHASE II CLINICAL TRIALS			* 29.00*	7,191	
LEUK.-ACUTE GRAN.	CHEM.		2.00	496	
LEUK.-ACUTE LYMPH.	CHEM.		2.00	496	
GENERAL	CHEM.		5.00	1,240	
BREAST	CHEM.		5.00	1,240	
COLORECTAL	CHEM.		5.00	1,240	
LUNG	CHEM.		5.00	1,240	
MELANOMA	CHEM.		5.00	1,240	
YALE UNIVERSITY		N CB7419100B CTEP			78,923
PHASE II CLINICAL TRIALS			*100.00*	78,923	
LUNG	SURG.		25.00	19,731	
LUNG	IMM.		75.00	59,192	
YALE UNIVERSITY SCHOOL OF MEDICINE		N CM0733900 CTEP			57,382
PHASE IV CLINICAL TRIALS			* 90.00*	51,644	
STOMACH	SURG.		25.00	14,346	
STOMACH	RAD.		25.00	14,346	
STOMACH	CHEM.		40.00	22,953	
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 10.00*	5,738	
STOMACH	MARKERS				
YAMANOUCHI PHARMACEUTICAL CO.		N CM9730700 D.T.P.			210,000
FORMULATION			* 20.00*	42,000	
DEVEL. OF EXP. FORMULATIONS					
PROD. AND FORM. FOR CLINICAL TRIALS			* 80.00*	168,000	
FORMULATION			70.00	147,000	
ANALYTICAL AND QUALITY CONTROL			10.00	21,000	

TABLE IV

DESCRIPTION OF CONTRACTS

IN THE

DIVISION OF CANCER TREATMENT

AEROJET STRATEGIC PROPULSION COMPANY (NO1-CM1-7490)

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

AGRICULTURE, DEPARTMENT OF (TRANSFER OF FUNDS) (YO1-CM4-0001)

Under the Transfer of Funds Agreement with the Economic Botany Laboratory of the U.S.D.A., worldwide general plant collections and recollections are made. The general plant collections (approximately 1,000 plants) are shipped to Research Triangle Institute for extraction and the extracts are screened *in vitro* and *in vivo* for antineoplastic activity. Confirmed active plants are recollected in large quantities (100 to 300 lbs.) on a priority based on the needs of the DCT for shipment to the fractionating chemists. The nomenclature of all plants acquisitioned is being continuously reviewed and corrected, and a computer file of all plants is being maintained. This Agreement will expire on September 30, 1982 and will not be renewed.

ALABAMA, UNIVERSITY OF (NO1-CMO-7355)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

ALBANY MEDICAL COLLEGE (NO1-CB5-3940)

This contract evaluated the therapeutic efficacy of intrapleural BCG in patients with lung cancer. Following surgical resection, patients were randomly assigned to receive intrapleural BCG + INH or INH alone. The design was subsequently altered so that Stage I patients received either BCG + INH, followed by additional doses of BCG, and control patients received BCG + INH alone. This contract is being phased out.

ALBANY MEDICAL COLLEGE (NO1-CM5-7032)

This contract, currently in phase out, is designed to support prospective, randomized controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. Conversion to Cooperative Agreement is scheduled in this fiscal year.

ALBERT EINSTEIN SCHOOL OF MEDICINE (NO1-CM1-7340)

This contract will support a Phase I evaluation of topical retinoids, a vitamin A derivative, to be applied directly onto the surface of the cervical mucosa. After a careful evaluation of toxicities, a Phase III trial will be initiated, which shall attempt to identify the role of vitamin A analogs as chemopreventive agents. Women with abnormal pap smear cytology will be prospectively analyzed in a double blind trial to see if retinoids can improve dysplastic cervical morphology. This chemopreventive trial shall try to demonstrate that cellular differentiation and maturation can be induced by vitamin A analogs.

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

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

AMERICAN COLLEGE OF RADIOLOGY (NO1-CM8-7219)

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

AMERICAN TYPE CULTURE COLLECTION (NO1-CMO-5725)

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

ARIZONA, UNIVERSITY OF (NO1-CM1-7497)

This is one of four contracts devoted to the development of a human tumor clonogenic assay for drug screening. Objectives include developing evaluation criteria for valid assays and for activity. Limited developmental studies such as comparisons of enzymatic and mechanical disaggregation techniques and comparisons of different media also are being conducted to improve the cloning and cost efficiency of the system for large-scale screening.

ARIZONA, UNIVERSITY OF (NO1-CM1-7500)

This contract will support a Phase I evaluation of topical retinoids, a vitamin A derivative, to be applied directly onto the surface of the cervical mucosa. After a careful evaluation of toxicities, a Phase III trial will be initiated, which shall attempt to identify the role of vitamin A analogs as chemopreventive agents. Women with abnormal pap smear cytology will be prospectively analyzed in a double blind trial to see if retinoids can improve dysplastic cervical morphology. This chemopreventive trial shall try to demonstrate that cellular differentiation and maturation can be induced by vitamin A analogs.

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. The five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

ARIZONA STATE UNIVERSITY (NO1-CM9-7297)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the U.S. Department of Agriculture. This contract is scheduled to terminate in August, 1982 and will not be recompeted.

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

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

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

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

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

ARTHUR D. LITTLE, INC. (NO1-CMO-7331)

The major objective of this contract is to develop three in vitro test systems for mass screening of natural products. When these in vitro screens are optimized and evaluated against 50 known pure natural products and 1000 unknown crude extracts, they will then be put either in the Natural Products Branch or Drug Evaluation Branch contract laboratories. This contract is in phase out status and will expire in September, 1982.

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

The current level of testing in mice of potential anticancer agents under this contract is approximately 15,000 L1210 equivalent tests per year. The contract provides for in vivo testing in the P388 leukemia pre-screen, for evaluation of materials in specified tumor panel models, for detailed evaluations requested by members of the NCI staff, and for characterizations and evaluations of tumor models as directed by the NCI Project Officer. All testing is carried out in accordance with the protocols of the NCI Developmental Therapeutics Program. Materials tested in the P388 leukemia pre-screen are new synthetic compounds and fractions of natural products provided by the NCI. Models of the conventional tumor panel now in use under this contract include the B16 melanocarcinoma, colon 38 carcinoma, and L1210 leukemia. Materials tested in the tumor panel models under this contract are selected by the Developmental Therapeutics Program and consist particularly of materials that have demonstrated activity in the P388 pre-screen. Detailed testing usually involved materials of potential clinical interest and includes schedule dependency studies, drug route studies, batch comparisons, and formulation comparisons.

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

The objective of this contract is to obtain basic information on the cytotoxic and biochemical effects of new antitumor agents that will aid DCT in deciding whether to develop drugs to clinical trial and in assigning priorities to those drugs that are under development. 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 obtain information on the biochemical properties of analogs at the request of the various Analog Coordinating Groups.

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

This contract provided cell culture assays for the natural products program of DCT, as an aid in the isolation and purification of potential antitumor agents of plant and animal origin. After termination of the plant products contract program in October 1981, the major emphasis of this contract shifted to completing developmental work on the astrocytoma assay as a pre-screen. Contract is extended from February 28, 1981 through August 1982 at a reduced level of effort without additional funds.

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

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

BANNER GELATIN PRODUCTS CORPORATION (NO1-CM1-7402)

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

BATTELLE MEMORIAL INSTITUTE (NO1-CM0-7266)

This contract provides for in vivo testing in the P388 leukemia tumor test system pre-screen for new materials. In addition, testing is conducted in the murine tumor test systems of the panel for materials of interest to the Developmental Therapeutics Program. This testing is conducted at a level of effort of approximately 23,500 L1210 equivalents per year. Special studies for the detailed evaluation of drugs of particular interest to the Program are carried out as requested by the Project Officer.

BATTELLE MEMORIAL INSTITUTE (NO1-CM1-7365)

This service type contract was recompeted in 1980 and a Prime Contract was awarded to Battelle Memorial Laboratories in November 1980 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 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 Testing; and Task IV - Automation of toxicity data, anomaly detection, scheduling of studies, and financial management. This contract is currently being recompeted.

BATTELLE MEMORIAL INSTITUTE (NO1-CM6-7099)

This contract is for the testing of materials in human xenograft tumor models using athymic mice. Currently, materials are tested with the tumor implanted in the subrenal capsule. Presently, one human lung, colon, and mammary xenograft models are employed. Approximately 265 compounds are tested per year in each of the three systems.

BEN VENUE LABORATORIES, INC. (NO1-CM9-7298)

This resource contract provides for the development and production of parenteral clinical dosage forms of antitumor agents. The contractor has the capacity for preparing large-scale produciton batches of dry filled, liquid filled and lyophilized sterile products. Specifically, the contractor performs the following services: (1) formulation development of sterile parenteral products; (2) production of sterile products; (3) quality assurance testing of finished products; and (4) stability surveillance of all dosage forms produced. All products are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BRISTOL LABORATORIES, INC. (NO1-CM0-7299)

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

BRISTOL LABORATORIES, INC. (NO1-CM0-7324)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation techniques of biotransformation and co-metabolism of antibiotics, chemicals and plant materials. This effort is to convert materials which have solubility, toxicity or marginal activity problems into worthwhile antineoplastic agents. A multitude of unique organisms is isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents. Each compound is exposed to a selected number of microbes to see if any worthwhile conversions occur. This contract will terminate in July, 1982. Due to budgetary restrictions the effort will not be recompeted.

BRISTOL LABORATORIES, INC. (NO1-CM3-3751)

This was a no-cost contract concerned with the marketing of the clinically useful nitrosourea, BCNU. The contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the Food and Drug Administration. The NDA was approved in March 1977, and the drug is currently on the market. This contract continued for five years from the NDA approval and thus expired March, 1982.

BRITISH COLUMBIA, UNIVERSITY OF (NO1-CM8-7236)

This contract is to evaluate the ability of plant cells to grow and to produce the antineoplastic agent in submerged fermentations. Often, plants which produce antineoplastic agents are found in foreign countries. In order to insure supply of these plants, one must be able to cultivate them or find worldwide sources. Weather and political conditions often make it prohibitive to obtain plant materials in sufficient quantity to isolate adequate amounts of the antineoplastic agent for NCI evaluation. The contract is evaluating the possibility of propagating plant cells in submerged culture to produce antineoplastic agents of interest. This would insure local supply of such agents and allow NCI to move quickly to obtain these materials. Eight plants of interest to NCI have been assigned and one has already successfully been propagated to produce the antineoplastic agent of interest. This contract terminated in December, 1981

BUREAU OF RADIOLOGICAL HEALTH, FDA (YO1-CO1-0700)

This is a follow-up study on patients who had 131-iodine and other diagnostic procedures during childhood and adolescence. The study population is 6,500 patients under age 20 who received diagnostic 131-iodine between 1946 and 1967 to test thyroid gland function and for thyroid imaging. The control is 13,000 (6,500 clinical comparison patients and 6,500 siblings of the exposed group). Determine whether exposures are associated with subsequent risk of developing benign or malignant thyroid neoplasms.

CALIFORNIA, UNIVERSITY OF (NO1-CBO-4344)

This contract investigated the effects of specific and non-specific immunotherapy as an adjunct to chemotherapy in skeletal and soft tissue sarcoma. This contract is being phased out and the final report has been received.

CALIFORNIA, UNIVERSITY OF (NO1-CB1-5525)

This contract investigated the effects of BCG in the treatment of Stage II melanoma patients. Patients were randomized following surgical lymphadenectomy in three groups. Each group received one of the following postoperative therapies: no additional therapy, BCG, or BCG plus allogeneic tumor-cell vaccine. This contract is being phased out; the final report has been received.

CALIFORNIA, UNIVERSITY OF (NO1-CB8-4250)

The objective of this contract is to produce xenogeneic monospecific antibodies to human lymphoma and leukemia associated antigens. The approach will be to fuse antibody-producing cells of mouse origin with mouse myeloma cells in order to produce hybrid cell lines capable of continuous production of monospecific antibodies against tumor associated antigens in vitro. Two different hybridization techniques will be compared. The specificity of the presumed monoclonal antibodies and the pattern of antigenic tumor-specific determinants on malignant lymphoid cells will be evaluated. This contract expired December 29, 1981.

CALIFORNIA, UNIVERSITY OF (NO1-CM0-7416)

A member of the Lung Cancer Study Group: This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a randomized surgical trial of lobectomy versus limited resection for Stage I lung cancer. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of post operative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cisplatinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. A new protocol proposed for activation by the group evaluates preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of Lung Cancer Study Group have been accepted in four major journals.

CALIFORNIA, UNIVERSITY OF (NO1-CM0-7420)

This is one of four contracts devoted to the development of a human tumor clonogenic assay for drug screening. Objectives include developing evaluation criteria for valid assays and for activity. Limited developmental studies such as comparisons of enzymatic and mechanical disaggregation techniques and comparisons of different media also are being conducted to improve the cloning and cost efficiency of the system for large-scale screening.

CALIFORNIA, UNIVERSITY OF (NO1-CM0-7439)

This is a Phase I/II clinical trial utilizing human lymphoblastoid interferon. Studies are in progress to determine the maximum tolerated dose and maximum biological response modifying dose. The Phase I part of the study is nearing completion and the Phase II portion of the study is expected to begin shortly.

CALIFORNIA, UNIVERSITY OF (NO1-CM0-7444)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. Studies are in progress to determine maximum tolerated dose and maximum biological response modifying dose. This Phase I study is nearing completion and will define the thymosin fraction 5 and alpha-1 dosages for Phase II trials. This contract will expire June 30, 1982.

CALIFORNIA, UNIVERSITY OF (NO1-CM6-7097)

This contract was designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. This contract expired December 31, 1981.

CALIFORNIA, UNIVERSITY OF (NO1-CM9-7239)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service of Baltimore. Typing data on over 15,000 NIH donors and recipients are on computer file. All typings are performed and entered into the computerized file on the same date that the blood was received. Thus, HL-A typing results for persons bled in Bethesda are consistently available through the computer terminal by the end of the next day. On the average, 600 typings are performed annually. This contract has been transferred to the NIH Clinical Center for funding and administration.

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. The construction drawings are essentially complete and pending approval of the drawings by the Government and identification of additional construction funds, the construction contract will be initiated in the spring of 1982. The facility should be completed in early 1983 and fully operational in mid 1983.

CALIFORNIA, UNIVERSITY OF (NO1-CM9-7318)

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

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

This is one of four contracts devoted to the development of a human tumor clonogenic assay for drug screening. Objectives include developing evaluation criteria for valid assays and for activity. Limited developmental studies such as comparisons of enzymatic and mechanical disaggregation techniques and comparisons of different media also are being conducted to improve the cloning and cost efficiency of the system for large-scale screening.

CDP ASSOCIATES (NO1-CM9-7143)

This project provided technical support services to the Office of the Director, DCT, as well as to the program areas of DCT in the performance of the planning and analytical tasks and general logistical support in the development of related or otherwise required documentation and conference support activities of the Division. This contract terminated in December, 1981.

CHARLES RIVER BREEDING LABOATORIES (NO1-CM1-7498)

This procurement contract is designed to furnish 156,000 six-week old first-generation hybrid mice for Drug Research and Developmental Therapeutics Program contract studies.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM5-0598)

This procurement contract is designed to furnish 156,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for Drug Research and Developmental Therapeutics Program contract studies. The breeding animals originate from the genetic center at this site.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM7-7141)

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

CHARLES RIVER BREEDING LABORATORIES (NO1-CM8-7199)

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

CHARLES RIVER BREEDING LABORATORIES (NO1-CM8-7212)

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

CHARLES RIVER BREEDING LABORATORIES (NO1-CM9-0163)

This procurement contract provides for the supply of 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for the Drug Research and Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers.

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 March 31, 1982; however, it is expected to be renewed for another two years and seven months.

CHEMICAL ABSTRACTS SERVICE (NO1-CM4-3722)

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

CHICAGO, UNIVERSITY OF (NO1-CMO-7411)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer. Conversion to Cooperative Agreement is scheduled in this fiscal year.

CHILDREN'S HOSPITAL FOUNDATION, OHIO STATE UNIVERSITY (NO1-CMO-7464)

This contract supported a Phase I toxicology and pharmacokinetic study of the agent indicine-n-oxide in pediatric patients. The contract terminated December 31, 1981; it was a one-time task order contract and was not recompeted.

CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CMO-7467)

This contract supported a Phase I study of the agent AZQ in pediatric patients. It terminated October 31, 1981 but was given a no cost extension through March 2, 1982 to allow additional patient accrual. It will not be recompeted as it was a one-time task order contract.

CINCINNATI, UNIVERSITY OF (NO1-CM-87222)

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

COLLABORATIVE RESEARCH, INC. (NO1-CMO-7358)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

COR BEL LABORATORIES, INC. (NO1-CB1-5565)

This is a contract to support large-scale experiments in rodents initiated by the former Laboratory of Immunodiagnosis, DCBD, now Biological Research and Therapy Branch, BRMP, DCT. This contract is currently in phase-out status.

DUKE UNIVERSITY (NO1-CMO-7436)

This is a Phase I/II clinical trial utilizing human lymphoblastoid interferon. Studies are in progress to determine the maximum tolerated dose and maximum biological response modifying dose. The Phase I part of the study is nearing completion and the Phase II portion of the study is expected to begin shortly.

DUKE UNIVERSITY MEDICAL CENTER (NO1-CM1-7477)

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

DUKE UNIVERSITY MEDICAL CENTER (NO1-CP8-5656)

This is one of four contracts on the subject of nutritional assessment which are funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is evaluating static and dynamic tests of muscle strength in cancer patients. These investigators are also evaluating the effects of nutritional status on drug metabolism.

DYNAMAC CORPORATION (FORMERLY ENVIRO CONTROL, INC.) (NO1-CMO-7332)

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

ELKINS-SINN, INC. NO1-CM6-7103)

The objectives of this contract are to provide facilities and capabilities for the production of parenteral investigational dosage forms for the Division of Cancer Treatment. The contractor is responsible for conformity to FDA Current Good Manufacturing Practices and is responsible 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. This contract terminated in February 1982.

EMMES CORPORATION (NO1-CM1-7371)

This contract was awarded September 29, 1981 as a result of a recompetition. This contract provides operations office support for the Gastrointestinal Tumor Study Group, the Head and Neck Contracts Project, the Intergroup Testicular Cancer Studies, and the Lung Cancer Study Group. Functions include coordination of protocol development, editing and preparing final form of protocols, forms design, randomization, quality control of data, editing and preparing meeting agenda which include clinical trials reports, writing and preparing minutes of meetings, preparing correspondence, record-keeping, and files maintenance.

EMMES CORPORATION (NO1-CM8-7193)

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

EMORY UNIVERSITY (NO1-CM1-5804)

This is one of three collaborating institutions which will assess the effects of augmented versus conventional versus no nutritional intervention in advanced colorectal and non-small cell lung cancer patients. The augmented nutritional support will consist of a higher percentage of daily caloric intake as protein, as well as additional mineral supplementation. Patients will be serially followed with extensive nutritional assessment to evaluate the effectiveness of the intervention in maintaining lean body mass. The nutritional assessment will include: total body nitrogen by prompt gamma emission techniques, total body potassium and water by isotope dilution, anthropometric measurements, and biochemical analyses.

EMORY UNIVERSITY (NO1-CM2-5603)

This study is evaluating the use of direct and indirect calorimetry in the cancer patient. In addition, other parameters of nutritional status are being performed and will be correlated with the results of the calorimetric studies.

EMORY UNIVERSITY (NO1-CP8-5651)

This is one of four contracts on the subject of nutritional assessment which are funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is evaluating the use of CT scans to measure body fat and muscle mass. This contract is being phased out.

ENERGY, DEPARTMENT OF (Y01-C00-0320)

This is a long-term study to determine the late effects in dogs of predetermined total doses of whole body gamma radiation when given at various daily dosage rates. The three main parts of study are: a) 180 dogs receive continuous (22 hr/day) gamma radiation at low dose rates; b) 190 dogs receive similar exposure until predetermined total dose received ("terminated" exposure group); and c) 180 colony controls.

ENERGY, DEPARTMENT OF (N01-C00-0321)

This is a long-term study to evaluate genetic and late somatic effects resulting from long term ingestion of low concentrations of triated water. Specifically: a) to evaluate genetic effects of tritium exposures to mice, including effects on reproductive efficiency and cytogenetic effects on liver cells; b) to evaluate somatic effects of tritium on mouse bone marrow; c) to establish RBE (Relative Biological Effectiveness) of tritium compared to cesium - 137 for several biological endpoints; and d) to determine exchangeable/nonexchangeable tritium in various cellular fractions (especially nucleus) and establish radiation dose to DNA.

ENERGY, DEPARTMENT OF (N01-C01-0711)

This study of low level radiation effects conducted at the Brookhaven National Laboratories is to explore dependence of mutation induction on dose rate and dose fractionation using both high and low LET radiations.

ENERGY, DEPARTMENT OF (N01-C01-0712)

This study, conducted at the Brookhaven National Laboratories, is to provide information on incidence of radiation-induced leukemias and to help determine whether linear extrapolation from high doses to low doses or low dose rates is an appropriate method for estimating hazards of small doses of radiation to man.

ENERGY, DEPARTMENT OF (Y01-CM2-0110)

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

ENERGY, DEPARTMENT OF (YO1-CM2-0111)

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Radiation-induced Myelogenous Leukemia." This project is concerned with the dose-response relationships and the mechanisms of neutron radiation-induced myelogenous leukemia in mice. Neutrons and other high-LET radiations are considered more effective for induction of tumors than low-LET radiations. Since survivors of the atomic bomb at Hiroshima were exposed to both gamma rays and neutrons, attempts have been made to deduce the leukemogenic effects of neutrons from the Japanese data, but the neutron dose estimates for Hiroshima have recently been questioned and may be revised. It is essential to obtain data for neutron radiation-induced leukemia in experimental animals. This study will be completed in four years.

ENERGY, DEPARTMENT OF (YO1 CM2-0112)

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Extrapolation of Radiation Risk." This study will address a question of fundamental importance to the understanding of mechanisms of carcinogenesis and to risk estimation, namely, whether the natural incidence of cancer influences the susceptibility to induction by radiation or by other carcinogenic agents. The investigators will determine whether the natural incidence of specific cancers influences the susceptibility to induction by gamma radiation in two different strains of mice. This study will be completed in five years.

ENERGY, DEPARTMENT OF (YO1-CM2-0113)

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Co-Carcinogenesis: Ionizing and Ultraviolet Radiation." This study will determine quantitatively the interactions of ultraviolet radiation and ionizing radiation (x-rays and fission neutrons) in terms of production of skin cancer in hairless mice. The persistence of initiated cells in skin will be investigated after exposure to doses of ionizing radiatin that alone do not result in an increase in the incidence of skin cancer. These cells may be promoted later to cancer cells by other agents. This study will be completed in five years.

ENVIRONMENTAL PROTECTION AGENCY (YO1-CM2-0109)

Under this Interagency Agreement, via EPA Contract 68-01-4831, work was initiated in 3/82 for the design and construction of an interactively accessible Drug Information System (DIS). This system will carry all the chemical and biological data pertaining to the screening of chemicals as anticancer agents. It will allow for continual updating as new data are acquired and will at all times be accessible in real time. It will support both routine data retrieval and also ongoing data acquisition.

FLORIDA, UNIVERSITY OF (NO1-CM9-7320)

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

FLOW LABORATORIES, INC. (NO1-CMO-7370)

This contract is concerned with the development of a practical scale-up procedure to manufacture 50 billion units of human fibroblast interferon during the two year period of the contract. The interferon will be 1×10^7 units/mg of protein.

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-CM9-7254)

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

FOX CHASE CANCER CENTER (NO1-CMO-7330)

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

tion of the radiation dose required for curing 50% of the tumors. All testing will be compared with the standard WR-2721. The contract also provides for the option of assessing potential long-term tissue injury by performing histopathology on a limited number of tissues and compounds. This contract should provide new radioprotectors or leads in developing new types (classes) of radioprotective compounds.

FOX CHASE CANCER CENTER (NOl-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 of Fox Chase Cancer Center to house the neutron therapy system was completed in October, 1981. After several delays, the DT generator tube was shipped to Philadelphia in March, 1982. Initial testing is under way and the facility should become fully operational in mid 1982.

FRED HUTCHINSON CANCER RESEARCH CENTER (NOl-CMO-7336)

A member of the Lung Cancer Study Group: This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a randomized surgical trial of lobectomy versus limited resection for Stage I lung cancer. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of post operative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cisplatinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. A new protocol proposed for activation by the group evaluates preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of Lung Cancer Study Group have been accepted in four major journals.

FRED HUTCHINSON CANCER CENTER (NOl-CMO-7445)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. Studies are in progress to determine maximum tolerated dose and maximum biological response modifying dose. This Phase I study is nearing completion and will define the thymosin fraction 5 and alpha-1 dosages for Phase II trials. This contract will expire June 30, 1982.

GEORGETOWN UNIVERSITY (NO1-CM0-7437)

This is a Phase I/II clinical trial utilizing human leukocyte interferon. Studies are in progress to determine the maximum tolerated dose and maximum biological response modifying dose. The Phase I part of the study is nearing completion and the Phase II portion of the study is expected to begin shortly.

GEORGE WASHINGTON UNIVERSITY (NO1-CM0-7446)

This is a Phase I evaluation of thymosin alpha-1. Studies are in progress to determine maximum tolerated dose and maximum biological response modifying dose. This Phase I study is nearing completion and will define the thymosin alpha-1 dosage for Phase II trials.

GEORGETOWN UNIVERSITY (NO1-CM1-7501)

This contract is for pathologic support of the clinical trial of chemoprevention. This Pathology Reference Center reviews cytology and culposcopy directed biopsies of the cervix.

GEORGETOWN UNIVERSITY (NO1-CM6-7094)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. The contract expired during this fiscal year.

GEORGETOWN UNIVERSITY (NO1-CM6-7110)

This contract supports a comprehensive program for research in treatment and care of patients with gastrointestinal cancer. The program includes a fully staffed and functioning GI unit, with participation by the surgery, radiology, clinical, pathology, and medical departments. Specifically, the GI Research Program includes studies in: early detection, staging, new and established anticancer agents, combinations and multidisciplinary approaches, pharmacology, evaluation of markers and professional training. A new major effort is devoted to the study of implications of nutritional deficiencies and symptomatic manifestations of malignancies, and efforts to correct them. Specific areas of investigation being pursued include development of drug regimens in gastric cancer, introduction and study of a new nitrosourea targeted towards gastrointestinal cancer, evaluation of effort of plasmapheresis on tumor growth, and efficacy of heroin on pain control.

GEORGETOWN UNIVERSITY (NO1-CM9-7208)

This contract is designed to contract Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of the lung, breast, and colon. This contract will be terminated this year, since this institution did not receive a new contract in the recompetition.

GEORGETOWN UNIVERSITY (NO1-CM9-7310)

This contract is designed to carry out Phase II studies in gastric and pancreatic cancer. The major areas included under the contract are: (1) systemic investigation of new agents in gastric and pancreatic cancer; (2) development of new combinations of agents; and (3) Detailed pharmacologic evaluation if single and combined agents.

HARLAN INDUSTRIES (NO1-CM0-7362)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in a closely controlled environment. All foundation colonies are rederived from NIH stock and maintained in associated flora isolators. All expansion colonies are maintained in the barrier environment.

HARLAN INDUSTRIES (NO1-CM5-0591)

This contract furnishes approximately 156,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or production colonies.

HARLAN INDUSTRIES (NO1-CM9-7242)

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 also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of DBA/8, BALB/c-CMC, and CD8F1 mice. This contract terminated March 31, 1982, and is not expected to be renewed.

HARLAN INDUSTRIES (NO1-CM9-7243)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratories. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies. The breeding stock is received from the Primary Genetic Centers. This contract terminated January 31, 1982, and was not renewed.

HAZLETON LABORATORIES AMERICA, INC. (NO1-CM1-5770)

This contract provides for the supply and medical support of dogs for leukocyte transfusion and autologous marrow rescue experiments. The specific experiments underway seek to examine the synergy of reconstitution of marrow function when mixes of peripheral blood and bone marrow stem cells are used to rescue lethally irradiated dogs. In addition, the efficacy of granulocytes with antimicrobial antibodies on their surface will be examined in experimental bacterial and fungal diseases. Furthermore, the possible enhancement of tumor rejection by viruses to which the host is immune will be explored. Also, efforts will continue that attempt to identify the mechanism by which staphylococcal protein A plasma perfusion result in tumor regression in certain instances.

HAZLETON LABORATORIES AMERICA, INC. (NO1-CM2-5601)

This contract is designed to provide comparative data on the response of nonhuman primates to known rodent carcinogens and to materials suspected of being carcinogenic in man; in addition, the long-term effects of antineoplastic and immunosuppressive agents currently in clinical use are being evaluated. Other objectives of the contract include the development of model tumor systems in primates for evaluating new and established antitumor agents; the provision of normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical, and immunological studies; the development of biological markers and diagnostic tests for detecting preneoplastic changes as well as frank neoplasia; the development of models for prevention of carcinogenesis, especially for protecting against potent primate carcinogens and against the carcinogenic effects of clinically useful antitumor agents; and the maintenance of a breeding colony of various species of primates so that these animals may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, NCI.

HAZLETON RESEARCH ANIMALS, INC. (NO1-CM6-0125)

This contract provides 144 beagle hounds for toxicological assays. These animals are raised under controlled conditions in accordance with specifications delineated in the contract. Breeding animals are supplied by the contractor.

HEALTH RESEARCH, INC. (NO1-AI0-2657)

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

HEALTH RESEARCH, INC. (NO1-CB6-4007)

This contract investigated the therapeutic effects of immunotherapy with tumor vaccine given adjuvant to curative surgical resection in squamous cell carcinoma of the lung by a prospective randomized study. Several serial tests with DNCB, PPD, and tumor associated antigen have not proven to be of prognostic value. This contract has been phased out.

HEALTH RESEARCH, INC. (NO1-CM0-7410)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer. Conversion to Cooperative Agreement is scheduled in this fiscal year.

HEALTH RESEARCH, INC. (NO1-CM5-7034)

This contract was designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. It expired during this fiscal year.

HEALTH RESEARCH INSTITUTE (NO1-CM9-7311)

The objective of this program is to evaluate in a Phase II study, photo-radiation 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.

IIT RESEARCH INSTITUTE (NO1-CM0-7359)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

IIT RESEARCH INSTITUTE (NO1-CMO-7359) - (CONTINUED)

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

IIT RESEARCH INSTITUTE (NO1-CM9-7213)

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

IIT RESEARCH INSTITUTE (NO1-CM9-7316)

Crude natural products, purified fractions of confirmed in vivo natural products, and synthetic materials are tested for efficacy in the P388 pre-screen tumor system. Upon request, materials are tested in the complete tumor panel. These are L1210; Lewis lung; B16 melanoma; Colon 38; $CD8F_1$ mammary; and colon, lung, breast xenograft systems. Testing is conducted at the 35,000 P388 test equivalent level.

ILLINOIS, UNIVERSITY OF (NO1-CM9-7259)

The objective of this contract is to do a worldwide survey of all the natural products literature, identifying new structures and reports of specific biological activity which may be related to cancer, and reports of biological activity of plant and animal extracts. This is a key contract for acquisition of new agents for the DTP screening program. This contract expires in September, 1982, and a recompetition is underway.

ILLINOIS, UNIVERSITY OF (NO1-CM9-7295)

This contract provides for the fractionation of confirmed active plant extracts in order to isolate in pure state and identify the active compounds. Plant materials used in this work are obtained, for the most part, through the U.S. Department of Agriculture. This contract expires in August, 1982, and will not be recompeted due to budgetary restraints.

ILLINOIS CANCER COUNCIL (NO1-CMO-7415)

A member of the Lung Cancer Study Group: This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a randomized surgical trial of lobectomy versus limited resection for Stage I lung cancer. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of post operative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cisplatinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. A new protocol proposed for activation by the group evaluates preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of Lung Cancer Study Group have been accepted in four major journals.

ILLINOIS CANCER COUNCIL (NO1-CMO-7470)

This contract supports a Phase I study of the agent dyhydroxyunthracenedione (metoxantrone) in pediatric patients. This study is expected to enroll 25-30 patients at various doses to determine the toxicity and pharmacology of the agent. Pharmacologic studies will evaluate the blood kinetics by appropriate measurements *in vitro*.

INDIANA UNIVERSITY FOUNDATION (NO1-CM1-7475)

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

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

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

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

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

INSTITUT JULES BORDET (NO1-CMO-7350)

Materials collected in Northern Europe are screened against animal tumors in accordance with the established in vivo 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 equivalents per year. More detailed evaluation of drugs of interest to NCI is conducted upon request or agreement of the Project Officer.

INSTITUT JULES BORDET (NO1-CM5-3840)

This contract expired in March, 1982 after satisfactorily performing its function in compound collection and exchange of scientific information. Compound collection will continue in the NCI-EORTC Liaison Office in Brussels funded by another mechanism.

INSTITUTE OF CANCER RESEARCH (NO1-CM-17502)

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

INSTITUTE OF CANCER RESEARCH (NO1-CM4-3736)

This project involves (1) validation of human tumor xenografts as models for cancer chemotherapy; (2) the use of human tumor xenografts and transplantable mouse tumors for testing new compounds of interest to DCT, NCI; (3) toxicology, pharmacology, and initial clinical trials of new drugs developed in this project; and (4) studies of the biochemical basis for treatment response or failure aimed at the design, synthesis, and detailed evaluation of new drugs.

IOWA, UNIVERSITY OF (NO1-CM0-7303)

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

IOWA, UNIVERSITY OF (NO1-CM0-7334)

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

IOWA, UNIVERSITY OF (NO1-CM0-7412)

The major objective of this fermentation contract is the preparation of novel antibiotics using the fermentation techniques of biotransformation and co-metabolism. This effort is to convert materials which have solubility, toxicity or marginal activity problems to more active antineoplastic agents. A multitude of unique organisms is isolated and used to obtain these microbial conversions in an effort to obtain antineoplastic agents. This contract will expire in September,1982, and will not be recompeted.

IOWA, UNIVERSITY OF (NO1-CM1-7476)

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

IOWA, UNIVERSITY OF (NO1-CM9-7319)

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

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 received world-wide attention. The Istituto has recently shown an improved overall survival for premenopausal patients treated with CMF. They also recently reported that 12 months of CMF is no more effective than 6 months. A re-analysis of disease-free survival among postmenopausal patients showed a clear advantage for patients receiving an average >75% drug dose compared to those with <75% drug dose.

ISTITUTO NAZTIONAL PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM3-3714)

The major thrust of this contract has been the multidisciplinary approach to breast cancer of all stages. In other disease areas, this contractor has participated within the Gastrointestinal Tumor Study Group in colon adjuvant studies and has developed new protocols to test concepts of late intensification in testicular, small cell lung, and breast cancer, and diffuse histiocytic lymphoma. This contract is being phased out.

INSTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM4-3726)

Clinical trial No. 6 has been concluded and shows no benefit for adjuvant BCG, DTIC, or BCG + DTIC for the treatment of malignant melanoma. This contract was terminated in June, 1982.

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

The objective of this contract is the maintenance of a chemotherapy liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs, and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer therapy. A small testing facility is also maintained for the screening and further evaluation of selected new compounds. This contract expired June, 1982.

JWK ASSOCIATES (NO1-CM2-5602)

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

KANSAS, UNIVERSITY OF (NO1-CM0-7304)

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

KENTUCKY, UNIVERSITY OF (NO1-CM0-7381)

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

KANSAS UNIVERSITY OF, MEDICAL CENTER (NO1-CM9-7272)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contract will be terminated this year, since this institution did not receive a new contract in the recompetition.

KING ANIMAL LABORATORIES (NO1-CM1-7499)

This procurement contract is designed to furnish 156,000 six-week old first-generation hybrid mice for Developmental Therapeutics Program contract studies.

LABORATORY SUPPLY COMPANY (NO1-CM5-0577)

This contract furnishes approximately 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are being furnished from genetic centers and/or rodent production centers.

LABORATORY SUPPLY COMPANY (NO1-CM9-7244)

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 investigators. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments. The breeding stock is received from primary genetic centers. This contract terminated January 31, 1982, and was not renewed.

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

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

LITTON BIONETICS, INC. (NO1-CM0-5724)

The major objective of this contract is to provide a well-equipped animal facility to satisfy the needs of various laboratories in the Developmental Therapeutics Program. Services provided are utilized for studies relating to the effects of drugs in the production of tumors, the study of metabolites of various drugs, the production of antisera and studies of the pathogenesis of leukemia. The facility can maintain 3,500 mice, 30 rabbits, 20 guinea pigs, 50 rats, 15 goats, 4 gibbon apes and 10 dogs with essential veterinary care available 365 days a year. The contract also provides technical assistance for performance of routine procedures and professional assistance for surgical procedures and post-mortem examinations.

LITTON BIONETICS, INC. (NO1-CM0-7326)

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

LITTON BIONETICS, INC. (NO1-CM0-7347)

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

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

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

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

The objective of this contract is to confirm the stated biological properties of BRM preparations. Studies are carried out to evaluate and verify the potential of each BRM to reduce tumor growth in in vivo animal tumor models, to augment the immunizing capability in animal tumor models, to evaluate the mechanism of each BRM in in vitro tests, to determine an effective time and dose of administration and a nontoxic effective dose.

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

The major objective of this contract is to provide purified type C viral proteins and antibodies, human hematopoietic growth factors and cell cultures of diverse hematopoietic origin. These reagents will be used in experiments concerned with developing a rationale for the treatment of cancer by first understanding its cause on a fundamental basis. The present studies will lead to information on normal or abnormal mechanisms for the control of cell growth and function by specific growth factors, to the identification of those cells which may be particularly prone to the presence of the HTLV virus in human patients.

LITTON BIONETICS, INC. (NO1-CM6-7067)

This contract supports Surgery Branch research by providing appropriate amounts of human immune 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. Additionally, the contractor operates and maintains a serum repository of specimens provided by the Surgery Branch. This bank serves as a valuable resource in conducting immunologic assays of tumor host immune interactions that result from therapy.

LITTON BIONETICS, INC. (NO1-CM8-7187)

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-CO7-5380)

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

Biological Response Modifiers Program (BRMP)

This contract provides support to the intramural program of the BRMP through the purchase of supplies, equipment and the renovation and maintenance of physical facilities. In addition, it also provides support for the preclinical efforts for potential new biological agents.

Chemotherapy Fermentation Laboratory (CFL)

The DCT supports a fermentation pilot plant facility at the NCI-FCRF for the large-scale production and isolation of microbial products of interest to the Chemotherapy and Biological Response Modifiers Programs. Facilities are also available for the production of other natural products. Large quantities of Interferon, Interleukin-III and other biologicals were produced for BRMP research projects during the last contract year. Fermentation and development is underway to increase yields of largomycin FII and toyocamycin. These materials will be produced in larger quantities for use in the program. Two novel antineoplastic agents have come from the earlier research effort and larger amounts of material will be produced for NCI evaluation.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGIC RESEARCH (NO1-CM9-7250)

This contract provided a multidisciplinary approach to drug development. The three main tasks included (1) the collection and screening of anti-neoplastic agents obtained from Southern Europe, (2) the screening of immuno-chemo therapeutic agents, and (3) pharmacologic studies of new agents with emphasis on the relationship of pharmacokinetics with in vivo antitumor effects. This contract expired on December 31, 1981.

MARSHALL RESEARCH ANIMALS (NO1-CM6-0123)

This contract provides 144 beagle hounds for toxicology assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the contractor.

MARYLAND, UNIVERSITY OF (NO1-CM4-3748)

Under this support services contract, the University of Maryland, at Baltimore, provides clinical and laboratory facilities, administrative support, and ancillary support services and personnel for the clinical and preclinical research program of the Baltimore Cancer Research Program. These activities include the Inpatient and Outpatient Departments, Cell Component Therapy, Clinical Research Pharmacy, Infection and Microbiological Research Sections, Laboratory of Clinical Biochemistry, and the Laboratory of Molecular Biology. This contract will be phased out during FY-1982.

MARYLAND, UNIVERSITY OF (NO1-CM8-7223)

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

MASON RESEARCH INSTITUTE (NO1-CMO-7325)

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

MASON RESEARCH INSTITUTE (NO1-CM6-7011)

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 Division of Cancer Treatment.

MASON RESEARCH INSTITUTE (NO1-CM8-7164)

This contract has as its major goal the maintenance of approximately 20,000 frozen tumor vials. This contractor furnishes needed tumors to the various DTP laboratories, as well as to other research institutions, both domestic and foreign. The tumors are supplied both in vivo and in vitro.

MASON RESEARCH INSTITUTE (NO1-CM9-7317)

Employing the P388 in vivo tumor system, synthetics and materials of natural product origin are assayed for efficacy. Materials, which are assigned to the tumor panel, are tested in the B16 melanoma; the colon 38; Lewis lung; L1210 murine tumor systems; and the colon, lung, and mammary human xenograft systems. Methodology research is conducted at the request of the Project Officer.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM1-7481)

This contract is part of a collaborative effort among three institutions to develop guidelines for the intraoperative radiotherapeutic treatment of abdominal tumors and tumors in others parts of the body. The collaborative effort will facilitate the investigation of this new radiotherapy modality by other institutions, both in terms of 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-CP8-5657)

This is one of four contracts on the subject of nutritional assessment which are funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is studying lipid mobilization and the response in fasting in cancer patients. This contract is being phased out.

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. The five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

MATHTECH, INC. (NO1-CM9-7195)

The objective of this contract is to provide a clinical trials monitoring service for clinical trials conducted by the Phase I and II Working Group. This service has two components: (a) to provide a central data management resource for both the Investigational Drug Branch (IDB) and for the clinical investigators conducting these studies and (b) to provide a monitoring resource to meet the Food and Drug Administration (FDA) regulatory requirements and to complement the data management objectives. This contract is currently being extended to allow for the recompetition of the contract which will probably take place in November, 1982.

MAXIMA CORPORATION (NO1-CM1-7400)

The objective of this small business contract is to perform computer searches in support of several Drug Synthesis and Chemistry Branch chemical search needs. These chemical searches support acquisition, preselection, task order syntheses, structure-activity studies, and literature monitoring. The substructure search feature furnishes data to determine program relevance, explore new areas of development, and identify areas for de-emphasis.

MAYO FOUNDATION (NO1-CM0-7414)

A member of the Lung Cancer Study Group: This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a randomized surgical trial of lobectomy versus limited resection for Stage I lung cancer. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of post operative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cisplatinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. A new protocol proposed for activation by the group evaluates preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of Lung Cancer Study Group have been accepted in four major journals.

MAYO FOUNDATION (NO1-CM0-7419)

This is one of four contracts devoted to the development of a human tumor clonogenic assay for drug screening. Objectives include developing evaluation criteria for valid assays and for activity. Limited developmental studies such as comparisons of enzymatic and mechanical disaggregation techniques and comparisons of different media also are being conducted to improve the cloning and cost efficiency of the system for large-scale screening.

MAYO FOUNDATION (NO1-CM2-7528)

This contract is part of a collaborative effort among three institutions to develop guidelines for the intraoperative radiotherapeutic treatment of abdominal tumors and tumors in others parts of the body. The collaborative effort will facilitate the investigation of this new radiotherapy modality by other institutions, both in terms of safety and efficacy.

MAYO FOUNDATION (NO1-CM9-7268)

This contract calls for a Phase II evaluation of therapies in advanced gastrointestinal cancer. More than 175 patients have been entered onto this contract during the past year. The role of the soft agar clonigenic assay as a prospective means of selecting chemotherapy will be analyzed in some colon cancers during FY 1982.

MAYO FOUNDATION (NO1-CM9-7273)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies have been conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast, and colon. This institution has been awarded a new Phase I contract. The Phase II portion of this contract is being phased out because the workscope of the RFP does not include Phase II studies.

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

The purpose of this contract is to provide effective inventory, distribution and quality assurance confirmation for biological response modifiers. The contractor is responsible for receipt, dispensing, storage, distribution and inventory control of biological agents. Quality assurance evaluation involves specific assays to confirm sterility and assays to determine pyrogenicity and endotoxin levels. The contractor performs general safety tests for biological agents in compliance with Government regulations intended for clinical use.

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

This resource contract provides for the production and purification of 5 billion units of human Type II (immune) interferon from human buffy coats with a specific activity of at least 2×10^7 units per mg of protein.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM-07337)

This contract is for Phase II/III studies to detect useful therapeutic effects of new drugs alone as well as other drugs in combination in solid tumors. The previous contract (NO1-CM-57043) completed a 5-year contract. During the period 1977-80 a total of 1,159 patients were entered into Phase II trials, and 1,590 patients into Phase III trials for a total of 2,749 entries. Between 2/1/79 and 1/31/80, a total of 233 patients were entered into polydrug studies. The results of these studies were reported in 56 papers and 19 abstracts.

This new Phase II/III contract is for a period of 9/15/80 to 2/14/86. Between 9/15/80 and 9/1/81, 10 protocols were carried over from the previous period and 8 protocols were activated during this period. About 270 patients were entered into protocols of which 196 were evaluated. Protocols continue to emphasize studies in testicular cancer, colorectal cancer, lung cancer, and other "signal tumors". This group has made significant contributions and has more than complied with the terms of the contract.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM1-5735)

This is one of three collaborating institutions which will assess the effects of augmented versus conventional versus no nutritional intervention in advanced colorectal and non-small cell lung cancer patients. The augmented nutritional support will consist of a higher percentage of daily caloric intake as protein, as well as additional mineral supplementation. Patients will be serially followed with extensive nutritional assessment to evaluate the effectiveness of the intervention in maintaining lean body mass. The nutritional assessment will include: total body nitrogen by prompt gamma emission techniques, total body potassium and water by isotope dilution, anthropometric measurements, and biochemical analyses.

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

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

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

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group has 44 patients on this study. Two more years of follow-up are planned.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM9-7274)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies have been conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast, and colon.

This institution has been awarded a new Phase I contract. The Phase II portion of this contract is being phased out because the workscope of the RFP does not include Phase II studies.

MEMORIAL SLOAN-KETTERING CANCER CENTER (NO1-CMO-7463)

This contract supported a Phase I study of AZQ in pediatric patients. The contract terminated on October 31, 1981, but was given a no-cost extension through June 30, 1982 to allow for additional patient accrual. It will not be recompeted as it is a one-time order contract.

MIAMI, UNIVERSITY OF (NO1-CMO-7409)

This contract is designed to explore in prospective, randomized, controlled protocol sutdies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer. Conversion to Cooperative Agreement is scheduled in this fiscal year.

MIAMI, UNIVERSITY OF (NO1-CM6-7093)

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

MIAMI, UNIVERSITY OF (NO1-CM9-7290)

Prior to November 1981, all crude natural products and fractions of crude natural products that had demonstrated efficacy in the 9ASK astrocytoma in vitro tumor model were tested in this system. Crude natural products that had presumptive cytotoxicity in either the 9KB, 9PS, or 9LE in vitro system as well as fractions of confirmed cytotoxic natural products were tested in the system of confirmed cytotoxicity. Synthetic materials were tested in one of the in vitro systems by special request or if submitted in too small a quantity for meaningful in vivo testing. Commencing November 1981, only fractions of crude natural products, presumptive cytotoxic materials, and synthetics submitted in either too small a quantity for meaningful in vivo testing or by special request were tested. This contract is terminated on June 30, 1982.

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 175 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherpy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

MICHIGAN, UNIVERSITY OF (NO1-CM-87225)

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

MICHIGAN TECHNOLOGICAL UNIVERSITY (NO1-CM-07293)

The objectives of this project are the rational design and synthesis of effective inhibitors of purine nucleoside phosphorylase (PNPase) and hypoxanthine guanine phosphoribosyl transferase (HGPRTase), key enzymes in the purine salvage pathway. Another target enzyme is inosinic dehydrogenase, a key enzyme that is rate-limiting in the synthesis of GMP and catalyzes an irreversible reaction. This contract will be phased out by the end of August, 1982.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (NO1-CM5-7009)

The major objective of this contract is the preparation of fermentations of marine, psychophilic, and thermophilic organisms. These fermentations are screened against various enzyme 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 compound from this work is in Phase II clinical trials in the U.S.A. Another compound has just passed Decision Network 2.

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CMO-7369)

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

MICROBIOLOGICAL ASSOCIATES (NO1-CM9-7246)

This rodent production center supplies inbred rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of four strains of rodents. This contract terminated March 31, 1982; however, it is expected to be renewed two years and seven months.

MICROBIOLOGICAL ASSOCIATES (NO1-CM9-7287)

This contract functions in four major areas:

1. To operate and maintain a virus serum diagnostic laboratory. Serum samples are submitted from contract animal suppliers and testing laboratories. Approximately 90,000 virus tests are performed annually for this portion of the effort.
2. To test experimental tumors (animal and human) for viral contaminants. An estimated 1,000 tumors will be tested annually.
3. To perform an estimated 4,000 ELISA tests annually for the detection of Mouse Hepatitis Virus (MHV).
4. To produce vaccinia virus which is used for immunizing mice against infectious ectromelia. Approximately 100,000 doses of vaccine are produced annually.

MIDWEST RESEARCH INSTITUTE (NO1-CM8-7234)

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

MILES LABORATORY, INC. (NO1-CM9-7172)

This was a no-cost contract concerned with the joint development of the antitumor agent, Chlorozotocin. Under this agreement, the contractor supplied the drug and provided other services to the NCI (at its own expense) in support of NCI-sponsored clinical studies. Because of a lack of promising clinical results, trials are closing. This contract expired February 15, 1982.

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 facility in a contract research facility. All testing is scheduled by the Project Officer.

MISSOURI, UNIVERSITY OF (NO1-CM9-7211)

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

MONSANTO RESEARCH CORPORATION (NO1-CM9-7255)

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

MONTEFIORE HOSPITAL (NO1-CM1-7474)

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

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

This contract evaluated the usefulness of neuraminidase-treated allogeneic AML cells in acute myelocytic leukemia. Patients were randomized to receive vaccine alone or vaccine plus MER following chemotherapy (cytosine, arabinoside, and daunorubicin). The MER arm has been deleted. Patients have been studied both *in vivo* and *in vitro* with a variety of immunological testing parameters.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM0-7407)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer. Conversion to Cooperative Agreement is scheduled in this fiscal year.

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

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

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM9-7275)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contract will be terminated this year, since this institution did not receive a new contract in the recompetition.

MUHIMBILI MEDICAL CENTER (NO1-CMO-7344)

This contract is to initiate the study of the demography and epidemiology of cancer in albino Africans at the University of Dar Es Salaam. Efforts will be made to establish an experimental model of the skin cancer/albino problem and its prevention with various retinoids or other materials.

MURPHY BREEDING LABORATORIES (NO1-CM5-0579)

This contract furnishes approximately 156,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

NATIONAL ACADEMY OF SCIENCES (NO1-CM5-3850)

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

NATIONAL CENTER FOR HEALTH STATISTICS (YO1-CM8-0109)

The objective of this Interagency Areement is to develop, implement and install a completely operational, functioning and computerized expandable exportable PROMIS System into the Baltimore Cancer Research Program clinical cancer treatment research setting. This contract will be phased out during FY-1982.

NATIONAL NAVAL MEDICAL CENTER (YO1-CMO-0103)

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

NAVAL MEDICAL RESEARCH INSTITUTE (YO1-CBO-0319)

This contract supported the opperation of a laboratory to perform detailed immunological studies on patients with cancer, particularly those receiving interferon. This contract terminated March, 1982.

NAVY, DEPARTMENT OF (Y01-CM1-0200)

This contract is for the design of plans for renovation of space in Building 1 (The Tower), and Building 8 of the National Naval Medical Center (NNMC), Bethesda, Maryland, to accommodate the NCI-Navy Medical Oncology Branch. This Branch replaced the NCI-VA Medical Oncology Branch and is staffed primarily by the personnel of the former NCI-VA Medical Oncology Branch. The transfer of clinical responsibilities occurred in July, 1981, and the full transfer of the Branch from the VA to the Navy occurred on October 1, 1981. The consulting architectural firm of Ellerbe, Dalton, Dalton and Newport (Mr. H. C. Allison and staff) has conducted numerous meetings with the senior medical and nursing staff of the NCI-VA Medical Oncology Branch and the responsible individuals of the NNMC conducting the retrofitting of the NNMC to coordinate and plan this project. This has led to the design of plans for renovating 6 floors of space (2 in Building 1 and 4 in Building 8) to be coordinated over a 2-3 year period. Initial plans were completed and received technical approval; however, additional design work is required to permit a modification of the space in Building 8 to accommodate a change in programatic requirements.

NAVY, DEPARTMENT OF (Y01-CM1-0201)

This contract is to cover the renovation and retrofitting of space in the National Naval Medical Center (NNMC), Bethesda, Maryland, to be occupied by the NCI-Navy Medical Oncology Branch. The NCI-Navy Medical Oncology Branch began clinical responsibilities in July, 1981, and transferred officially to the NNMC on October 1, 1981. Preliminary to this: (1) a "Memorandum of Understanding" was signed between the Surgeon General of the Navy annd the Director, NIH, and the Director, NCI; (2) meetings were held between NCI-VA, other NCI officials, Navy staff and with the Navy consulting architects firm of Ellerbe, Dalton, Dalton, and Newport (see contract Y01-CM1-0200); (3) a draft agreement (prepared by Dr. J. Minna, NCI, and Capt. D. Pasquale, NNMC) governing the interaction of the NCI and NNMC was approved in principle by the Commanding Officer, NNMC, and the Director, NCI. As part of this contract: (1) the NNMC will provide a completely designed and functional clinical ward of 30 beds (ward 6 West) in their new hospital facility in Bethesda which was completed and had occupancy January, 1981; (2) two floors of temporary "swing space" in Building 1 (floors 4 and 5) for housing the outpatient clinic, offices, laboratories, and conference rooms; (3) four floors of "permanent" space in Building 8 (floors 3, 4, 5 and 6) to house the same functions; (4) the Navy retrofitting facility (Dept. of the Navy, Chesapeake Division, Naval Facilities Engineering Command, Washington Navy Yard), will supervise the retrofitting and renovation of the space on site and submit monthly reports as to the status of the work. The swing space in Building 1 has been completed and occupied while the renovation of Building 8 has now begun. A change order is being prepared for the Building 8 renovation to accommodate a change in programatic requirements. This renovation is part of the larger renovation of the NNMC. This allows the NCI to have the lowest cost for renovation, saving the NCI from negotiating a separate, more costly, contract. The contract has been signed and approved by the Director, NCI, and the Commanding Officer, NNMC, Bethesda, Maryland.

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

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

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

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

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

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

NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CM0-7443-1)

This is a Phase I evaluation of thymosin fraction 5. Studies are in progress to determine maximum tolerated dose and maximum biological response modifying dose. This Phase I study is nearing completion and will define the thymosin fraction 5 dosage for Phase II trials. This contract expired June 30, 1982.

NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CM0-7443-2)

The purpose of this contract is to evaluate the toxicity, dose and modifications of biological response in patients by leukocyte interferon in a Phase I clinical trial. The maximum acceptable dose, dose-toxicity relationships of interferons in patients with various malignancies, duration of response and survival time will be determined in this trial. This contract expired June 30, 1982.

NORTHERN CALIFORNIA ONCOLOGY GROUP (NO1-CM8-7154)

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

NORTHROP INDUSTRIES, INC. (NO1-CM0-7286)

This contract has as its major purpose serological diagnosis for the presence or absence of murine viruses. During a contract period of one year, approximately 40,200 virus serology tests are performed. This contract will be phased out early in FY 1983 and will not be recompeted.

NORTHWESTERN UNIVERSITY (NO1-CM1-7363)

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

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM0-7442)

This contract was for a Phase I study to evaluate maximum tolerated dose and maximum biological response modifying dose of the pyran copolymer MVE-2. The contract expired December 31, 1981.

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

The general objectives of the project are concerned with the acquisition of pharmacokinetic data in patients on new and established antitumor agents. Coupled with information on the quantitative toxicology and the clinical response to the drug, the pharmacokinetic studies are used to design an optimum therapeutic dosage regimen. An analytical method for 2'-deoxycoformycin (NSC-218321, 2'-DCF) in plasma and urine was developed based upon adenosine deaminase (ADA) inhibition. Enzyme degradation was found to occur monoexponentially with time during the assay so the method developed for 2'-DCF quantitation takes into consideration the relative activity of the enzyme in the incubation mixtures. The pharmacokinetic analysis evaluated the results of 36 courses of 2'-DCF administered intravenously to 25 patients which consisted of 3 courses at 2 mg/M^2, 14 at 4 mg/M^2, and 19 at 10 mg/M^2. The following studies were performed in a limited number of patients: (a) whole blood pharmacokinetics of 2'-DCF, (b) renal clearance of 2'-DCF, (c) time-course inhibition of ADA in plasma following intravenous bolus administration of 2'-DCF. The plasma concentration of 2'-DCF as a function of time showed first-order elimination with a biphasic decay characteristic of a two-compartment open model. The rapid disposition phase is short with the harmonic mean t 1/2 α = 8.72 min; the terminal half-life is 4.93 hours; the mean central compartment volume is 5.94 $\pm$ 3.29 L/M^2; the mean apparent volume of distribution is 23.1 $\pm$ 6.16 L/M^2; and the total body clearance is 52.4 $\pm$ 16.6 ml/min/M^2. 2'-DCF is largely eliminated in the urine within 24 hours (95.9 $\pm$ 12.2% of the administered dose). 2'-DCF is not converted to a metabolite which is inactive as an ADA inhibitor. The time profiles for ADA in plasma demonstrated an initial mean harmonic half-life of ADA activity of 5.3 minutes and a terminal half-life of 8.6 hours. The terminal phase half-life reflects the total body enzyme recovery. Complete recovery of plasma ADA occurred within 24 hours of 2'-DCF administration in all the patients.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM9-7264)

The purpose of this contract is to study the pharmacologic properties of new antitumor agents in experimental animals (rats, mice and dogs). Assay methods are developed, and information is then obtained on the absorption, plasma clearance, distribution, plasma protein binding, metabolism and urinary and biliary excretion of such agents. Comprehensive pharmacokinetic studies with potential predictive value for subsequent clinical application are stressed. Studies are presently under way with the following compounds of high priority program interest: the C-nucleoside riboxamide (NSC-286193), an isopropyl pyrrolizine compound (NSC-278214) and dihydro-5-azacytidine (NSC-264880). This contract terminated June, 1982.

ONTARIO CANCER INSTITUTE (NO1-CM0-7418)

A member of the Lung Cancer Study Group: This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a randomized surgical trial of lobectomy versus limited resection for Stage I lung cancer. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cisplatinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of post operative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cisplatinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. A new protocol proposed for activation by the group evaluates preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of Lung Cancer Study Group have been accepted in four major journals.

ONTARIO CANCER INSTITUTE (NO1-CM9-7267)

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

PAN AMERICAN HEALTH ORGANIZATION (NO1-CM2-7391)

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

PAN AMERICAN HEALTH ORGANIZATION (NO1-CO6-5332)

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

PAPANICOLAOU CANCER RESEARCH INSTITUTE (NO1-CM8-7230)

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

PENNSYLVANIA, UNIVERSITY OF (NO1-CM2-7529)

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

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

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

PITTSBURGH, UNIVERSITY OF (NO1-CB2-3876)

This contract is designed to support the study of adjuvant treatment in breast cancer. Currently, protocols B-04, B-05, B-07, B-08, and B-09 are closed to patient accrual although all patients are still in followup. New protocols for Stage II breast cancer testing the role of adjuvant adriamycin, and for Stage I breast cancer have recently been activated. Conversion to Cooperative Agreement is scheduled in this fiscal year.

PITTSBURGH, UNIVERSITY OF (NO1-CM7-7177)

This contract is designed to support randomized controlled studies using adjuvant therapy in the treatment of colo-rectal cancer. Present studies include C-01 which randomizes patients having stage B+C colon cancer between no treatment, chemotherapy, and immunotherapy, and R-01 which randomizes patients having stage B+C rectal cancer between no treatment, radiotherapy and chemotherapy. Conversion to Cooperative Agreement is scheduled in this fiscal year.

POLYSCIENCES, INC. (NO1-CM0-7300)

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.

PURDUE RESEARCH FOUNDATION (NO1-CM9-7296)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the U. S. Department of Agriculture. This contract terminated July, 1982.

RESEARCH TRIANGLE INSTITUTE (NO1-CM0-7352)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

RESEARCH TRIANGLE INSTITUTE (NO1-CM1-7396)

This is a service contract to prepare extracts of plant materials for screening by the screening laboratories. The majority of plant extracts screened by NCI originate with this contract. The majority of the contractor's plants are obtained through a Transfer of Funds Agreement between the NCI and the U. S. Department of Agriculture. Special studies on methodology of plant extraction are conducted in order to maximize the yield of confirmed active plants. Due to program changes, this contract was terminated at the end of the first year on July 31, 1982.

RESEARCH TRIANGLE INSTITUTE (NO1-CM9-7261)

This procurement contract collects plants in the United States. The plants are then subjected to aqueous extraction and the samples lyophilized and sent to NCI screeners for testing. Actives are submitted to the NCI fractionation contractors. This contract was terminated in February 1982.

RESEARCH TRIANGLE INSTITUTE (NO1-CM9-7313)

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 radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

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

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

SASCO (NO1-CM9-0164)

This procurement contract provides for the supply of 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for the Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers.

SIDNEY FARBER CANCER INSTITUTE (NO1-CM0-7433)

This is a Phase I/II clinical trial utilizing human leukocyte interferon. Studies are in progress to determine the maximum tolerated dose and maximum biological response modifying dose. The Phase I part of the study is nearing completion and the Phase II portion of the study is expected to begin shortly.

SIDNEY FARBER CANCER INSTITUTE (NO1-CM5-7035

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

SIDNEY FARBER CANCER INSTITUTE (NO1-CM9-7276)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contract will be terminated this year, since this institution did not receive a new contract in the recompetition.

SIMONSEN LABORATORIES (NO1-CM5-0578)

This contract furnishes approximately 156,000 six-week old B6C3F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers.

SIMONSEN LABORATORIES (NO1-CM7-7166)

The contractor maintains a primary genetic center of inbred strains of rodents. Small quantitites of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production colonies from pedigreed expansion colonies. All pedigreed foundation colonies are maintained in associated flora isolators.

SIMONSEN LABORATORIES (NO1-CM9-7247)

This contract provides for the maintenance of a rodent production center. This produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirement for specific investigations. This contract furnishes breeding animals for largescale production colonies. The breeding stock is received from the primary genetic centers. This contract terminated March 31, 1982; however, it is expected to be renewed for another two years and seven months.

SISA, INC. (NO1-CM0-7354)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

SLOAN-KETTERING INSTITUTE FOR CANCER CENTER (NO1-CB5-3875)

This is a trial evaluating levamisole as a therapeutic adjunct in squamous cell carcinoma of the head and neck. This contract is being phased out.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CB5-3970)

This contract evaluated selected immunotherapeutic agents for acute and chronic manifestations of toxicity, maximum tolerated dose, and effects on several immune parameters. Agents studied included BCG, poly ICLC and endotoxin. This contract evaluated the effects of these agents on lymphokine production, T-cell function, and granulocyte function. This contract is being phased out.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CB7-4145)

The objective of this contract is to determine whether the cells of renal cancers have cross-reacting tumor-associated antigens. The contractor will attempt to analyze the specificity of the serological reactions and to investigate the patterns of antigen expression in terms of culture time, growth characteristics, and cell cycle kinetics. Periodic assessment of tumor immunity will be performed on sera from patients with Stage I renal cancer receiving conventional therapy and on sera from patients with Stage II, III, and IV renal cancer who are immunized with BCG and allogeneic renal cancer cells. This contract expired March 29, 1982.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CB7-4146)

This contract evaluated the usefulness of levamisole in the treatment of patients with squamous cell head and neck cancer. Patients were randomized following surgery to receive levamisole or a placebo in a double blind study. This contract has been phased out.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CMO-7435-1)

This is a Phase I evaluation of thymosin fraction 5. Studies are in progress to determine maximum tolerated dose and maximum biological response modifying dose. This Phase I study is nearing completion and will define the thymosin fraction 5 dosage for Phase II studies. This contract expired June 30, 1982.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CMO-7435-2)

This contract is for a Phase I/II clinical trial utilizing human fibroblast interferon. It is estimated that the interferon for these trials will be available in July, 1982, at which time patient accrual will start.

SMALL BUSINESS ADMINISTRATION (NO1-CM4-3719)

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

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM1-7521) (FORMERLY INFORMATION PLANNING ASSOCIATES, INC. NO1-CM7-7104)

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 amending information as necessary. The contractor is also involved with the preparation of IND annual reports, the establishment of drug master files, the distribution of clinical brochures, and the dissemination of adverse drug reaction information.

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

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

SOUTH FLORIDA, UNIVERSITY OF (NO1-CM-87220)

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

SOUTHERN ANIMAL FARMS (NO1-CM5-0599)

This contract furnishes approximately 156,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies.

SOUTHERN ANIMAL FARMS (NOl-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 March 31, 1982; however, it is expected to be renewed for another two years and seven months.

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

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

SOUTHERN RESEARCH INSTITUTE (NOl-CMO-7260)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

SOUTHERN RESEARCH INSTITUTE (NOl-CM9-7309)

This project is designed to provide to DCT a maximally flexible single instrument for the rapid conduct of Program directed developmental and applied studies pertinent to all pre-clinical, therapy related, tasks. Principal tasks are (1) to apply fundamental biological principles to the development of new and improved laboratory models for the discovery of more effective antitumor drugs; (2) to evaluate, in detail, drugs in development to NCI sponsored clinical trial to determine their optimum conditions of usage; (3) to conduct *in vivo* screening against transplantable animal tumors and human tumor xenografts; and (4) to conduct studies leading to the refinement of screening protocols.

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

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

SRI INTERNATIONAL (NO1-CMO-7351)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

SRI INTERNATIONAL (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. The five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

SRI INTERNATIONAL (NO1-CM-17485)

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

SRI INTERNATIONAL (NO1-CM8-7183)

SRI International is the major contractor 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. Also solubility, stability and other physical-chemical properties are determined to provide information as a guidance to the effective use, formulation and storage of the materials. 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 analytical testing provide data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring. This is the larger of the two contracts for providing this service.

SRI INTERNATIONAL (NO1-CM9-7256)

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

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

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

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

This contract is in support of the Drug Synthesis and Chemistry Branch's fundamental responsibility to acquire selected novel synthetic compounds for evaluation as potential anticancer agents the initial step in the National Cancer Institute's Linear Array for drug development. The major focus of this contract is the active solicitation, acquisition 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.

STARKS, C. P., INC. (NO1-CMO-7357)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. A majority of the compounds selected for re-synthesis is requested by the DTP Prescreen Committee. Task Order synthesis also serves to support the radiosensitizer and radioprotector screening contracts. This mechanism is also used for the resynthesis of a limited number of panel compounds. Unique compounds which are novel and potential anticancer agents emerging from the literature are also synthesized by this mechanism in quantities sufficient for program needs.

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

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

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

TEXAS INSTITUTE FOR REHABILITATION AND RESEARCH (NO1-CP8-5618)

This is one of four contracts on the subject of nutritional assessment which are funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contract supports studies of stereometric photography as a measure of body surface area, body volume, and body density. From body density, body fat and muscle may be estimated. This contract is being phased out.

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. The five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER (NO1-CB8-4248)

The objective of this contract is to evaluate, in a controlled clinical study, the effect of intralesional injection of BCG crude cell walls on canine breast carcinoma. Dogs clinically free of detectable metastatic disease are randomly assigned to intralesional immunotherapy prior to surgery. The BCG crude cell walls are provided by the NCI. Tumor regression, tumor recurrence, disease-free interval, and survival data from the two are compared. Selected assays of humoral and cellular immunity are performed and results correlated with clinical course.

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

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

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

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

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CB3-3888)

This contract encompassed several prospectively randomized Phase I and II trials of biologic response modifiers. Agents studied were MER, C. parvum, BCG, CWS, P_2, endotoxin, and thymosin V. Immunologic studies were also performed both in vivo and in vitro. A draft of the final report has been submitted, and this project is being phased out.

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

This contract conducts Phase II/III studies in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, testicle, ovary, endometrium, cervix, head and neck, stomach, pancreas, and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. A minimum of 175 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CMO-7417)

Lung Cancer Study Group Pathology Reference Center: A special task contract to review cytology, surgical biopsies, and autopsy material provided by the Lung Cancer Study Group. Data is accrued and processed to develop models for study of the natural history of limited stage lung cancer.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND 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-CM9-7277)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast, and colon. This institution has been awarded a new Phase I contract. The Phase II portion of this contract is being phased out because the workscope of the RFP does not include Phase II studies.

TORONTO GENERAL HOSPITAL (NO1-CM1-5803)

This is one of three collaborating institutions which will assess the effects of augmented versus conventional versus no nutritional intervention in advanced colorectal and non-small cell lung cancer patients. The augmented nutritional support will consist of a higher percentage of daily caloric intake as protein, as well as additional mineral supplementation. Patients will be serially followed with extensive nutritional assessment to evaluate the effectiveness of the intervention in maintaining lean body mass. The nutritional assessment will include: total body nitrogen by prompt gamma emission techniques, total body potassium and water by isotope dilution, anthropometric measurements, and biochemical analyses.

UPJOHN COMPANY (NO1-CM0-7380)

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

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. The five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

VANDERBILT UNIVERSITY MEDICAL CENTER (NO1-CM0-7438)

This contract was for a Phase I study to evaluate maximum tolerated dose and maximum biological response modifying dose of the pyran copolymer MVE-2. The contract expired December 31, 1981.

VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE (NO1-CM9-7278)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations of regimens mutually agreed upon. This institution has been awarded a new Phase I contract.

VETERANS ADMINISTRATION (YO1-CM7-0107)

The VA Surgical Oncology Group is supported under this Interagency Agreement. The Group, in existence for some 23 years, conducts trials evaluating chemotherapy, radiotherapy, and/or immunotherapy as adjuncts to surgical treatment of lung, gastric, pancreatic, esophageal, rectal, head and neck, and colon carcinomas. Ancillary studies include the identification of prognostic factors, the significance and evaluation of hormonal markers and CEA, and histopathologic classification. The Board of Scientific Counselors recommended termination of this Agreement, and phaseout funding will continue until September, 1982. The Group has closed all of their studies and are in the process of preparing final manuscripts.

VSE CORPORATION (NO1-CMO-7251)

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

VSE CORPORATION (NO1-CM8-7192)

The Drug Distribution and Protocol Monitoring System (DDPMS) is an automated procedure of verifying the accuracy of requests made by clinical investigators for investigational and commercial anticancer drugs. This verification is mandated by the Food and Drug Administration since federal law requires that drug used for investigational purposes only be given in sufficent quantity to authorized users for approved protocols. Verified data is retained and forms a drug distribution history which is used to monitor protocol progress as clinical trials progress. This protocol monitoring function is also a Food and Drug Administration requirement imposed on the National Cancer Institute.

The DDPMS is composed of numerous computerized files containing investigators' names and addresses, the protocol information file (investigator name, number, approved protocols, drug dosage form, registration record), the IND Drug Activity File (drug distribution history by protocol) and the patient report file. In addition, the system has reading capability to retrieve inventory and drug cost data from a system operated by the Pharmaceutical Resources Branch.

WARNER-LAMBERT COMPANY (NO1-CMO-7379)

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) a small biotransformation program; (4) the isolation work required to obtain the active component from the confirmed active beers; (5) the production of large quantities of antineoplastic agents approved for clinical trials; and (6) the preparation of appropriate dosage forms of such agents.

WARNER-LAMBERT COMPANY (NO1-CM1-7491)

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

WASHINGTON, UNIVERSITY OF (NO1-CB8-4247)

The objective of this contract is to determine the conditions and operative mechanisms for maximizing the therapeutic effect of adoptive cellular immunotherapy used alone or in combination with chemotherapy in the treatment of tumors of C57BL/6, BALB/c and CB6F1 (BALB/c female x C57BL/6 male) mice. The approach is to utilize, in adoptive cellular immunotherapy experiments, cells immunized both *in vitro* and *in vivo*. Studies will be undertaken to characterize the effector cell(s) responsible for tumor therapy as well as cells which suppress the therapeutic effect. The role of the antigens for the major histocompatibility complex in sensitization to tumor associated antigens and in the generation of cells therapeutically effective against established syngeneic tumors will be explored.

WASHINGTON, UNIVERSITY OF (NO1-CM9-7282)

This contract provides a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support a clinical neutron therapy research program at the University of Washington. The fabrication of the cyclotron and other components of the neutron therapy system is nearing completion. Construction is underway and the facility should be completed in late 1982 or early 1983. The facility should be operational in mid 1983.

WAYNE STATE UNIVERSITY (NO1-CMO-7404)

The contractor conducts Phase II/III studies in patients with solid disseminated tumors. A minimum of 175 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

WAYNE STATE UNIVERSITY (NO1-CMO-7408)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer. Conversion to Cooperative Agreement is scheduled in this fiscal year.

WAYNE STATE UNIVERSITY (NO1-CM9-7279)

This contract is designed to conduct Phase I and Phase II studies with new anticancer drugs sponsored by the Division of Cancer Treatment. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast, and colon. This institution has been awarded a new Phase I contract. The Phase II portion of this contract is being phased out because the workscope of the RFP does not include Phase II studies.

WELLCOME FOUNDATION, LTD. (NO1-CM1-7489)

This contract was for delivery of 50 billion units of lymphoblastoid interferon, 3×10^7 units/mg protein, and has been successfully completed. This contract expired January 1, 1982.

WISCONSIN, UNIVERSITY OF (NO1-CMO-7434)

This contract is for a Phase I/II clinical trial utilizing human fibroblast interferon. It is estimated that the interferon for these trials will be available in July, 1982, at which time patient accrual will start.

WISCONSIN, UNIVERSITY OF (NO1-CM9-7280)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This institution has been awarded a new Phase I contract.

WISCONSIN MEDICAL COLLEGE (NO1-AIO-2658)

The Biological Response Modifiers Program funded this project entitled "Immune Interferon Standards" in Fiscal Year 1980. The contract is administered by NIAID and expires on August 31, 1982. The objective of the contract is to provide NIH with 1,200 ampules of (1) a suitably stable, potent, freeze-dried immune interferon prepared from human lymphocytes and (2) an equal number of a similarly suitable preparation prepared from mouse lymphocytes for use as standard reference reagents.

YALE UNIVERSITY (NO1-CB7-4191)

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

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1-CMO-7339)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer. Conversion to Cooperative Agreement is scheduled in this fiscal year.

YALE UNIVERSITY (NO1-CM5-3843)

This contract funds participation in the Gastrointestinal Tumor Study Group. It is currently in phaseout status.

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

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

International Agreements for Collaborative Preclinical and Clinical Research

Through a series of bi-national agreements, the Division of Cancer Treatment undertakes certain preclinical and clinical research programs which can best be accomplished by international collaboration. These Agreements permit the monitoring of worldwide developments in therapeutic research and facilitate the exchange of information, personnel, and various research materials. In particular, international Agreements permit the identification and procurement of synthetic agents and natural products from abroad that are of potential clinical interest to the NCI.

The international aspect of the DCT's activities is coordinated closely with the Office of the Associate Director for International Affairs, NCI; the NCI liaison office in Brussels; and with such important international organizations as the European Organization for Research and Treatment of Cancer (EORTC), the International Union Against Cancer (UICC), the World Health Organization (WHO), the Pan American Health Organization (PAHO), and the International Agency for Research on Cancer (IARC). The NCI's Cancer Chemotherapy Research Office in Brussels is a center of reference which makes available to investigators in the US and Europe all pertinent information concerning ongoing cancer research programs on both continents. The Brussels office has been especially useful in the areas of experimental and clinical pharmacology, clinical trials, exchange of scientists, and in the organization of symposia jointly conducted by American and European investigators. The Brussels office also relates closely to the European pharmaceutical industry, providing a flow of new agents with potential anticancer activity.

An office in Japan similar to the NCI's operation in Brussels is under consideration, in view of the important contributions already made to cancer treatment by the Japanese academic establishment and pharmaceutical industry.

France

The French-American Agreement for Cancer Clinical Research involves the DCT and the Institut National de la Sante et de la Recherche Medicale (INSERM), as well as various French institutions funded by INSERM. The US-France Agreement for scientific and technical cooperation has included a cancer research activity since 1975. The clinical research program includes an exchange of information and collaborative research in the areas of chemotherapy radiation oncology, biologic response modification, immunotherapy, endocrine therapy, clinical pharmacology, clinical trials, and clinical epidemiology. The annual meeting of the Steering Committee for this Agreement was held in Paris in June 1982. Ongoing projects were reviewed in the areas of clinical trials (new drugs); clinical pharmacology; biologic response modifiers; radiation therapy; and clinical epidemiology. There are 18 such projects conducted jointly by French and American investigators. These projects include studies of new nitrosourea analogs, anti-estrogens, the chemotherapy of G.I. tract malignancies, the mechanism of action of ellipticines, new derivatives of anthracyclines, new platinum coordination compounds, the pharmacokinetics of fluoropyrimidines, and a new series of DNA-bisintercalating dimers. Other investigations concern drug-carrying

liposomes coupled to monoclonal antibody and chemically defined immunomodulators. An important epidemiologic study is concerned with hepatitis B virus and its relationship to primary carcinoma of the liver in tropical Africa. Most of these projects have been productive and a majority were rated highly at the Steering Committee Meeting. Future plans include a continuing exchange of American and French scientists, a number of joint workshops, and a new collaborative epidemiologic study concerned with the geographic epidemiology of the human T-cell leukemia virus (HTLV).

Japan

The US-Japan Cooperative Cancer Research Program is in its eighth year, and has been particularly active in the area of new drug development and evaluation. The Agreement has facilitated a continuing flow of drugs from Japan to the United States, enhancing the chemotherapy potential of the DCT's overall program. A number of joint clinical trials have also been instituted; a major study on the chemotherapy of gastric carcinoma is nearing completion, and is among the first cancer clinical trials to investigate whether there are ethnic/racial differences in response to chemotherapeutic agents.

During the past year, workshops were held under the sponsorship of the therapy portion of this Agreement on "The Development and Evaluation of New Combined Modality Regimens" (Gettysburg, PA); the Evaluation of Immunotherapy (Hawaii); and the Treatment of Bladder Cancer (Tokyo). A number of scientist exchanges occurred in the areas of radiation oncology and urology. A continued exchange of drugs for preclinical investigation was also carried out.

During the past year, much emphasis has been given to the sharing of information on new synthetic compounds, such as 4-carbamoylimidazolium-5-olate and M-83; mitoxantrone; new antibiotics (CC-1065 and Pip 1B); pentostatin and neothramycin; aclacinomycin, and pepleomycin. In the area of immunotherapy there has been particular interest in Bestatin, and in the area of radiation therapy, in misonidazole. The major focus of clinical trial information exchange within this Agreement has been on small cell carcinoma of the lung, carcinoma of the stomach, and carcinoma of the bladder. With respect to the joint clinical trial in advanced gastric cancer, as noted previously, there were no differences in response rate nor duration between the patients of the two countries; toxicity was also comparable, but the therapy itself (adriamycin + 5 FU) did not yield a therapeutic improvement.

With respect to carcinoma of the bladder, information was exchanged on the use of karyotype analysis in diagnosis, intravesical chemotherapy, and the development of new approaches to invasive bladder carcinoma using in vitro sensitivity assays, the nude mouse model, and biochemical parameters.

Plans for future meetings include: An annual program review in Bethesda (November, 1982); a workshop on radiation oncology to be held in Japan (October, 1982); and a workshop in Hawaii on the Genetics of Drug Resistance and Sensitivity (summer of 1983). In a further attempt to increase cooperation in the area of new drug development, a number of Japanese investigators have been invited to attend the NCI's Phase I and II Working Group Meetings and will

receive NCI Decision Network proceedings. As noted previously, the establishment of a liaison office in Japan is under consideration.

The DCT also supports a contract with the Cancer Chemotherapy Center of the Japanese Foundation for Cancer Research which provides us with comprehensive information on all new drug screening in Japan, new drugs undergoing preclinical preclinical and clinical trials in Japan, and other clinical investigations. In the last year, the contractor submitted 910 discrete informational items including 418 abstracts, and the proceedings of 34 meetings held in Japan. The results of Phase III clinical trials in 242 patients were provided, and the NCI received complete details on Phase I-II studies in Japan of mitoxantrone, neothramycin, fibroblast and lymphoblastoid interferon, prednimustine, hexamethylmelamine, pepleomycin, and vindesine. The Cancer Chemotherapy Center contract will continue to function during the coming year, increasing the input into the chemotherapy program.

Italy

The Cooperative Cancer Program between the NCI and the Italian National Cancer Institute was formed in 1977, and provides for the sharing of research information in the areas of clinical trials, basic and clinical pharmacology, and cancer-related epidemiology. A program review was held in November, 1981 in Bethesda involving 20 American and 20 Italian scientists, and provided a detailed exchange of information in the areas described above.

In the past year, the scientist exchange aspect of the clinical porition of that Agreement has been very active, with Drs. Kanter, Bernacki, Pizzo, Bast and Rustum visiting Italian institutions, and Drs. Cornaglia, Canobbia, Bonadonna, Bernardi, Amadosi, Salmona, Giutani and Vecchi visiting the NIH or other American institutions. Until now, much of the thrust of the joint workshops and symposia has been to establish a foundation for truly collaborative scientific projects. In the next year, it should be possible to define such specific joint projects in cancer clinical and preclinical research. The possibilities for truly collaborative investigation seem strongest in the following areas: clinical trials in breast cancer; clinical trials in neuroblastoma; studies of supportive care; studies of new anthracyclines and analogs; and studies of biologic response modifiers. The latter two areas of interest are particularly strong in Italy, and the NCI stands to profit significantly from this collaboration. Moreover, with the arrival of Dr. Pistenmaa, we are hopeful of launching a collaborative program in Radiation Therapy during the coming year.

The next meeting of the Steering Committee will be held in Sicily in October, 1982, and will include a bi-national update on combined modality treatment trials in selected tumors, the integration of radiotherapy in combined modality regimens, new approaches to treatment such as monoclonal antibody, the genetics of drug resistance and sensitivity, and new insights from basic science with applicability to clinical research. Issues of common interest within the area of supportive care will also be discussed, and one-half day of th day of the workshop will be taken up with radiobiology and new directions in radiotherapy.

Latin America

The NCI's Collaborative Cancer Treatment Research Program with Latin American countries is mediated through the Pan American Health Organization. Unlike our other bi-national Agreements, the PAHO member institutions perform clinical trials under contract with the Cancer Therapy Evaluation Program. Formal protocols guide the conduct of these trials in hematologic malignancies, childhood malignancies, sarcomas, carcinoma of the stomach, breast cancer, head and neck cancer, and miscellaneous solid tumors. At this time, the feasibility of multi-national protocols is under consideration; this would involve a number of Latin American countries in collaboration with American institutions on specific protocols in order to increase patient accrual. This program is entering its fourth year and about 600 new patients per year are accrued to the NCI-sponsored PAHO clinical trials. There are nine major Latin American centers active in the program with the satellite participation of 45 additional institutes and 92 investigators. A program review committee assesses the performance of the individual institutions and is advisory to the Clinical Oncology Review Committee, DCT as well as to PAHO. Detailed results of the PAHO clinical trials are presented in the annual report of the Cancer Therapy Evaluation Program.

Germany

The NCI-German Research Ministry Program in Cooperative Cancer Research was formed as a subset of a DHHS-Germany Agreement signed in 1976. The Agreement in cancer therapy emphasizes drug development and has been in existence since February, 1981. The program includes the exchange of information and materials, tumor screening methodology and data, studies in animals on the chemotherapeutic and long-term toxic effects of specific classes of cancer chemotherapeutic agents (e.g., nitrosoureas), and Phase I-II trials of new drugs. The next bi-national Steering Committee meeting will take place in Germany in September of 1982, and at that time we hope to establish the logistics for exchange of scientists, reagents, and collaborative studies. The areas of interest to be emphasized include: human tumor xenografts in drug screening, agents which influence differentiation and dedifferentiation, regional detoxification, water souble nitrosoureas, and Phase I-II trials. From the NCI side, this Agreement largely involves the Developmental Therapeutics Program (Dr. John Driscoll). The pharmaceutical and chemical industries in West Germany are very strong and have historically provided a number of important candidate antitumor agents; thus this Agreement has great potential for the NCI's Drug Development Program.

USSR

The sixth annual American-Soviet program review was held in Bethesda during September of 1981. At this meeting, it was agreed to restructure the program to include the following three subject areas:

A. Cancer Treatment

B. Carcinogenesis

C. Cancer Prevention

At that meeting, it was jointly decided to emphasize the exchange of scientists and to discourage large meetings and delegations. Since that meeting, activity within the US-USSR Agreement has been minimal. Dr. Daniel Hoth, CTEP, DCT, visited Moscow in November 1981 to review Soviet data accrued on tamoxifen as an adjunct to the surgical therapy of breast cancer, this having been a joint trial. Drs. Gorbunova and Sudzhyan, Moscow, visited the Baltimore Cancer Research Center and the Sloan Kettering Institute in February, 1982, and a joint Phase I trial of CBDCA was initiated in May with CBDCA recently sent to Moscow. There is also a limited drug flow between the two countries for use in their respective preclinical screening programs.

United Kingdom

The NCI has no formal Agreement with the United Kingdom, but there is extensive collaboration and informational exchange between the DCT and various British institutions, particularly in the area of drug development and screening. The DCT has an especially close relationship with the Institute of Cancer Research (Dr. K. Harrap). Dr. John Venditti functions as Project Officer on a contract with Dr. Harrap which has contributed much information to our understanding of DNA-reactive drugs and DNA repair processes as they influence cancer chemotherapy.

Hungary

A US-Hungary Agreement was signed in 1979, but activity so far has been minimal. The Agreement covers the exchange of candidate antitumor agents, (including synthetic and natural products), collaborative assay of selected agents in tumor test systems, and Phase I-II clinical trials. Liposome encapsulation of anti-tumor drugs is of particular interest since there is expertise in Hungary as well as in the U.S. with this system. Selected aspects of biologic response modification are also of interest in Hungary, particularly involving characterization of alloantisera specific for T-cell subpopulations.

Peoples' Republic of China

The US-PRC program in cancer research is still being formalized. The intention is to emphasize combined modality therapy; screening; and pharmacologic as well as toxicologic evaluation of selected new drugs. There is much interest in the treatment of tumors that are prevalent in China, e.g., carcinoma of the esophagus, lung, liver, nasopharynx, stomach, and breast. The exchange of potential anti-tumor agents will include conventional drugs (natural and synthetic origin), and herbal derivatives. There have been a number of scientist exchanges in preparation for the inception of this Agreement. Finally, because of the recent discovery of an endemic human T-cell leukemia virus (HTLV) in southwest Japan, much effort will be placed on initiating collaborative arrangements to facilitate an examination of the seroepidemiology of this candidate human leukemia virus in the Peoples' Republic.

Poland

The NCI's cooperative cancer program with Poland involves the Maria Sklodowskiej-Curie Memorial Institute of Oncology and the National Research Institute of Mother and Child. The Agreement with the Institute of Oncology was signed in

1976, and that involving the National Research Institute of Mother and Child was signed in 1980. To this time, the clinical cancer research aspects of this Agreement have been within the area of pediatric oncology and have included scientist exchanges. The first extended visit of a Polish investigator to the NIH is that of Dr. Danuta Perek, who has spent six months during the present year with the Pediatric Oncology Branch, DCT. Dr. Wieslaw Czerwinski is scheduled to spend three months with that same Branch in the autumn of 1982. The Pediatric Oncology Branch has established joint Phase II clinical trials in neuroblastoma with the National Research Institute for Mother and Child. These ongoing trials involve streptozotocin and single-dose actinomycin D.

Egypt

The NCI has a formal bi-national Agreement with the Republic of Egypt, which in the past has been supported by PL-480 funds. The Cairo Cancer Institute is a well-organized facility and has an accrual of patients with bladder cancer probably greater than that of any other single institution in the world. Most recently, a Phase II study of chemotherapeutic agents was completed, having been initiated in 1976. Twelve drugs have been studied in about 400 patients having squamous and transitional cell bilharzial bladder carcinoma. The drugs include: hexamethylmelamine, VM 26, methotrexate, bleomycin, adriamycin, platinum, cytoxan, flurouracil, trenimone, m-Amsa, vincristine, and pentamethylmelamine. Notable response rates include: HMM, 38%; PMM, 40%; VCR, 44%; trenimone, 60%; cytoxan, 28%; FU, 15%; and DDP, 13%. Trenimone is no longer manufactured and platinum administration has been limited by nephrotoxicity in Egypt.

On the basis of these results, an active chemotherapy combination might include vincristine, PMM, and FU. The durable response rate after radical cystectomy in bilharzial bladder carcinoma is 35% (five year disease-free survival), not improved by pre- or post-operative radiotherapy. Thus, it is timely to initiate a controlled trial of radical cystectomy ± adjuvant chemotherapy. The combination described above is under active consideration, as are other combinations. It is anticipated that 100 patients can be entered on each arm of a two-armed randomized study within two years, and the drugs will be made available by the NCI for this latest study of the US-Egypt Agreement.

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8. Riccardi, C., Bartocci, A., Puccetti, P., Spreafico, F., Bonmassar, E., and Goldin, A.: Combined Effects of Antineoplastic Agents and Anti-Lymphoma Allograft Reactions. European Journal of Cancer 16: 23-33, 1980.

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ANNUAL REPORT - SCIENTIFIC INFORMATION BRANCH
1981 - 1982

The Scientific Information Branch was established in 1980 to provide overall management and direction to the publications of the DCT and its information gathering and reporting services. The SIB is located in the Office of the Director, DCT, NCI and contains two major components: the Publications Section and the Literature Research Section. Its activities can be divided into three major areas: the publication of scientific and technical information in Cancer Treatment Reports, Cancer Treatment Reports Symposia, and the DCT Bulletin; the preparation of information surveys, reports and reviews for the scientific staff of the DCT and its committees and panels; and the operation of the DCT library. In addition, the Chief, SIB, participates with senior investigators of the Medicine Branch in the evaluation and analysis of various ongoing clinical trials.

PUBLICATIONS

Cancer Treatment Reports (CTR), a primary source scientific journal dealing with preclinical and clinical cancer treatment is in its twenty-second year of continous publication. From 1959 to 1968 CTR, then known as Cancer Chemotherapy Reports, was issued 6-10 times a year depending on the acceptance of manuscripts submitted. Several types of manuscripts were published, including program information, study protocols, experimental studies, and clinical reports. Most but not all manuscripts were reviewed by outside referees as well as by the editorial board. In 1968 the journal expanded to three parts: Part I (original research, both experimental and clinical), Part 2 (comprehensive, lengthy chemotherapy studies involving a great deal of tablular material), and Part 3 (program information including study, protocols, clinical brochures, toxicology reports, and review articles).

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

1. Full length manuscripts containing the results of clinical or preclinical research.
2. Brief Communications
3. Letters to the Editor
4. Clinical Trials Summaries
5. Guest editorials and commentaries
6. Current Controversies in Cancer Management
7. Meeting reports

All material submitted for consideration in CTR is subject to review as appropriate by two outside reviewers and a member of the editorial board. In

addition, the journal publishes meeting announcements and program information for oncology programs throughout the world.

Submissions in 1981 and 1982

During 1981, the journal received 499 research manuscripts. During the first 6 months of 1982, 280 manuscripts have been submitted. During the summer of 1981, CTR began to publish issues on time and the journal has continued to be published on a normal monthly schedule time since then. The International Symposium on Methotrexate was published in early 1982 and the 4 remaining symposia are scheduled for publication and distribution this month. In addition, the staff of the SIB has continued to upgrade and streamline office operations using the Records Management System to facilitate more efficient processing, indexing, and tracking of manuscripts submitted for publication in CTR and Symposia.

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

TABLE 1

Year	Manuscripts Received	Manuscripts Accepted	Issues Published	Pages Published	Reviewers
1973	244	207	7	1100	167
1974	286	180	11	1823	213
1975	365	261	9	1888	295
1976	478	302	12	2021	402
1977	422	313	9	1771	432
1978	578	364	12	2168	557
1979	740	667	10	2175	695
1980	576	269	4	827	595
1981	499	212	6	1159	339
1982	280*	135*	6*	1453*	---

* From January 1 to June 30, 1982

Sections of the Journal

Brief Reports - In 1973, a section entitled Brief Reports and Preliminary communications was added to the journal. This section contains short clinical manuscripts and abbreviated reports of preliminary research. Brief Reports can be processed by the Publications Section more quickly than full length manuscripts. They are cost efficient in terms of editorial effort and journal space and increase the number of research studies that can be disseminated to the readership. Submissions to this section continue to be excellent.

Editorial Commentaries - In 1974, a section containing invited editorials and commentaries was initiated. Authoritative scientists are invited by the editorial board to comment on subjects of current interest in cancer treatment. In the past 7 years, many such manuscripts have been received and published. In 1982, the SIB invited prominent investigators to provide editorial commentaries on the management of patients with non-small cell bronchogenic carcinoma, histologic progression in non-Hodgkin's lymphoma, and statistics in medical research. Pertinent manuscripts will be grouped in the issue by disease and/or therapy for discussion by the guest editor.

Letters to the Editors - During the past several years, CTR has observed a significant increase in the number of Letters submitted and accepted for publication. Letters usually refer to previously published articles or represent unique case reports.

Clinical Trials Summaries - In January 1981, this new section was introduced. Clinical Trials Summaries are brief summaries of clinical trials that have produced negative results. Authors are limited to 300 words of text but are encouraged to present patient characteristics, response data, toxicity, and survival in tabular form. This new format, which is concise and can be processed and published quickly and efficiently by the editorial staff, has permitted publication of results from an increased number of clinical trials that contain essential, albeit negative, research data. The response to this new format has been positive and the journal is receiving an increasing number of submissions in this format. At the end of each year, the editorial staff prepares two tables that contain the information presented in the Clinical Trials Summaries published during the year. The tables breakdown the results by tumor type, drug, or drug combination.

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

1. The management of patients with stage I non-seminomatous testicular cancer. Cancer Treat Rep 66(1): 1-18, Jan. 1982.

2. The management of patients with nodular non-Hodgkin's lymphoma. Cancer Treat Rep 66(4): April 1982.

3. Randomization in controlled clinical trials. Cancer Treat Rep 66(5): 1077-1105, May 1982.

Proceedings of Symposia

During the past several years, CTR has been approached frequently to publish the proceedings of scientific meetings sponsored by the Division in 1981 the following Symposia was published:

1. International Symposium on Methotrexate. Cancer Treat Rep 65(Suppl 1): 1-189, 1981.

Within the next two months the following proceedings will be available:

1. Symposium on Nutrition and Cancer

2. The 8th New Drug Seminar on L-Asparaginase and Daunorubicin

3. Third Conference on Brain Tumor Therapy

4. Advanced Seminar on Clinical Biochemical Pharmacology

In order to ensure timely publication the proceedings of the following meeting were published as a regular issue of CTR:

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

Editorial Board

The Editorial Board includes the editor-in-chief and twelve associate editors. Each year three editors rotate off the board and three new members are added. Provisions have been made to allow editors to have an additional year on the board at the discretion of the editor-in-chief and the Director, DCT. An Advisory Editorial Board of 15 members has also been established to supplement the areas of expertise represented by the associate editors. New Advisory Board members were appointed this year for a two year period. The official policies of the journal are contained in the official charter which was established in 1975.

Coverage of CTR in Current Contents and Related Publications

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

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

Office of Management and Budget Approval

In January of 1982 a request for continuation of funds was submitted to the OMB. This document outlined the purpose and scope of CTR, the publication costs incurred by the staff, a breakdown of the distribution of each issue, including the categories of subscribers, and the justification for continuing the publication. In July 1982, OMB approved continued production through January 1983. The Department has approved our request and forwarded it to the Office of Management and Budget with the recommendation that cost-recovery be improved prior to the next request for continued approval of publication.

TABLE 2

Distribution

1. Official Use (distributed at no cost)	
a. Federal Government	
NIH employees	193
NCI contractors and grantees	840
FDA employees	17
VA employees	41
VA libraries and hospitals	79
PHS employees	10
Armed services (employees and libraries)	24
b. State agencies	9
c. Research institutes (including libraries)	551
d. Medical schools and universities (including libraries)	216
e. Hospitals (including libraries)	384
f. Special advisory groups	24
2. Free distribution	
a. Foreign investigators and institutions	390
b. Foreign libraries	101
c. Pharmaceutical and related industries	59
d. Individuals (such as medical practitioners)	287
3. Superintendent of Documents	
a. Paid subscriptions	3400
b. File copies	73
c. Depository Libraries	465
TOTAL	7163

LITERATURE SERVICES

The Literature Research Section was also relocated twice during the year. However, it received and filled approximately 170 requests for information. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy, and the related chemical and biomedical disciplines are used by the staff in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluation of toxicological and clinical studies. Responses were provided as comprehensive or selected bibliographies, computer print-outs, abstracts and copies of articles. More than half of the requests entailed manual literature searches supplemented by the various automated bibliographic retrieval systems such as Medline, Toxline, and Cancerline. Comprehensive searches with copies of articles were done for clinicians of the DCT on such subjects as the two stage surgical procedure in breast cancer, treatment of multiple myeloma, vomiting in cancer patients, and the clinical application of specific drugs. Bibliographies were prepared, or updated, for such agents as WR-2721 and other radiation protectors and sensitizers, several platinum compounds, 3-deazaguanine, AT-125, homoharringtonine, the tricyclic nucleoside, and m-AMSA. Comprehensive searches were also performed on nasopharyngeal tumor models, mutagens in urine, whole body hyperthermia, bacterial screening of metal complexes, various enzyme systems and the toxic effects of specific compounds. Although the Section no longer prepares the agent folders, bibliographies are prepared for the compounds to be discussed at Decision Network meetings. A total of 37 were compiled for five meetings.

The Section also co-ordinates NCI access to the on-line data bases of the Medlars automated bibliographic research system for all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for staff on specific subjects of continuing interest.

The Section also maintains the Cancer Therapy Library, a collection of books and journals for the use of NCI staff. Copies of over 70 journals are regularly received including abstracting and indexing secondary sources.

Collaboration with Clinical Oncology Program

Other major accomplishments in 1980-1981 include participation of the Chief, SIB, in the clinical therapeutic trials performed by the Medicine Branch, DCT, NCI. General accomplishments and publications are summarized under the report entitled Clinical Trials and Miscellaneous Clinical Investigations (Project Report 201-CM-03403-16M).

Other Publications Include:

1. Hubbard SM: The role of the nurse in cancer therapy and cancer treatment research. NITA, Official Journal of the National Intravenous Therapy Association 4: 259-265, 1981.

2. Hubbard, SM, Macdonald, JS: An introduction to current controversies in cancer management: Stage I testicular cancer a case in point. Cancer Treatment Reports 66(1): 1-3, 1982.

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5. Hubbard, SM: Evolving roles for oncology nurses in cancer clinical trials. Recent Advances in Clinical Oncology, chapt. 30. London, Churchill Livingston, Inc., 1982, pp. 385-400.

6. Hubbard, SM: Hospice. The Encyclopedia Americana Vol. 14, Danbury, Grolier, Inc., 1982, p. 276.

7. Hubbard, SM: Neoplasia, Chapter 5. In Jones, DA, Jirovec, MA, and Dunbar, CM (Eds.). Medical Surgical Nursing: A conceptual approach. 2nd Ed. New York, McGraw-Hill, 1982, pp. 141-186.

8. Johnson BL and Hubbard SM: Leukemias and Lymphomas. Chapter 7. In Jones DA, Jirovec M, and Dunbar CA (Eds.), Medical Surgical Nursing: A Conceptual Approach, 2nd Ed., New York, McGraw-Hill, pp. 275-316, 1982.

9. Hubbard, SM: Cancer treatment research: The nurse's role in clinical trials of cancer therapy. In Woods, ME and Kowalski, J. (Eds.). Nursing Clinical of North America (In press)

10. Hubbard, SM and Jenkins, J: An overview of current concepts in the management of patients with testicular tumors of germ cell origin. Nursing '82 (In press)

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1981 - September 30, 1982

I. Introduction

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

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

The intramural program is conducted through five Laboratories: Medicinal Chemistry and Biology, Molecular Pharmacology, Tumor Cell Biology and Chemical Pharmacology. The fifth laboratory, Experimental Therapeutics and Metabolism, was organized during FY-82 to study the molecular toxicology of new anticancer drugs. 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 Developmental Therapeutics Program 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 during the report period is shown in Table I.

II. Accomplishments

A. Extramural Program

1. Acquisition of new materials as potential anticancer drugs

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

The main function of the DS&CB is the acquisition of synthetic compounds of new structural types for antitumor screening. Approximately 12,000 new compounds were acquired from the 25,000 which were offered for donation by various companies and universities. Over 800 synthetics were found to have reproducible P388 activity (T/C > 120%). In addition, 300 new suppliers were added this year.

The task order mechanism has continued to be a useful way to acquire the one to ten gram samples which are sometimes required for prescreen confirmation testing. A total of 267 compounds were obtained this way during the reporting period with an additional 33 new projects consisting of 308 compounds assigned. The DCT storage and distribution contractor shipped over 1,600 research samples per month to university investigators and DTP contractors in 30 countries and 44 states.

b. Natural Products Branch (NPB)

A major change in the natural products drug discovery effort took place during FY-82. Large reductions occurred in the contract-supported program to isolate pure new crystalline materials for testing. Attention was shifted towards a more aggressive worldwide surveillance program with emphasis on obtaining donated pure materials.

Over 10,000 cultures were fermented under contract during 1981 with 120 in vivo active cultures discovered. Thirty-nine pure fermentation compounds were provided from our contract effort with 139 donated from outside sources. The structure was determined for Fredericamycin, a novel compound from our contract at the Frederick Cancer Research Facility (FCRF). This material is active in the human tumor clonogenic assay. A total of 141 and 40 crystalline materials of plant and animal origin, respectively, were obtained for testing. Two natural products, homoharringtonine (plant) and echinomycin (fermentation) started Phase I clinical trial. A total of 55 fermentation and 14 plant derived compounds were put into the DTP tumor panel.

2. Biological evaluation

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

The AG&PB provides healthy animals with properly defined genetic characteristics for large-scale testing done by DTP and certain other parts of NCI, NIH, and the Federal Government. The majority of the animals provided are mice, but significant numbers of dogs are also required for toxicology protocol testing. Both breeding and diagnostic contracts are utilized to produce and monitor the health and genetic characteristics of the animals required. Recent advances in technology now

permit the AG&PB to provide "super clean" animals to those facilities able to maintain a disease-free environment. Animals of this type are required for tests using human tumor xenografts and for use with biological response modifiers.

b. Drug Evaluation Branch (DEB)

This Branch managed 16 contracts devoted to the screening and detailed evaluation of compounds acquired as potential antitumor agents. During the period 4/81-3/82, almost 19,000 materials, including 14,000 synthetics, were screened for the first time in vivo. A decrease in natural products screening occurred this year as the result of a decision to stop screening crude plant extracts.

More than 330 new compounds were assigned to the tumor panel bringing this total to over 2,000 compounds. An analysis of the results for 1,100 compounds completed in all eight tumor panel models allowed a number of conclusions to be presented to the DCT Board of Scientific Counselors in June. A positive correlation was observed between the degree of P388 prescreen activity and the probability of achieving DN2 level activity in at least one tumor panel model. Three models (L1210, B16, and the mammary xenograft) predicted 94% of the actives found using all eight panel tumors. Of the 979 compounds which failed to meet the DN-2 criterion for L1210 leukemia, 119 met the criteria in one or more alternative models. Human tumor xenografts selected 26 compounds which failed the mouse screens. A number of tumor panel modifications are planned based on the analysis of data from the first 1,100 compounds.

Substantial progress was made toward the development of the human tumor clonogenic assay into a screening tool. The tumor types most amenable to the analysis were identified (ovarian, malanoma, lung, breast, colorectal) and a number of variables critical to scaling-up the procedure were determined.

3. Formulation and bulk chemical procurement

The Pharmaceutical Resources Branch (PRB) provides comprehensive pharmaceutical services for the various programs of DCT. Large-scale synthesis for clinical formulation provided over 308 kilograms of material. Among the high priority compounds synthesized, tiazofurin (ICN nucleoside) and methotrexate were prepared in 15 kg and 38 kg amounts, respectively. One a smaller scale, prep lab synthesis of tumor panel compounds provided 110 materials. Seventeen radiolabeled compounds were procured through our contracts in that area.

A computerized reference sample file and bulk drug container inventory was set up to facilitate obtaining the GMP data required by the FDA. Pharmaceutical production activity was high with 431,000 injectables and over 1,000,000 oral units prepared.

A "Notification of Expiration" system was devised and implemented as part of our FDA-required clinical product monitoring and control program.

4. Pre-clinical toxicology

The Toxicology Branch (TB) has been operating under new FDA approved protocols for the past year. INDA toxicology reports were submitted to the FDA for homoharringtonine, dihydro-5-azacytidine, Henkel compound, echinomycin and N-methylformamide under the new protocol. In all Phase I studies started thus far, the new protocol has predicted a safe clinical starting dose. Histopathology data is being supplied to the FDA within 90 days of an INDA filing. A study of the mouse as a predictive species with 19 drugs of established antitumor properties showed that the predictiveness of qualitative toxicity may be similar to that from other species if the sample time intervals are appropriately chosen. Work continues to develop a rodent model as a predictor of qualitative toxicity.

A number of new protocols were devised and submitted to the FDA regarding compounds very difficult to test in the mouse. Compounds which are very insoluble or very non-toxic are vehicle volume limited in intravenous (IV) mouse toxicity tests. Intraperitoneal (IP) mouse tests as well as IV and IP rat testing has been proposed.

5. Information technology

The newly established Information Technology Branch (ITB) provides modern data management capabilities for both DTP and DCT. Its initial task was to design a modern drug information system which encompasses all the critical current capabilities while greatly improving them and making DTP self-sufficient in this area. Work is progressing rapidly under an interagency agreement with the Environmental Protection Agency. The final design for the first of four modules was completed during the initial three months contract period.

The ITB works interactively with other DCT Programs. ITB is committed to developing an interactive search capability for a data base being built by the Biological Response Modifiers Program. The ITB also provides general DCT support through timely reports on topics of current interest. It prepared 15 technical reports ranging from the use of partition coefficients in drug design to a literature review of the cardiotoxicity of the anthracyclines. The design of a data system for the mass of data generated by the clonogenic assay contracts is underway. A new multiplexed telecommunications line, installed to overcome problems communicating with a contractor in Columbus, Ohio, is saving DTP $30,000 per year in telephone costs.

6. Grants in pharmacology and biochemistry

The Extramural Research and Resources Branch (ERRB) provides scientific guidance for extramural projects mainly through the grants mechanism. This branch supported 306 grant projects representing over 31 million dollars in total costs. Projects of NCI interest, with special relevance to the drug discovery and other objectives of the DCT, are administered. These grants deal mainly with the following antitumor agent areas: synthesis and chemistry, natural products, experimental therapeutics, pharmacology and mechanism of action.

B. Intramural Program

1. Laboratory of Chemical Pharmacology (LCP)

The pharmacology of misonidazole and desmethylmisonidazole continues to be actively studied. Data suggest that desmethylmisonidazole is approximately two times more toxic than misonidazole and that a similar metabolite is responsible for the observed neurotoxicity. The pharmacokinetics of riboxamide were determined in mice at two doses (25 and 250 mg/kg). At the higher dose, an initial plasma concentration of 1.38 mM was determined which rapidly declined with a T 1/2 of 21 min. The terminal half-life was much longer (at least 260 min), resulting in a nearly constant plasma concentration of approximately 0.11 mM. The kinetics of the 25 mg/kg dose were similar; the initial plasma concentration was 0.11 mM which declined with an initial half-life of 11 min and the plasma concentration in the terminal phase was nearly constant at 0.08 mM with a T 1/2 of at least 310 min.

Attempts are underway to improve the cellular uptake of liposome entrapped materials into lymph nodes by using liposomes to which monoclonal antibodies (Ab) are coupled. In a preliminary study, I^{125} labelled Ab directed against specific surface antigens on lymphocytes was injected into the footpads of C57B10A mice in which the Ab is expressed and in control mice in which the Ab is not expressed. At 3 hr after injection, animals were sacrificed and various tissues and lymph nodes removed. In the control mice, less than 2% of the radioactivity was associated with lymph nodes, whereas more than 50% was present in corresponding nodes of C57B10A mice. Our findings suggest that coupling Abs to the surface of liposomes may provide a means of more precisely targeting drug-containing liposomes to specific sites such as the lymph node; they also open the possibility that Abs raised against experimental tumors and coupled to the liposome membrane may have therapeutic activity against lymph node metastases.

Liposome studies were also applied to radiolabeled Ara-C. Clearance of Ara-C equivalents from the plasma was found to be slower for liposome-entrapped Ara-C than for the free drug. The rate of metabolic breakdown of Ara-C, as indicated by the ratio of Ara-C to Ara-U in plasma, was found to be slower in the monkeys receiving the liposome-entrapped drug than in animals given free Ara-C.

Urinary excretion of Ara-C and its metabolite was significantly reduced in animals receiving liposome-entrapped Ara-C (27%) as compared to monkeys given the free drug (40%). Liposome-entrapment also markedly altered the tissue distribution of Ara-C. Tissue concentrations of Ara-C equivalents were higher by 50 and 8 fold respectively in the spleen and liver of animals receiving the liposome-entrapped drug than in corresponding tissues of monkeys given free Ara-C. Tumor concentrations of Ara-C equivalents were significantly higher by 2 fold in animals treated with the liposome-entrapped drug as compared to monkeys receiving the free drug.

A method was developed to measure the flux through the de novo pyrimidine pathway in tumors and organs in vivo. This method quantitates the incorporation of stable-labelled isotopes of carbon and nitrogen into nucleosides by GC-MS. It is the first method for making such determinations in vivo and allows, for the first time, an evaluation of the differential effects of inhibitors of de novo pyrimidine synthesis in normal vs. tumorous tissues. The technique quantitates the incorporation of inhaled $^{13}CO_2$ and parenterally administered ^{15}N-glutamine or ^{15}N-NH_4Cl into pyrimidine nucleosides. Also, the technique has enabled us to investigate the utilization of ^{15}N-NH_4Cl and ^{15}N-glutamine by by isolated hepatocytes (i.e. the relative role of carbamyl phosphate synthetases I and II in pyrimidine biosynthesis). Initial in vivo studies with standard inhibitors of de novo synthesis revealed that treating animals with 400 mg/kg PALA 24 hrs before measurement of the flux through the pyrimidine biosynthetic pathway resulted in 70-90% inhibition of this pathway in Lewis lung tumors.

Thymidylate synthetase and deoxycytidylate deaminase are two critical enzymes in the de novo synthesis of thymine deoxynucleotides. Several important antitumor agents, prominently the fluoropyrimidines and methotrexate, are known to strongly inhibit thymidylate synthetase based on information from in vitro studies using isolated enzymes and from observations of drug effects on DNA synthesis. We developed a method to measure the activities of these two enzymes in intact cells using ^{3}H-5-deoxycytidine and ^{3}H-5-deoxyuridine. It appears that the deaminase pathway is approximately 50% as active as the kinase in providing the cell with deoxyuridylate.

Anthracycline-induced cardiotoxicity is an important problem in the clinical use of these agents. Studies were continued into the biochemical mechanism for this toxicity. Utilizing the isolated rat heart cell system developed in this Laboratory to test the relative cardiotoxicity of anthracycylines, studies were directed at identifying the intracellular sites of action. A protein was isolated from heart tissue that appears to be an intracellular receptor for anthracyclines. Identification of this high molecular weight, high affinity mitochondrial protein may provide insight into the long sought mechanism of cardiotoxicity of this important class of antitumor drugs.

2. Laboratory of Experimental Therapeutics and Metabolism (LETM)

A major focus of interest continues to be the biochemical toxicology of the lung. The furan derivative, 4-ipomeanol, is a highly selective lung alkylating agent. The toxic mechanisms of action of this compound and cyclophosphamide are under intense study. The discovery that pulmonary Clara cells are a major site of cytochrome P-450 enzymes in lung, a conclusion reached initially through our studies with 4-ipomeanol, has been confirmed subsequently by related investigations in several other laboratories. Isolated lungs and lung slices, in conjunction with high-resolution autoradiography, proved to be a useful way to investigate the cell-specific activation of 4-ipomeanol, and this technique will be tested with other pulmonary cytotoxins. A recently completed set of adjunct studies in an avian species showed that bird lungs were practically devoid of the cytochrome P-450 system, and that 4-ipomeanol did not cause lung toxicity in these species. Instead 4-ipomeanol caused severe damage to the liver of avians, a site where the agent is rapidly activated by metabolism. Interestingly, avian lungs do not have Clara cells nor ciliated bronchiolar cells, and lung tumors are exceedingly rare in this species. Studies with 4-ipomeanol also have indicated the potential importance of pulmonary glutathione (GSH) as a protective factor against lung damage by highly reactive alkylating agents or metabolites. GSH presumably protects by combining with the reactive species to form less reactive, less toxic conjugates that can be eventually excreted. Our studies have been extended to other potentially cytotoxic and/or carcinogenic furan compounds. 3-Methylfuran reportedly is a major component present in certain urban smogs. We have found that this compound, like 4-ipomeanol, is metabolized in situ in the lung to a highly reactive product that destroys the bronchiolar epithelium.

Studies of mechanisms of lung injury by known anticancer drugs also are currently receiving emphasis. Investigations are centered on BCNU and bleomycin, the two antineoplastic agents responsible for the majority of drug-induced lung disease in cancer patients. A reliable animal model for BCNU-induced lung fibrosis has been developed and biochemical/morphological correlates are being defined. BCNU causes a marked depletion of pulmonary glutathione, suggesting this substance may have a protective role against lung damage by BCNU. The latter finding is the basis for current studies of the effects of sulfhydryl depleting agents (e.g., diethylmaleate) and sulfhydryl enhancing agents (e.g., cysteine, WR-2721) on BCNU lung injury.

A major new initiative undertaken this year in collaboration with the Surgery Branch, NCI, is the development of an in situ lung perfusion protocol for treatment of metastatic soft-tissue sarcomas and osteogenic sarcomas in lung. The initial drug under study is adriamycin. The pulmonary pharmacokinetics have been defined in the preclinical experiments, and an initial starting dose for patient studies determined. Clinical trials have begun and three patients have thus far been perfused using a starting concentration

of 1 μg/ml. Although no tumor regressions have yet been observed, the procedure seems to hold great promise since surgical and pharmacologic/toxicologic problems appear to be manageable. Dose escalations are continuing with adriamycin, and future investigations will also explore the possible value of other types of drugs used in this setting.

With regard to target tissue toxicity by anticancer drugs, another organ of major interest is the kidney. Similar to our experimental approaches to biochemical toxicology of lung we are developing relevant animal models of drug induced renal injury and are attempting to elucidate the chemico-biologic events underlying their pathogenesis. Methyl-CCNU is a nephrotoxic drug of current interest. We have developed a reliable model of Me-CCNU renal damage in mice and rats and have shown that histopathological changes produced by the drug are closely paralleled by marked changes in biochemical parameters (e.g., PAH uptake and TEA transport) measurable in vitro in biopsy specimens, as well as by certain in vivo functional tests (e.g., urinary osmolality, kidney-derived urinary enzymes). Further refinement and study of these models may lead to improved approaches for predicting, monitoring, or treating such reactions in patients.

3. Laboratory of Medicinal Chemistry and Biology (LMCB)

Several compounds which originated in the LMCB are in various stages of the DCT drug development process. AZQ is in Phase II clinical trial with reports of some activity against brain tumors, the target for which the drug was designed. BZQ, an analog with a somewhat different spectrum of pre-clinical activity, was given to EORTC for initial Phase I trials which should start during 1982. Spirohydantoin mustard, an agent also designed for brain tumors, should complete toxicology during this year and start Phase I trials in 1983. An INDA was filed on dihydro-5-azacytidine which started Phase I trials in mid-1982. Ara-AC, a molecular hybrid between ara-C and 5-azacytidine, was chosen for advanced pre-clinical development (DN2A) this year based on its activity against all three xenografts in the DCT tumor panel.

Progress continues to be made in the preparation of cytidine deaminase inhibitors for potential use in combination with antitumor agents such as ara-C and 5-azacytidine, whose clinical utility is limited by enzyme-catalyzed deamination in vivo. Several compounds of the diazepinone riboside series have been prepared with K_i values in the range 1-5 x 10^{-8}M, i.e., approximately one order of magnitude more active than the reference compound tetrahydrouridine, the most potent cytidine deaminase inhibitor described prior to the present studies. The most active member of this series, NSC-322096, when combined with ara-C, produced increases in T/C values against murine L1210 leukemia comparable to those obtained with three times the dose of ara-C alone. The dose of the inhibitor was one-tenth that of tetrahydrouridine required to produce a comparable increase in T/C values.

Ongoing studies with sangivamycin, a pyrrolopyrimidine antibiotic, have confirmed its time-dependent lethality in HT-29 cells, in association with its marked incorporation into mRNA. In view of the unusual activity of sangivamycin in the human colon carcinoma line, a collaborative Phase I/II study of this agent in colorectal cancer has been initiated with the Medical College of Wisconsin (Dr. Paul Ritch), with this laboratory providing bioassay support as required.

A new project initiated this year has been a thorough biochemical and cytokinetic study of human fibroblast interferon (IFβ) in several human tumor cell lines. We have determined whether the IFβ mediated induction of (2',5') oligo(A) synthetase, an enzyme which is unique to IFN-treated cells and is associated with antiviral activity, is related to the growth inhibitory properties of IFβ and of double-stranded RNA (poly(I·C); $I_n \cdot C_n$). It is planned to measure the levels of (2',5')oligo(A) synthetase in patients with CLL being treated with IFβ, as a measure of their responsiveness to treatment.

The Biochemistry Section devoted a large effort to elucidating the mechanism of action of tiazofurin, a C-nucleoside with exceptional activity against Lewis lung carcinoma. Cytofluorimetric analysis showed cell arrest in the S-phase. When an examination was made of the ability of a comprehensive series of preformed purines and pyrimidines to overcome the inhibition of thymidine incorporation provoked by exposure to the thiazole nucleoside, the guanines were notably effective, but xanthosine also was shown to be an active antidote. Confirmation that the drug was producing a state of guanine deprivation was provided by HPLC analysis of acid-soluble extracts: a time-dependent fall in the concentration of GMP and GTP ensued upon exposure to the drug; on the other hand, IMP concentrations increased ca. 15 fold. Further studies revealed the site of inhibition to be IMP dehydrogenase, rather than GMP synthetase. Kinetic studies revealed that neither the parent compound nor its 5'-monophosphate was sufficiently active as an IMP-dehydrogenase inhibitor to account for the results observed. The anabolite responsible for the inhibitory effect has now been isolated from homogenates of the tumor by dialysis and ion-exchange HPLC, and its presence monitored by enzyme inhibition assay. Exposure of the molecule to snake-venom phosphodiesterase or nucleotide pyrophosphatase destroyed its inhibitory potency, whereas other phosphoesterases were either less effective or inert. Since these results suggested that the anabolite might be a dinucleotide with a phosphodiester linkage of the kind found in NAD, attempts were made to synthesize such an NAD analog from the 5'-monophosphate of the thiazole nucleoside and ATP-Mg^{++}. Using a purified preparation of NAD pyrophosphorylase, modest yields were obtained of a compound with chromatographic, spectrophotometric and enzyme-inhibitory properties identical to those of the material isolated from P388 tumor nodules. This enzyme-synthesized material was radioactive when [^{3}H]ATP was used as co-substrate, and yielded both AMP and thiazole nucleoside-5'-monophosphate on treatment with phosphodiesterase. It resisted attack by NAD glycohydrolase.

Mass spectral analysis and nuclear magnetic resonance studies were compatible with a dinucleotide structure in which the nicotinamide of NAD has been replaced by thiazole-4-carboxamide, i.e., adenine-D-ribose-phosphate-phosphate-D-ribose-thiazole-4-carboxamide. Versus IMP dehydrogenase, the dinucleotide exhibited a K_i of ca. 0.05 μM and was competitive with NAD as the variable substrate. This dinucleotide was independently synthesized for the Biochemistry Section by the Drug Design and Chemistry Section, LMCB, and proved identical to the biologically isolated compound.

Using purified rat liver mitochondria as a model biological system, it was found that NADPH-dependent lipid peroxidation, measured by the 2-thiobarbituric acid method, was stimulated as much as 4-fold by adriamycin. Superoxide dismutase, catalase, 1,3-dimethylurea, reduced glutathione, EDTA, and α-tocopherol inhibited adriamycin-augmented mitochondrial lipid peroxidation, indicating that multiple forms of reactive oxygen and possibly trace amounts of endogenous iron participated in the peroxidation reactions. Thus, it is likely that the in vivo effects of adriamycin on mitochondrial morphology and function are mediated by adriamycin-enhanced reactive oxygen-mediated lipid peroxidation.

Based on earlier studies from this laboratory which demonstrated conditions in which tubulin-dependent GTP hydrolysis was totally dependent on taxol (NSC125973), we were able to develop an assay for the drug sensitive to 0.1 μM. Conditions were established suitable for measuring serum concentrations, and a preliminary pharmacokinetic study was performed in rabbits. Although taxol appears to be protein-bound in serum, it was rapidly cleared with α-phase and β-phase half-lives of 2.7 and 42 min, respectively. This assay is suitable for human pharmacokinetic studies, and will be made available to clinical research groups conducting Phase I/II trials with this agent.

4. Laboratory of Molecular Pharmacology (LMP)

Previous work had shown that a particular DNA repair defect affecting removal of O^6-alkyl-guanine lesions confers sensitivity of cells to chloroethylnitrosoureas. Some human tumor cells have this defect, whereas others do not. Repair-defective cells (designated Mer- phenotype), when treated with chloroethyl-nitrosoureas, produce increased amounts of interstrand crosslinks. We had previously found this repair defect to be specific for nitrosoureas, and not to apply to other classes of interstrand crosslinking drugs. During the current year, we have examined 13 cell strains derived from surgical specimens from glioblastoma patients. The cell strains were provided by the Surgical Neurology Branch, NINCDS. These cell strains were found to vary widely in the extent of interstrand crosslinking produced by chloroethyl-nitrosoureas. DNA-protein crosslinking varied relatively little among these cell strains and served to eliminate factors, such as drug uptake and intracellular drug inactivation. The working hypothesis is that the differences among the cell strains derives from differences in ability to repair DNA-chloroethyl monoadducts

prior to their conversion to interstrand crosslinks. The cell strains were also tested for interstrand crosslinking following treatment with cis-Pt(II). A range of crosslinking potentials was observed among the cell strains, but the potential for crosslinking by cis-Pt(II) and by chloroethylnitrosourea were independent of each other. A further difference between the drugs was that, whereas Pt(II) generated a uniform distribution of crosslink frequencies among the cell strains, chloroethylnitrosourea gave a bimodal distribution in that some strains showed substantial crosslinking while others showed almost no detectable crosslinking. The production of interstrand crosslinks in glioblastoma cells thus is governed by different and potentially predictable factors. The possibility of predicting clinical response will be tested in cooperation with the Surgical Neurology Branch, NINCDS.

Previous work had identified a new type of DNA alteration in mammalian cells treated with DNA intercalating agents. Several classes of intercalating agents were shown to produce DNA strand breaks associated with a protein which is probably linked covalently to one terminus of the strand break. During the current year, this work was extended in several respects. It was found that the characteristic protein-associated strand breaks can be generated in isolated nuclei treated with the intercalators, m-AMSA, 5-imino-daunorubicin and ellipticine. The strand breaks and DNA-protein crosslinks were produced in approximately equal quantities, as was the case in intact cells. It will now be possible to characterize the intercalator-induced effect in a subcellular system and to study the enzyme(s) which are presumed to be responsible for the effect.

In the course of the intercalator studies, a new uptake phenomenon was discovered and characterized for m-AMSA. This compound exhibited an anomalous uptake as its concentration was raised. The anomaly was characterized by cooperativity, saturability, hysteresis, temperature-dependence and lack of interaction with DNA. This phenomenon was attributed to a sequestration of drug in a cell compartment from which the drug does not access the DNA and does not produce major cytotoxicity.

The kinetics of the formation and reversal of the DNA alterations produced by m-AMSA, observed in our experiments, may have pharmacologic implications. The frequency of these alterations rises rapidly to a steady state level and then remains constant until drug is removed, whereupon the alterations rapidly disappear. This suggested that the drug could be considered to behave pharmacodynamically in a manner associated with some antimetabolites rather than with DNA-damaging agents. In accord with this idea, a clinical protocol was designed in which m-AMSA is delivered as a prolonged continuous infusion. A Phase I-II trial has been completed, and a new trial on non-lymphocytic leukemia has been initiated.

It had generally been assumed that histone synthesis is closely linked to DNA synthesis. While true for the major histone variant species, we had found that certain minor variants, discovered in this Laboratory and called H2A.X and H2A.Z, are synthesized throughout the cell cycle and are not suppressed as the major histone species are when DNA synthesis is inhibited with hydroxyurea. The same was true also for the previously known variant species, H3.3. During the current year, this work was extended to quiescent (G_0) cells. It was found that quiescent cells also continue to synthesize histones at a reduced but significant rate. The pattern of histone variant synthesis in quiescent cells was found to be different from that of either S-phase or G_1-phase cells. The main difference between the G_0 and G_1 patterns is that that the major variants, H2A.1 and H2A.2, continue to be synthesized at a slow rate in G_0, but are totally suppressed in G_1; this slow continued synthesis is not attributable to the presence of a small number of S-phase cells because it is not blocked by hydroxyurea. Thus three different patterns of histone synthesis have been defined which are characteristic of of the S, G_1 and G_0 states.

5. Laboratory of Tumor Cell Biology (LTCB)

T-cell growth factor (TCGF) has been purified to homogeneity. Glycosylated, sialylated, and non-modified forms have been identified. The mRNA for TCGF has been purified. The frog oocyte system has been developed for translation of the mRNA. Numerous new neoplastic human T-cell lines have been developed using partially-purified TCGF.

Ten additional new human T-cell leukemia virus (HTLV) isolates have been obtained from eight different areas of the world (there are now a total of 13 isolates). All isolates have been from adult T-cell leukemia or lymphoma or in some people in a "pre-leukemic" phase. Antibodies to HTLV p19 and p24 have been found in sera from a large number of T-cell leukemia/lymphoma patients and in some healthy normals. Seroepidemiology studies show that HTLV is endemic in Japan, West Indies, South East U.S.A., China, Alaska, various regions of South America, and possibly in Israel. Molecular cloning of HTLV has been achieved. Two subgroups of HTLV have been identified. Most isolates fit subgroup I. The biological activity of HTLV has been demonstrated. HTLV can directly transform human mature cord blood T cells. These cells are OKT-4 positive, lose their requirements for exogenous TCGF for growth, turn on production of endogenous TCGF, develop several new surface markers including modified HLA, gain the ability to grow on agar, grow to high saturation density, and produce tumors in nude mice. The preliminary data on the molecular mechanism of transformation of human T-cells by HTLV appears to be very similar to that in avian B lymphomas induced by avian type-C leukemia viruses. In addition to HTLV p24, p19 and reverse transcriptase, p15 has also been purified. Amino acid sequence analysis of HTLV p24 and much of the p15 has been completed. Assays for HTLV antigens in sera of patients with T cell malignancies have been developed. ELISA assays for HTLV

antibodies and antigens for clinical laboratory testing have been developed. Some normal blood bank donors (2 of 286) have been found to be HTLV positive.

Four human onc genes have been cloned (myb, myc, sis, fes). The study of their expression in human hematopoietic cells of various types has been completed. Complete nucleotide sequence analysis of the sis gene has been achieved. The first example of an amplified onc gene in man has been found, namely the myc gene in HL-60. The amplification is 15 to 32 fold and was found in fresh cells as well as the cell line HL-60.

A DNA transfection system has been established and DNA from some fresh uncultured human lymphoid tumors was shown to successfully transform NIH 3T3 cells. Endogenous retrovirus related sequences (not onc genes) have been identified in human DNA and successfully cloned. The sequences include reverse transcriptase gene and other regions.

Myeloid cells and monocytes from normal donors have been successfully grown in culture in many instances for close to one year and in one case a permanent normal human monocyte cell line has been established. Retinoic acid and cAMP (e.g., prostaglandin E, cholera toxin, dibutyl cAMP) act synergistically in inducing terminal differentiation of HL60. A differentiation inducing factor (DIF), produced by T cells, acts synergistically with retinoic acid in inducing terminal differentiation of both HL60 and promyelocytes from a patient with acute promyelocytic leukemia. Two human monoblastic-like cell lines (U937 and THP-1) have been induced to differentiate with retinoic acid to monocyte-like cells.

TABLE I

COMPOUNDS THAT PASSED DECISION NETWORK 2A, 2B, 3 and Special (4/1/81 - 3/31/82)

NSC Number	Name	Compound Type*
	Decision Network 2A	
156492D	Discreet	S
261726	4H-Imidazo[4,5-c]pyridin-4-one, 6-amino-1,5-dihydro-; 3-Deazaguanine	S
267213	Acetic acid, [[(4-methoxyphenyl)sulfonyl]hydrazono]-	S
281272	1,3,5-Triazin-2(1H)-one, 4-amino-1-β-D-arabinofuranosyl-; ara-AC	S
293015	4H-1-Benzopyran-8-acetic acid, 4-oxo-2-phenyl-, 2-(diethylamino)ethyl ester, hydrochloride; Lipha compound	S
303861D	Discreet	SS
322921	Phenol, 4-[5-(4-methyl-1-piperazinyl)[2,5'-bi-1H-benzimidazol]-2'-yl]-, trihydrochloride; Bisbenzamide	S
325014	Propanamide, 2-amino-N-[3-dichloromethyl)-3,4,4a,5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-1-oxo-1H-2-benzopyran-4-yl]-, [3S-[3,4α(R*), 4aβ,5β,6α]]-; Bactobolin	NP
328426	β-D-Glucopyranose, 2-O-(3-O-acetyl-6-deoxy-β-D-glucopyranosyl)-6-deoxy-, 3-acetate 1-[decahydro-5''-methyl-4''-[(1-oxo-3-phenyl-2-propenyl)oxy]dispiro[oxirane-2,3'(2'H)-benzofuran-2',2''-[2H]pyran]-6'-carboxylate]; Phyllanthoside	NP
330500	Geldanamycin, 18,21-didehydro-6,17-didemethoxy-18,21-dideoxo-18,21-dihydroxy-15-methoxy-6-methyl-11-O-methyl-, (6S,15R)-; Macbacin II	NP
333856D	Discreet	NP
	Decision Network 2B	
226080	Rapamycin	NP
253272	Acetamide, N-[(methylamino)carbonyl]-N-[[(methylamino)carbonyl]oxy]-; Dow compound	S

NSC Number	Name	Compound Type*
269148	2,6-Epoxy-2H-naphthaceno[1,2-b]oxocin-9,16-dione, 4-(dimethylamino)-3,4,5,6,11,12,13,14-octahydro-3,5,8,10,13-pentahydroxy-11-methoxy-6,13-dimethyl-; 7-O-Methylnogarol; 7-OMEN	SS
278214	Carbamic acid, (1-methylethyl)-, [5-(3,4-dichlorophenyl)-2,3-dihydro-1H-pyrrolizine-6,7-diyl]bis(methylene) ester; Anderson compound	S
305884	Acetamide, N-methyl-N-[4-[(7-methyl-1H-imidazo[4,5-f]quinolin-9-yl)amino]phenyl]-, monohydrochloride; Acodazole HCl; Norwich compound	S
	Decision Network 3	
3051	Formamide, N-methyl-	S
141633	Cephalotaxine, 4-methyl-2-hydroxy-2-(4-hydroxy-4-methylpentyl)butanedioate ester; Homoharringtonine	NP
241240	Platinum, diamine[1,1-cyclobutanedicarboxylato(2-)-O,O']-, (SP-4-2)-; Carboplatin; CBDCA	S
264880	1,3,5-Triazin-2(1H)-one, 4-amino-5,6-dihydro-1-B-D-ribofuranosyl-, monohydrochloride; Dihydro-5-azacytidine	S
280594	1,4,5,6,8-Pentaazaacenaphthylen-3-amine, 1,5-dihydro-5-methyl-1-(5-O-phosphono-β-D-ribofuranosyl)-; Triciribine phosphate; Tricyclic nucleotide	SS
296934	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-tris(oxiranylmethyl)-, (α)-; Teroxirone; Henkel compound	S
312887	9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-β-D-arabinofurnaosyl)-; Fludarabine Phosphate; 2-Fluoro-ara-AMP	S
526417	Echinomycin; Quinomycin A	NP
	Decision Network 4	
40774	9H-Purine, 6-(methylthio)-9-β-D-ribofuranosyl-, dihydrate . 2 HOH; 6-MMPR	S
163501	5-Isoxazoleacetic acid, α-amino-3-chloro-4,5-dihydro-; Acivicin	NP

NSC Number	Name	Compound Type*
337766	9,10-Anthracenedicarboxaldehyde, bis[4,5-dihydro-1H-imidazol-2-yl)hydrazone], dihydrochloride; Bisantrene Hydrochloride	S

Decision Network Special

20256	Uridine	NP
65346	7H-Pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-7-β-D-ribofuranosyl-; Sangivamycin	S

*S = synthetic
SS = natural product modified synthetically
NP = natural product

ANNUAL REPORT OF THE DRUG SYNTHESIS & CHEMISTRY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 - September 30, 1982

The Drug Synthesis and Chemistry Branch (DS&CB) occupies the front-end position in the Linear Array for drug development. As such, the fundamental responsibility of the Branch 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 acquisition; Task Order syntheses; contract syntheses; Cancer Research Emphasis Grants (CREGS); storage and distribution; and worldwide surveillance. In addition, DS&CB supports the Radiotherapy Development Program (RDP) and the European Organization for Research on Treatment of Cancer (EORTC) Program.

The DS&CB has undergone major shifts both in program and personnel. The Chemical and Drug Information Section was transferred to the newly formed Information Technology Branch (ITB). A new Acquisition Section was created to centralize all acquisition-related activities. The branch has phased out several research contracts as well as CREGS. We have fully implemented the new Task Order contract mechanism for the synthesis of compounds of interest. A new project, "Synthesis of Congeners and Pro-drugs," is being initiated. The computerized structure-activity model is being refined to include therapeutic index estimates and partition coefficient data. The staff has assumed new roles and responsibilities as dictated by the dynamics of a changing Program.

Program Objectives

The objectives of the DS&CB are: (a) to acquire unique chemical compounds for evaluation as potential antitumor agents through active solicitation and synthesis; (b) to explore and develop new classes of chemicals as preclinical candidates; (c) to develop computer assisted structure-activity models; (d) to manage the accession, storage and distribution of all chemical agents that enter the Program; (e) to maintain worldwide surveillance to ensure a steady flow of novel compounds to the Program; (f) to design and synthesize potential radiosensitizers and radioprotectors; (g) to identify compounds for development by EORTC, and (h) to disseminate developing structure-activity relationships to the scientific community.

Program Organization

To meet the above objectives, the DS&CB is organized on traditional lines, namely, the Office of the Chief, the Acquisition Section, and the Drug Synthesis Section. Overlaying the traditional hierarchy, the Branch has

adopted a matrix organizational structure wherein the staff is assigned tasks depending on the changing needs of the Program and on the expertise of individual staff members. The contracts managed by DS&CB are outlined in Tables I. Presently, the DS&CB is staffed with six professionals and three technical and clerical personnel.

Acquisition (Chart I)

The main function of the Acquisition Section is to ensure the continuous flow of approximately 12,000 selected compounds annually for the primary screen. Extensive contacts are maintained with industries, government laboratories, and scientists in academic and research institutions to fulfill this objective. The Section is engaged in the following activities: (1) developing and maintaining sources 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) the acquisition of 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: approximately (a) 25,000 chemical structures were acquired and processed through the preselection "gauntlet"; (b) 12,000 compounds were selected as candidates for acquisition; (c) 12,000 compounds were acquired and assigned NSC numbers; (d) 300 new suppliers were added; (e) 800 compounds showed confirmed activity against P388 leukemia, and (f) 49 compounds were purchased and 173 were acquired for Tumor Panel evaluation.

Four synthetic compounds assigned to DN2A came through selective acquisitions, namely; NSC-293015, NSC-D303861, NSC-322921 and NSC-D156492. NSC-293015 is a flavone acquired from France and has shown DN_2 activity against the extremely resistant C8 colon tumor. The compound is also active against CD8F1 mammary, B16 melanoma, and P388 leukemia. NSC-D303861 has shown DN_2 activity against the MX1 tumor and is also active against the L1210 and P388 leukemias. NSC-322981, purchased from a chemical catalog, shows DN_2 activity against L1210 leukemia. NSC-D156492 shows DN_2 activity against B16 melanoma.

The other three synthetic compounds assigned to DN2A are ARA-AC (NSC-281272), synthesized by LMCB, DTP; 3-deazaguanine (NSC-261726) and NSC-267213, a sulfonylhydrazone of glyoxalic acid synthesized by SRI International.

Pre-selection Model--an Evaluation

Computer methods for preselecting compounds for large-scale screening against P388 based on molecular fragment structure-activity statistics (Hodes model) have been in operation for the past three years. Now that approximately 20,000 compounds with "predicted activity scores" have been screened, we have begun to critically evaluate the actual performance of the model. A detailed report of this analysis, including the methodology, is available from the Acquisition Section.

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 development of the chemistry-biology interlink and its active implementation during this year.

Examples of structure-activity analyses include: benzoisoquinoline, 1,3-diones, cyclic triazines, tin compounds and cyclic methylene ketones, lactones and lactams.

Resynthesis--Task Order Contracts (Table I, Chart II)

The Quick Reaction Work Order Contract (Task Order) mechanism has emerged as an innovative and pragmatic approach to resynthesize compounds of program interest. A major effort was required to translate the basic concept into actual operating procedures including complex logistics, production and administrative details. The project team approach is used to manage this activity. The functional responsibility for this project rests with the Synthesis Section.

The Task Order mechanism makes available ten master contractors (Table I), who have the expertise to synthesize a wide variety of selected compounds. Each synthesis packet is sent to three master contractors and one contractor is selected for each task. The key element of technical excellence is optimized since three competing technical approaches can be evaluated for each packet. The incentive to continuously compete for each synthesis packet assures both technical excellence and cost-effectiveness of the Task Order mechanism.

The Task Order mechanism provides for the synthesis of a variety of organic and inorganic compounds which have been identified by the Program as meriting development. For this reporting period, we have awarded 33 individual projects consisting of 308 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 (see Chart II). During this reporting period, we received 267 compounds synthesized through this mechanism.

The selection of literature candidates for Task Order synthesis is made possible through the services of the Dynamac Corporation, the literature surveillance contractor. The contractor provides a continuing review and analysis of recent literature with emphasis on compounds with biological activity. In addition, it is essential to rapidly search the literature for detailed methods for synthesizing a large number of compounds. This capability is provided by Maxima Corporation which performs computerized searches of bibliographic data bases for synthesis methods.

Synthesis--Natural Product Analogs

This contract for the synthesis of natural product analogs as potential anticancer agents expired during this fiscal year.

The objective of this contract was to develop via chemical synthesis compounds related to products of natural origin with improved antitumor activity and decreased toxicity. The areas for synthetic modification included tricothecanes of the anquidine type, isoxazoline antibiotics, and benzodiazepines related to anthramycin, neothramycin and tomamycin. Two other areas for analog development, anthracenediones and maytansines, were not continued because the initial compounds were not promising.

During the period of this contract, Bristol Laboratories synthesized and submitted for evaluation a total of 59 tricothecanes, a project that was ongoing at the time of the contract initiation. The contractor has developed an in-depth structure activity correlation on tricothecanes.

In summary, the contractor has synthesized and submitted a total of 141 compounds during the total contract period.

Synthesis--Nucleosides

The objectives of the contract are to design and synthesize novel nucleosides that inhibit key enzymes in both the de novo and salvage pathways of nucleoside metabolism; specifically, PNPase and HGPRTase. During this period, the contractor synthesized 15 new N-nucleosides and one C-nucleoside. These compounds were screened for: (a) PNPase inhibition; (b) in vitro tissue culture inhibition of L1210 and P388; and (c) in vivo testing in L1210. Four of the nucleosides showed PNPase inhibitory activity (K_I= 1 x 10^{-5}M). They also inhibited L1210 and P388 at 100 mg/ml. However, only one of the compounds, NSC-409352, showed in vivo antitumor activity (T/C of 148). Thus it appears that for compounds of this category PNPase activity is not predictive of significant in vivo antitumor activity. This contract is being phased out at the end of FY'82.

Storage and Distribution

The objective of this project is the storage, distribution, inventory and documentation of synthetic materials, crystalline natural products and bulk clinical drugs.

During the past year, Flow Laboratories, Inc., the storage and distribution contractor, has shipped more than 1,600 compounds per month to contract screening laboratories, formulation laboratories, NCI and NIH researchers, and independent investigators in 44 states of the U.S. and 29 foreign countries. The quantities of the materials shipped ranged from milligram to kilogram amounts. The vast majority of compounds are shipped within 48 hours after receipt of the request and the packages normally arrive at their destination 48 to 72 hours later. Dry ice packages are transmitted only on Mondays and Tuesdays using the Government Bill of Lading or Federal Express. The bulk of the shipping is done using U.S.

franked labels. The above outlined procedures have shortened the time between shipping and receipt of the compounds, and at the same time have reduced the shipping costs.

As time permits, the contract is continuing the physical inventory of compounds (more than 400,000 containers) held in the DS&CB repository starting with DN, Panel and SAC compounds. Flow has completed the inventory of both bulk drugs and their reference and morgue samples to comply with FDA regulations. The contractor continues to shelve and inventory compounds returned by the screening laboratories and shelve reference samples. This activity is a continuing process and is proceeding very slowly because of budget constraints.

The contractor interacts closely with the DS&CB, DEB, the Natural Products Branch (NPB), PRB and ITB, as well as the acquisitions contractor and the chemical information contractor.

Chemical Searches

The 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 chemical information about the chemotherapeutic agents in the Program.

The full structure search capability of the Chemical Information System (CIS) determines the uniqueness of specific compounds, and the detection of duplicates in support of the Branch's selective acquisition program. For the reporting period, full structure searches totalling more than 1,300 were performed.

The substructure search feature of the CIS provides data necessary to determine Program relevance, the exploration of new areas for development and the identification of areas for deemphasis. The data are also useful in the development of new research projects and structure-activity studies. During this reporting period more than 500 substructure searches were performed.

In addition the Questel/Darc data base system was utilized to access synthesis methods for selected compounds.

Worldwide Surveillance

For years the NCI has acquired the majority of its compounds through the contributions of chemical and pharmaceutical industries, research organizations and government laboratories. Both domestic and foreign sources have contributed generously in the past. Now, these contributions continue as a result of DS&CB's aggressive acquisition and worldwide surveillance programs, sustained good public relations and service.

The development of new sources is a continuing process necessitated by the dynamic nature of the chemical community. The diversity and availability

of compounds for testing is heavily dependent upon existing research priorities in this community. The DS&CB must be able to anticipate and respond to such potential changes to ensure a continuous supply of unique compounds to the program. During the past year, several new suppliers have been added to our program.

We have developed a literature surveillance program, under contract, to expand and ensure the complete coverage of the chemical and biological literature to support our acquisition effort. The contractor previews the massive number of compounds published each month, estimated at 30,000 per month, and prepares a list of approximately 1,000 of the most interesting ones for review by DS&CB. These compounds are scored by the Hodes model and reviewed by chemists. The compounds that are finally selected are acquired either through mail requests or Task Order synthesis.

Radiotherapy Program Support

DS&CB works in close cooperation with Dr. Pistenmaa and his staff and the Radiosensitizer/Radioprotector working group. We actively support the Radiotherapy Development Program in several ways, including (a) monitoring two radiosensitizer contracts and (b) identifying and acquiring compounds for both the radiosensitizer and radioprotection screening contracts.

(a) Radiosensitizer Synthesis

The current focus of the two contracts is the design and synthesis of novel compounds, excluding 2-nitroimidazoles, as potential radiosensitizers.

SRI International has synthesized and evaluated a number of new structural types including compounds that deplete glutathione (maleimides). Several classes such as quinoxaline 1,4-dioxides, pyrazine 1,4-dioxides and pyridine N-oxides have shown promise. Of these NSC-30531, NSC-132883 and NSC-343744 have demonstrated *in vivo* radiosensitizer effectiveness. Currently the contractor is incorporating other desirable characteristics such as appropriate solubility and lipophilicity into these compounds. A DN candidate is likely to result from these efforts.

The Institute of Cancer Research has examined 150 compounds as potential radiosensitizers *in vitro*. These include (a) nitroimidazoles containing an alkylating group, oximes and hydrazides; (b) nitroimidazoles containing a carbohydrate side chain; (c) imidazothiazoles; (d) cyclic sulfones; and (e) quinones. One of the more promising compounds is NSC-347503, a nitroimidazole with an alkylating function. The compound is more effective than misonidazole both *in vitro* and *in vivo*. Two other interesting leads have emerged. NSC-178552, a nitroimidazole with a sulfone function; and NSC-342697, an imidazothiazole, have both shown excellent *in vitro* radiosensitizer effectiveness (10 times misonidazole). Recently the contractor has shifted his efforts to explore new areas such as quinones, napthoquinone-imidazoles, thiazoles and triazines containing electron-withdrawing groups other than the NO_2 function.

(b) Radiosensitizer and Radioprotector Screening

The DS&CB has selected and shipped 68 compounds to Arthur D. Little for physical chemical measurements and in vitro radiosensitizer studies. These compounds were mostly non-nitro triazines, imides, quinones, N-oxides and sulfones. In addition, 3 compounds, NSC-318502, NSC-347502 and NSC-351358 were resynthesized for in vitro studies.

In the radioprotector area there is very little structure-activity data available to aid in choosing classes of compounds for screening. Because of Walter Reed's expertise in the synthesis and testing of antiradiation drugs, we have used their compilation to select compounds for our radioprotector screening contract. In, addition 5 thiazolidineacetic acid derivatives, 6-flavenoids, 3 polyamines and 5 sulfones were also selected for screening as potential radioprotectors.

(c) EORTC Compounds for Development--a New Initiative

The DS&CB plays a key role in the identification of compounds and renders other support services in connection with the new NCI initiative to develop clinical compounds through EORTC. These encompass two categories of compounds, namely; (a) compounds passing our DN2A criteria but not necessarily scheduled for U.S. development because they are analogs of compounds under development, (b) compounds which have been studied in the tumor panel, not meeting DN2A criteria, but which do exhibit antitumor activity and have interesting structural features, particularly in relation to their possible metabolic conversion and speculated mechanisms of action.

EORTC has expressed immediate interest in advancing 5 compounds to Phase I study. These are NSC-47548, NSC-144229, NSC-205795, NSC-276375 and NSC-281617. Two compounds of immediate interest to EORTC belonging to category (b) are NSC-249321 and NSC-277521. Several other compounds for further exploration have also been identified, namely, NSC-263639, NSC-265534, NSC-273356, NSC-277484 and NSC-277834.

Synthesis of Congeners and "Pro-drugs"--a New Initiative

The objectives of this new project are twofold: (a) to synthesize congeners of compounds with activities just below the level of DN_2 with a view toward enhancing their activities and increasing their spectra of activity; (b) to design and synthesize "pro-drugs" and other ompounds that possess elements of both congener and pro-drug.

There are several compounds in our files where progress is hampered because of activity just below the DN_2 level, poor solubility and/or poor stability. In an attempt to overcome these problems, and to increase the number of new compounds specifically designed to mitigate these difficulties, we propose to initiate this project. The objective is to design and synthesize "pro-drugs" that are chemically altered transport forms in order to modify both biological and pharmaceutical properties. Included in these properties are: (1) improving bio-availability by increasing aqueous solubility; (2) increasing compound stability, and; (3) modifying pharmacokinetics to improve adsorption, distribution, metabolism, etc. Another objective

is the design and synthesis of relatively stable derivatives that possess elements of both congener and "pro-drug." Two contracts in the area will become operational by the end of the current fiscal year.

Seminars

The following seminars were sponsored by DS&CB:

1) "The Design and Reactivity of Metal Anticancer Drugs, Especially Platinum and Gold."
Dr. P.J. Sadler of Birkbeck College, University of London, England.

2) "Antitumor Activity and Bone Marrow Toxicities of Some Glucose Mustards."
Dr. James Cantrell, Ms. Dianna Grier and Dr. Philip Schein of Georgetown University Hospital, Washington, D.C.

3) "Computer-assisted Prediction of Xenobiotic Metabolism."
Dr. Jack J. Chou of the University of California, Santa Cruz.

4) "Synthesis and Stereochemistry of Diamine Platinum Complex."
Dr. James D. Morrison of the University of New Hampshire, Durham.

Chemical Structures

The chemical structures of compounds discussed in this report are shown in Figures 1 through 4.

CHART I

COMPOUND SOURCE ANALYSIS

MAY 1, 1981 TO APRIL 30, 1982

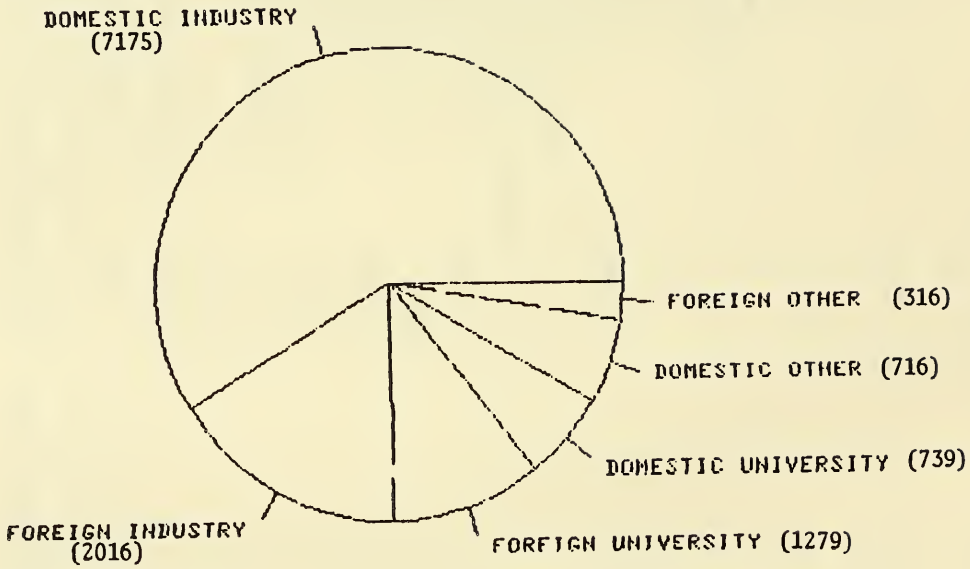


CHART II

Compounds Selected for Synthesis March 1981 - May 1982

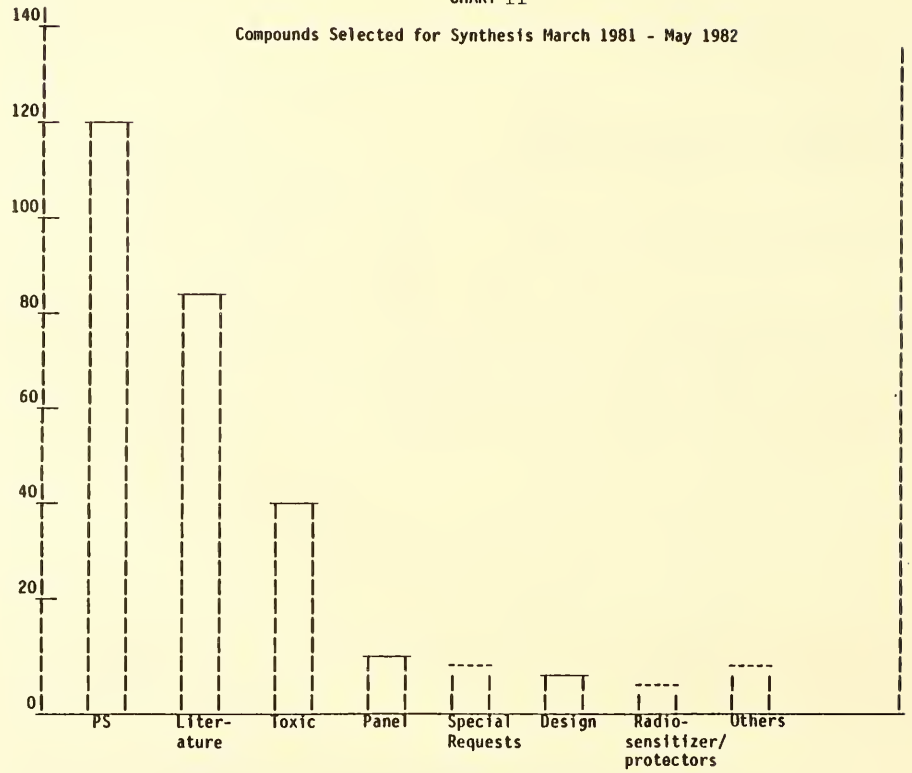


TABLE I

DRUG SYNTHESIS & CHEMISTRY BRANCH CONTRACTS

Contractor	Investigator	Contract No.
Bristol Laboratories	Doyle	NO1-CM-87180*
Chemical Abstracts Service	Myers	NO1-CM-43722
Dynamac (Enviro Control,Inc.)	Gray	NO1-CM-07332
Flow Laboratories, Inc.	Dorian	NO1-CM-97254
Institute of Cancer Research	Adams	NO1-CM-17502
Michigan Technological Univ.	El Khadem	NO1-CM-07293*
SRI International	Lee	NO1-CM-17485
Starks C. P., Inc.	Schultz	NO1-CM-87206

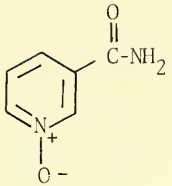
*Contract expired during this period

Quick Reaction Work Order Contracts:

Alabama, University of	Baker	NO1-CM-07355
Collaborative Research,Inc.	Friedman	NO1-CM-07358**
IIT Research Institute	Uchic	NO1-CM-07359
Mid-America Cancer Center	Cheng	NO1-CM-07353**
Research Triangle Institute	Seltzman	NO1-CM-07352
SISA, Inc.	Razdan	NO1-CM-07354
Southern Research Institute	Montgomery	NO1-CM-07260
Southwest Foundation for Research and Education	Rao	NO1-CM-07356
SRI International	Acton	NO1-CM-07351
Starks Associates, Inc.	Starks	NO1-CM-07357

** Contractor withdrew from competition during this period

Figure 1



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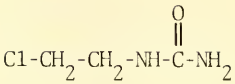

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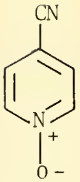

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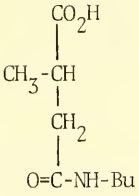

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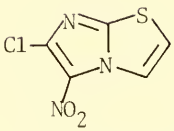

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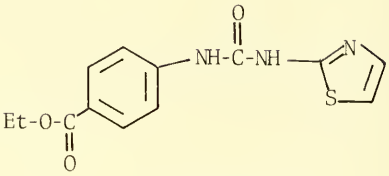

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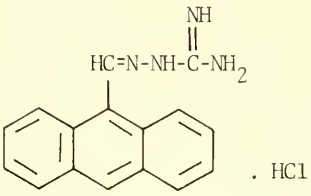

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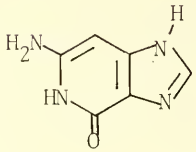

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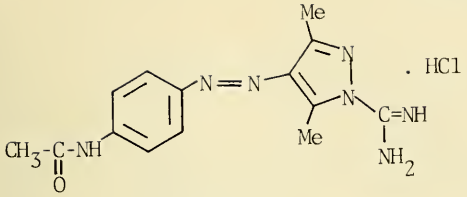

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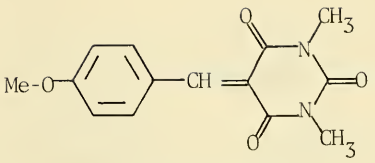

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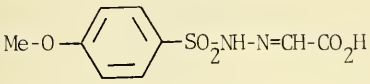

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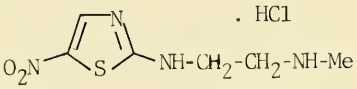

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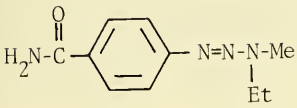

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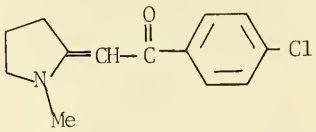

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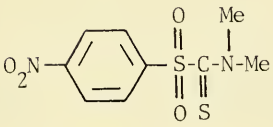

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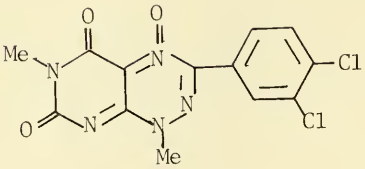

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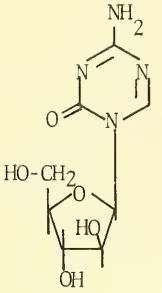

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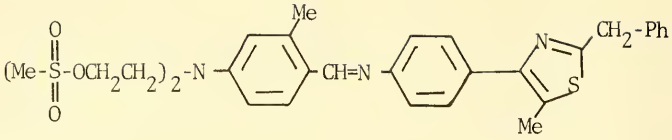

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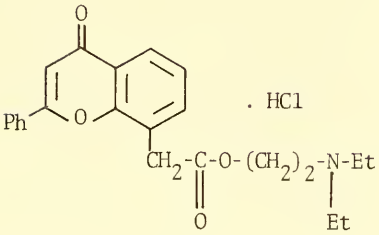

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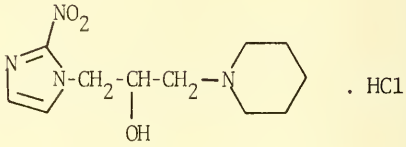

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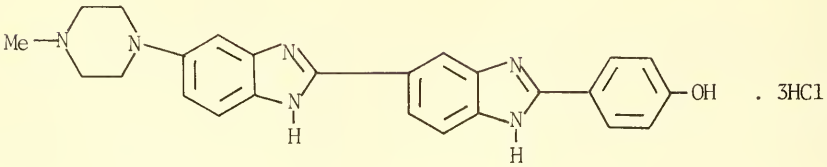

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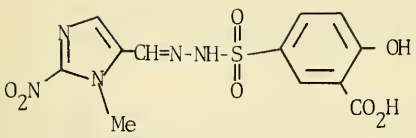

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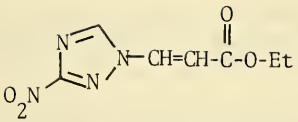

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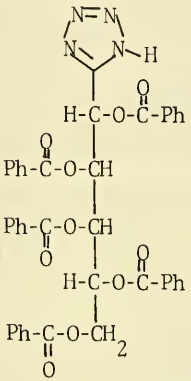

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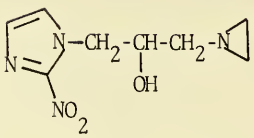

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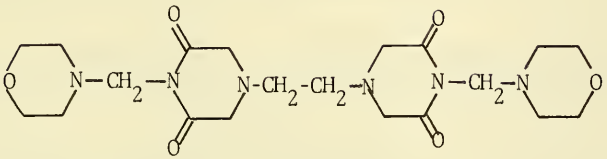

NSC 351358

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM-07101-07 DSCB

PERIOD COVERED October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Computer Methods for Drug Preselection Based on Structure-Activity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. Hodes	Research Mathematician	DSCB, NCI
Other:	K. Paull	Chemist	DSCB, NCI
	R. Geran	Biologist	DEB, NCI

COOPERATING UNITS (if any)

Chemical Abstracts Service

LAB/BRANCH

Drug Synthesis and Chemistry Branch

SECTION

Acquisitions Section

INSTITUTE AND LOCATION

NCI, NIH, Silver Spring, Maryland 20910

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Structure activity relationships have been adapted to aid in selecting compounds for large-scale screening in mouse lymphocytic leukemia (P388). Molecular fragment statistics from compounds tested in P388 are used to estimate antitumor activity, novelty and toxicity. These estimates are examined by a medicinal chemist along with the structures of over 30,000 potential acquisitions per year to decide which compounds to screen. Results from P388 on almost 20,000 compounds that have been through this system indicate that the estimates are useful. New estimates are planned for therapeutic index and activity with regard to physical parameters. In addition, these methods have yielded an automated literature surveillance project.

ANNUAL REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 - September 30, 1982

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

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

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

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

Objectives

As part of the overall Division of Cancer Treatment Program, the Natural Products Branch has three major objectives:

I. Fermentation

The acquisition of new antineoplastic agents from microbial fermentations is the primary responsibility of this area. A contract program exists to isolate unique organisms and to ferment and prescreen for biological activity. Active fermentations in the prescreens are tested in P388 leukemia *in vivo*. Confirmed active ferments are assigned to chemists and the active anticancer agent is isolated and evaluated by NCI. The capacity exists in these contracts to produce sufficient amounts of material for clinical studies. The program is using novel and/or improved techniques and unusual substrates to obtain unique organisms in the hope of generating numerous new structural leads. *In vitro* prescreens (enzyme inhibition, tubulin binding, microbial inhibition, phage induction and cytotoxicity against

various cell lines) have increased the efficiency of the program in selecting potential leads and decreased the in vivo testing load for primary fermentations.

II. Plant and Animal Products

The acquisition of new active antineoplastic agents from higher plants and from animal products (marine, primarily) is the major function of this area. The program has operated through two mechanisms, a coordinated contract program for collection, extraction and isolation of new agents, and donations of both crude extracts and purified materials from interested scientific collaborators. As of October 1981, a program decision was made to eliminate the contract program in this area based on the reasons that 1) this area was fairly well covered by acquisition of pure compounds 2) the costs of new leads were high in relation to the return and 3) the isolation and structure elucidation of new agents was equally suited to grants research as contracts. As of September 1982, only one contract will remain in this section which is for large scale isolation of plant and animal derived agents to meet NCI needs for bulk drug supply for formulation, toxicology and clinical trials. This contract is being recompeted.

III. 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.

Program Implementation

The objectives of the Natural Products Branch are carried out through the Sections and the Office of the Chief, and are:

I. Fermentation Section

The objective is the isolation and development of novel antineoplastic drugs derived from microbial fermentations. The section maintains seven contracts in support of these tasks. Four contracts are devoted to the isolation of unique organisms and to systematically evaluate the microbial world for its ability to produce novel anticancer agents. The work includes 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. These antineoplastic agents are supplied by the four contractors, and one other contractor is maintained to support production of active agents in quantities sufficient for toxicology and clinical trials. The Section directs two contracts de-

voted to biotransformation of known structures and cometabolism of various unusual compounds. Biotransformation is the microbial technique used to convert a compound to one of lower toxicity, broader spectrum of activity, increase solubility or make it pharmacologically more acceptable to man. A microbe can carry on as many as 20 chemical reactions, many at the same time, producing only one isomer and without destroying the primary structural moiety of a compound. Cometabolism fermentation involves the feeding in of an unusual chemical in the hope of influencing the microbe's metabolism and having it produce new antineoplastic agents. Twenty crystalline metabolites have been acquired this year using the biotransformation and cometabolism techniques. Five more metabolites are expected before the end of this current contract period. The biotransformation program has been productive, but because of budgetary constraints this will be terminated in September, 1982.

TABLE 1

CUMULATIVE FERMENTATION PROGRAM STATISTICS

Approximately 15,000 microbes isolated/year up to 1981. This was reduced in 1982 to concentrate on chemical isolation of existing leads.

(a) 193,618 culture broths (primary) tested in vivo since inception of program

(b) 15,600 confirmed actives since inception of program

(c) 2,181 crystalline compounds tested since inception of program

(d) 60 new compounds added to tumor panel (in 1981) 1/

1/ Tumor panel consists of in vivo testing against five murine tumors and three xenografts (human tumors in nude mice).

II. Plant and Animal Products Section

The objective is the development of novel anticancer drugs from higher plants and animals (including marine organisms). In support of this activity the Section maintains a world-wide program of acquisition of both crude and purified plant and animal materials. The acquisition of pure compounds is supplemental to the literature surveillance program in the Office of the Chief. The Section acquires for testing crude extracts reported to be active in in vivo or in vitro experimental models related to cancer, extracts used in folklore and traditional medicine, and extracts of biochemically or taxonomically unusual plants and animals for screening. When active leads are discovered the Section works closely with the investigators involved to assist in isolation of the pure active principles by coordinating information on related problems, suggesting procedures for isolation, and providing bioassay support to guide isolation. The Section acts as a worldwide resource to scientists active in this area as a result of having a large base of data on screening results with plant and animal materials and extensive knowledge and experience in chemotaxonomy of cytotoxic and antitumor agents. When pure compounds are isolated from crude

extracts they are tested and the Section is responsible for follow up of results and obtaining further supplies of compound for additional testing when required. The Section maintains a contract for pilot plant scale isolation of compounds which are needed in substantial quantities for advanced preclinical and clinical studies. Compounds are isolated in quantities of several grams to several kilograms as required. This contract has isolated 10 kilos of Indicine N-oxide from 10,000 pounds of Heliotropium plant and 250 grams of taxol from 3,000 pounds of Taxus plant during the current year as well as smaller quantities of other drugs.

TABLE 2

CUMULATIVE PLANT PROGRAM STATISTICS

(a) Approximately 35,000 plant species and 115,000 extracts tested since inception of program

(b) Plant extracts active - 5,046 since inception of program

1) 1,598 genera

2) 3,490 species

(c) 14 new compounds added to tumor panel (in 1981)

(d) 2,513 crystalline plant compounds tested

TABLE 3

CUMULATIVE ANIMAL PROGRAM STATISTICS

3000 animal species extracted and tested since inception of program

(a) 16,500 extracts tested since inception of program

(b) 675 confirmed actives

(c) 420 genera

(d) 571 species

(e) 630 crystalline animal compounds tested

(f) 7 animal compounds in tumor panel

Branch Staff

The Natural Products Branch is composed of a staff of five persons consisting of two senior professional scientists (GS 13 or above), one other professional staff member and a clerical staff of two.

The responsibility for the activity of the Branch is as follows:

Natural Products Branch

Acting Chief - Dr. Matthew Suffness

Plant and Animal Products Section

Head - Dr. Matthew Suffness

Fermentation Section

Head - Ms. Amelia Acierto

Program Operation

Fermentation Section

The fermentation program has been designed to hopefully obtain more novel organisms and ferment these cultures under various conditions which should produce novel antineoplastic agents. With reduced funding as approved by the Board of Scientific Counselors in October, 1981 the work under this program has been reprioritized in an effort to allow the contractors to finish on-going projects before the end of the remaining contract year. Work continues but at a lower rate to develop new prescreens (*in vitro*) in order to obtain new structures with antineoplastic activity. This year the prescreens allowed the major NCI fermentation contractors to evaluate 10,704 new cultures. This is less than previous years due to de-emphasis of new screening because of the many leads now in chemistry. Two thousand five hundred thirty-seven of these fermentation broths were active in one or more of the prescreens (antimetabolite, microbial screen, tubulin binding, antiviral, enzyme inhibition). Nine hundred of the *in vitro* actives have been regrown and tested *in vivo* vs P388 leukemia in the mouse. Normally when all crudes were tested directly *in vivo* (until 1975), approximately 3% were active. Using the prescreen, 10-25% of the *in vitro* actives were active *in vivo*. In addition, with presumptive chromatography now being done before the *in vivo* step, most of the selected actives are novel. We also found out in our experiment that concentrating the *in vitro* active broths allowed 42% to be *in vivo* active. Continued optimization of *in vitro* prescreening has allowed the program to find more novel *in vivo* active leads at lower cost. Cost savings have been achieved through decreased use of expensive *in vivo* testing for initial screening.

Results in Table 4 indicate a highly productive year even with a decreased budget. At present, we have 258 microbially derived compounds in the tumor panel. This year, 123 novel fermentations with *in vivo* P388 activity were assigned for chemical isolation studies.

Due to the budgetary reductions, we have had to cut our culture isolation, prescreen, fermentation and chemical efforts.

TABLE 4

1981 Fermentation Statistics

Company	Cultures Fermented	Active In Vitro	Tested In Vivo	Active In Vivo	Undergoing Chem. Fract'n	New Compounds Submitted	New Active Compounds
A	729	184	160	40	46	1	0
B	4621	1517	242	31	39	9	6
C	5354	836	538	49	48	9	6
Total	10,704	2537	940	120	133	19	12

Biotransformation and co-metabolism are two fermentation techniques being evaluated for their potential to produce interesting microbial metabolites (two contracts). The biotransformation and co-metabolism program is designed to modify compounds which have a structure nucleus of interest to NCI but the drug itself is either too toxic, has some ill-desired side effect, difficult to formulate, narrow spectrum of activity or is not quite active enough. It is hoped with subtle select modifications made microbially a superior compound will be obtained. Eighteen compounds were obtained from the biotransformation program this year and are being evaluated. Twenty-two compounds were exposed to this technique, and in many cases more than 100 microbes were used in an attempt to get a modification of the structure. Table 5 shows the submissions from this work.

TABLE 5

Biotransformation Submission (1981)

Parent	Metabolite	NSC #
4-hydroxyandrost-4-ene-3,17-dione	4,9alpha-dihydroxyandrost-4-ene-3,7-dione	350893
	4,9alpha-dihydroxy-5alpha-androstane-3,17-dione	350894
	4,11alpha,17Beta-trihydroxyandrost-4-ene-3-one	350851
	17alpha-oxo-D-homo-4-hydroxy-androst-1,4-diene-3,17-dione	351139
Aphidicolin	18-acetoxyaphidicolin	339660
	6Beta-hydroxyaphidicolin	340292
	Aphidicolin-18-carboxylic acid	342436
	3-ketoaphidicolin	346198
	3-epiaphidicolin	351140
		351909
Hedamycin	12-iminohedamycin	348119
Mitomycin C	Mitomycin metabolite	D341616
	Mitomycin metabolite	D341617
	Mitomycin metabolite	D343250
Quinaldine	N-decanoylquinaldinee	349154
	N-hexanoylquinaldine	351280
	6-desaminoquinaldine	351908
Quinazolinone-2, 3-dihydro-2-(1-naphthyl)	6-hydroxy 2,3-dihydro-2-(1-naphthyl) quinazolinone	342435

Table 6 shows a list of the current fermentation contracts and Table 7 shows the contracts that have expired or are due to expire.

This year, we obtained 39 pure antibiotic materials from our contractors and 139 from outside sources. A total of 113 fermentation antibiotics are undergoing special testing (in more than one animal tumor system), and 258 fermentation antibiotics are being tested in the entire tumor panel.

Our Litton Bionetics contract is primarily a pilot plant research and development contract where kilogram quantities of toyocamycin is being produced as an intermediate for the tricyclic nucleoside, tricribrine phosphate which is now in the clinic. The production and processing of largomycin has been developed to prepare sufficient quantities for schedule dependency studies, formulation and toxicology. Several leads (Gilvocarcin, PR 1350, luteoskyrin and anticapsin) are having processes developed for scale-up so we can produce sufficient material for the tumor panel. Fredericamycin, a novel compound discovered under this contract as a possible DEC candidate based on its DN2 level of activity and was found to be highly active in the stem cell assay. Roridin A is being produced as a new material to be converted to 8Beta-hydroxy 9Beta,10Beta-epoxyroridin A, which is a highly active lead having a DN2 level of activity.

Plant and Animal Products Section

The operation of this area has changed drastically since October, 1981 when the decision was made to abolish the contract supported drug discovery program in this area. Eight contracts have been terminated this fiscal year as shown in Table 8, and the emphasis in this Section has been on providing an orderly winddown of contract supported work to achieve maximum benefit from the program and to transfer all necessary data and materials to those organizations who wish to attempt to continue their work under NIH Grant support.

The efforts of the Section are now being directed toward a more aggressive program of acquisition of pure compounds for screening rather than acquisition, screening, fractionation and isolation of new agents from crude extracts. The Section will continue to screen limited numbers of crude extracts of special interest and to work with chemists on plant and marine animal leads in development, but this effort will continue to decline in favor of acquisition of already isolated materials.

The remaining contract (Table 9) continues to play an important role in supplying bulk quantities of drugs in development including indicine N-oxide, taxol and phyllanthoside, and it is expected that there will be a continuing need for scale up isolation as new leads progress through the program.

Despite the disruption encountered with phase out of the contract program, this has been a successful year with 44 pure plant products and 24 animal products being isolated and purified under contract, and 97 plant and 16 animal products being acquired by donation.

TABLE 6

Fermentation Contracts

Contractors	Investigators	Contract No.
Bristol Laboratories	Bradner	NO1-CM-07299
Litton Bionetics (FCRF Fermentation Program)	Flickinger	NO1-CO7-5380
Microbial Chemistry Research Foundation	Umezawa	NO1-CM-57009
Warner-Lambert	Dion	NO1-CM-07379
Upjohn Company	Neil	NO1-CM-07380
University of Illinois*	Loub	NO1-CM-97259

* Literature surveillance for all natural products

TABLE 7

Fermentation Contracts Expired or Due to Expire in FY 82

Contractors	Investigator	Contract No.	Expiration Date
Bristol Laboratories	Claridge	NO1-CM-07324	8/31/82*
British Columbia Univ.	Kutney	NO1-CM-87236	12/31/81
Kyowa Hakko Kogyo Co.	Misawa	NO1-CM-87190	9/19/81
University of Iowa	Rosazza	NO1-CM-07412	9/14/82*

*Early termination due to funding restrictions

TABLE 8

Plant and Animal Products Contracts Terminated in FY 82

Contractor	Investigator	Contract No.	Termination Date
Agriculture, U.S. Dept. of (plant collection)	Duke	YO1-CM-40001	9/30/82*
Arizona State University (plant chemistry)	Pettit	NO1-CM-97297	8/31/82
Arizona State University (marine chemistry)	Pettit	NO1-CM-97262	11/30/81
Illinois, University of (plant chemistry)	Farnsworth	NO1-CM-97295	8/31/82
Purdue University (plant chemistry)	Cassady	NO1-CM-97296	8/31/82
Research Triangle Institute (aqueous extraction)	Taylor	NO1-CM-97261	2/28/82*
Arthur D. Little, Inc. (prescreen development)	Broome	NO1-CM-07331	9/29/82*
Research Triangle Institute (organic extraction)	Taylor	NO1-CM-17396	7/31/82*

* Early termination

Table 9

Plant and Animal Contract

Contractor	Investigator	Contract No.
Polysciences, Inc.	Boettner	NO1-CM-07300

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees, academic institutions, research organizations and other government laboratories to maintain our knowledge of natural products research and development throughout the world. Also, this liaison allows us to obtain prime compounds soon after their discovery for evaluation in the NCI program (e.g. Macbecins I and II, Tetrocarcin, Neothramycin, Mazethramycin, THP, Mitomycin RR-150, Geldanomycin derivatives, etc.).

This year, 252 new compounds were obtained because of this liaison, many of which are active and are being obtained in larger amounts for evaluation. Close contact is maintained with the suppliers in order to provide interchange of chemical and biological data which is essential to insure efficient use of material.

A trip was made to Japan to cement relations between Japanese companies and NCI. At present over 30% of the new natural products acquired are coming from Japanese sources. The following organizations were visited:

Fujisawa Pharmaceutical Co.
Ajinomoto Co.
Otsuka Pharmaceutical Co.
Asahi Chemical Co.
Japan Antibiotics Research Association
Kureha Chemical Co.
Yamanouchi Pharmaceutical Co.
Institute of Microbial Chemistry
Kyowa Hakko Kogyo Co., Ltd.
Takeda Chemical Co.
Asahi Chemical Co.
Dainippon Pharmaceutical Co.
National Cancer Center
Green Cross Co.
Zenyaku Koygo Co.
Kaken Chemical Co.
Suntory Co.
Nippon Kayaku Co.
Cancer Research Inst. (Tokyo)
Meiji Seika Kaisha, Ltd.

Many natural products are obtained from all parts of the world and from all types of organizations. The following criteria are used to determine if NCI is interested in testing such materials or should request them:

1. Compound has some anticancer activity against any tumor, whether in NCI's system or not

2. A novel structure type never evaluated in our program

3. Activity against yeast, fungi or certain bacteria

4. Antiviral activity (human, animal or bacterial)

5. Reported enzyme inhibition of proteases lipases, CAmp phosphodiesterase, aminopeptidase, transmethylase, etc.

6. Other biochemical or biological activity which may be cancer related

Major Changes in the Branch during FY 82

The virtual abolishment of the contract program in the plant and animal area coupled with a large cut in the fermentation contract funds has reduced the number of contracts administered by the Branch from 19 to 7, which will undoubtedly result in a decrease in compounds acquired for screening.

Branch activities have shifted orientation towards attempting to increase worldwide surveillance and liaison efforts to maintain a good flow of novel compounds into the antitumor screening program. Acquisitions of donated compounds have increased in recent years relative to contractor isolated compounds and it is expected that the Branch can maintain good productivity in acquiring compounds which become clinical candidates. The resignation of the Branch Chief, Dr. John Douros, to take a job in private industry, makes this effort more difficult since he had made a large number of important personal contacts in the pharmaceutical industry during his tenure at NCI, which will take some time to build up again.

On the positive side, the smaller scope of Branch efforts should enable a greater concentration on the worldwide surveillance and acquisition program, which may lead to more donated compounds which will partially offset the loss of the contract drug discovery effort.

Major Accomplishments

1. The ultimate objective of the Branch is to supply the Division of Cancer Treatment with new drugs of natural origin for clinical trials. This year two drugs had Investigational New Drug Applications approved, homoharringtonine (NSC 141633), of plant origin, and echinomycin (NSC 526417) a fermentation product.

2. There are a considerable number of drugs currently in advanced preclinical development or clinical trials sponsored by NCI that have originated with the Natural Products Branch, and these are outlined in Table 10.

3. During the past year 4 compounds have passed DN2A and have been selected for advanced preclinical development.

4. A total of 359 new pure natural products meeting criteria for screening were acquired. These are further defined in Table 11.

5. Large scale production of taxol (300 g), indicine N-oxide (10 kilos) and toyocamycin (15 kilos) was achieved by NCI contractors to provide material for ongoing studies.

6. A total of 69 compounds, 55 from fermentaton and 14 from plant sources, 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 very encouraging and we anticipate having three or four new Decision Network 2A candidates in the next year for advanced preclinical development.

7. Taxol and rapamycin entered toxicology studies and are progressing. Both compounds are projected to be the subjects of IND filings early in FY 83.

Table 10

Natural Products in Advanced Development

NSC #	Drug	Origin	Status
163501	Acivicin	fermentation	Phase II Clinical trial
218321	Pentostatin	fermentation	Phase II Clinical trial
132319	Indicine N-oxide	plant	Phase II Clinical trial
208734	Aclacinomycin A	fermentation	Phase II Clinical trial
276382	Pepleomycin	fermentation	Phase II Clinical trial
526417	Echinomycin*	fermentation	Phase I Clinical trial
141633	Homoharringtonine*	plant	Phase I Clinical trial
226080	Rapamycin	fermentation	Toxicology
125973	Taxol	plant	Toxicology
269148	Menogarol	fermentation	Toxicology
330500	Macbecin II+	fermentation	formulation
325319	Didemnin B	marine animal	bulk procurement
328426	Phyllanthoside+	plant	formulation
234714	Aphidicolin	fermentation	formulation
325014	Bactobolin+	fermentation	bulk procurement
333856D	Discreet+	fermentation	bulk procurement
237020	Largomycin	fermentation	bulk procurement

*INDA's approved FY 82

+Passed Decision Network 2A FY 82

Table 11

Pure Compounds Acquired Calendar Year 1981

	Contract	Donated	Total
Fermentation	39	139	178
Plant	44	97	141
Animal	24	16	40
Total	107	252	359

Amonkar, A., Chang, C.-j., and Cassady, J.M.: 6-Geranyloxy-3-methyl-1,8-dihydroxy-anthrone, a novel antileukemic agent from Psorospermum febrifugum Sprach var. ferrugineum (Hook fil.). Experientia 37: 1138-1139, 1981.

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ANNUAL REPORT OF THE ANIMAL GENETICS & PRODUCTION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 to September 30, 1982

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 gram; (2) other NCI research contracts (DCCP, DCBD, etc.); (3) NIH Intramural Program; (4) Research Grants; (5) NIEHS, and (6) Veterans Administration Research facilities (surplus animals which are offered when available).

An additional function of this Branch is to coordinate the movement of transplantable experimental animal tumors from tumor bank sources into scientific laboratories.

The structure of the animal production program includes: four (4) Primary Genetic Centers; seven (7) Rodent Production Centers; ten (10) Hybrid Production Contracts; two (2) Beagle Hound Production Contracts; seven (7) Animal Disease Diagnostic Contracts; one (1) contract with the National Academy of Sciences; one (1) tumor bank contract; and one (1) rederivation contract. FY 1982 funding for the efforts listed above is included in this report.

The Branch staffing consists of one senior staff member, one professional associate, one animal contracts assistant, and one clerk-typist. Contracts and Branch functions are currently structured as follows:

PRIMARY GENETIC CENTERS

These centers serve as a breeding nucleus for the animal program. In order to insure that maximum quality control is maintained at this level, it is required that any animals received by such centers are derived into a germfree state, i.e., Caesarian sections are performed under isolator conditions. These animals are then maintained as foundation colonies in defined flora isolators. These foundation isolators serve as a source of breeders for the larger expansion colonies which are housed under barrier conditions.

RODENT PRODUCTION CENTERS

The purpose of these contracts is to accomplish large-scale production of pure strains of inbred mice which will be supplied to fix price contractors for hybrid mouse production. The rodent production contracts receive breeding stock from the genetic center expansion colonies. These breeders, therefore, represent direct offspring of brother x sister matings. This production also takes place under rigid barrier-controlled conditions. These contracts are essential for providing the large number of "super clean" breeders for hybrid production contracts.

Due to budgetary constraints and redirection of program effort, three of these contracts are being phased out. The total number of Rodent Production Centers will be reduced from seven (7) to four (4) during to FY 1982.

HYBRID MOUSE PRODUCTION CONTRACTS (Fixed-Price)

These contractors supply the large volume of first generation hybrid mice for the screening program. Breeding animals for these colonies are received from the rodent production contracts.

Due to budgetary constraints and redirection of program effort, total production from these contracts has been reduced by approximately 33 percent.

BEAGLE PRODUCTION CONTRACTS

These animals are produced on a fixed-price contract basis from qualified breeders and are primarily used by the toxicology program.

DIAGNOSTIC CONTRACTS

These are service contracts which are utilized to monitor and upgrade the quality of animals moving within the animal production program and to the laboratory. Presently, they consist of two (2) virus diagnostic services, three (3) pathology services, one (1) bacterial diagnostic service (primarily Salmonella and Pseudomonas species), and one (1) isolator monitoring (associated flora) contract.

HISTOCOMPATIBILITY CONTRACT

These contractors perform genetic monitoring of inbred mice and rats by means of skin grafts.

In order to provide assurance that replacement strains which are received from the NIH repository are free of pathogens and compatible with NIH foundation strains, an additional contracts is presently being competed. contract will provide a Biochemical a Biochemical Genetic Monitoring Service which will test the replacement strains for histocompatibility prior to distribution to the genetic centers.

TUMOR BANK CONTRACT

This contract provides experimental human and animal tumors (free of contaminating microbes) on a scheduled basis to screening/tumor panel laboratories and to qualified investigators both in the U.S.A. and abroad. Due to an increasing awareness of genetic drift problems, a number of investigative laboratories have asked to be placed on the screening schedules, thus, increasing the workload of this contract.

NATIONAL ACADEMY OF SCIENCES

A small amount of support is supplied to the National Academy of Sciences. This support involves development and publication of standards and guidelines in the field of animal care and breeding. The goal of this Branch is to achieve and to maintain quality animal production, while meeting the needs of the program.

FREDERICK CANCER RESEARCH FACILITY

The cooperation of the Frederick Cancer Reseach Facility (FCRF) again merits special recognition. The cooperative effort between DCT and FCRF enabled FCRF to remain afloat (interim breeder animals from DCT genetic centers) until rederived animals were expanded in sufficient numbers to meet FCRF needs. The rapid expansion of nude mouse production at FCRF enabled the AG&PB to meet DCT's critical needs for human tumor screening on schedule. Quality of animal production at FCRF continues to be equal or superior to that of any production facility in the U.S.A.

FCRF is also participating in the centralized rederivation effort for our program and is performing a pilot study to determine the feasibility of embryo cryopreservation as a means of preserving strains with little current usage, controlling genetic drift, etc.

The reimbursement program provides a service to other NCI divisions, intramural investigators, and to grantees since animals of the quality and quantity needed are not available with the NIH background. DCT benefits from the reimbursement program because a significant percentage of the animals supplied to other programs are surplus to DCT's needs (offsex, etc.), thus, providing a net savings to the DCT Animal Program Budget. The savings provided to the taxpayer by avoiding duplication of effort in laboratory animal production and genetic and health monitoring is also obviously significant.

The DCT/NCI Program is now in a position to provide "super clean" animals to those facilities that can maintain these animals in a comparatively disease-free condition. Special programs that necessarily require this quality level include human tumor testing, Biological Response Modifiers, NCI tumor bank, NCI intramural laboratories, etc. A number of testing laboratories are upgrading their facilities in order to receive and hold these animals, e.g., Southern Research Institute, Arthur D. Little, Inc., Roswell Park, Yale University, etc. Within the limits of budgetary conditions, it would appear appropriate to encourage to further upgrading of testing facilities with the consequent elimination of animal disease variables in cancer research data.

ANIMAL GENETICS & PRODUCTION BRANCH PROGRAM FUNDING
FY 1982

PRIMARY GENETIC CENTERS (4)	$4,573,000
Supply breeding nucleus for the animal program and athymic mice for drug evaluation.	
RODENT PRODUCTION CENTERS (7)	1,281,000
Large-scale production of inbred mice under both conventional and barrier controlled environment.	
HYBRID MOUSE PRODUCTION CENTERS (10)	1,619,000
Supply hybrid mic for the screening program.	
BEAGLE PRODUCTION CENTERS (2)	70,000
Supply dogs for large-scale toxicology.	
DIAGNOSTIC & HISTOCOMPATIBILITY PROJECTS (7)	980,000
To monitor animal health and genetic integrity.	
DEVELOPMENT OF STANDARDS & GUIDELINES (1)	29,000
For animal care and breeding.	
MAINTENANCE OF FROZEN TUMOR BANK (1)	225,000
PRODUCTION OF ATHYMIC & OTHER NEEDED MICE (FCRF)	1,246,000
CENTRALIZED REDERIVATION	NO COST
Rederiving new starts into the associated flora state from the NIH Repository	
TOTAL	$10,023,000
Less Reimbursements	1,635,000
TOTAL COST TO DCT	$8,388,000

ANNUAL REPORT OF THE DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 - September 30, 1982

I. Objectives

Objectives of the Drug Evaluation Branch are the recognition of new agents for development to clinical trial against cancer and the conduct of pre-clinical therapy related tasks essential or complementary to new anti-cancer drug development. In achieving objectives, DEB plans, directs and evaluates a comprehensive contract program for screening large numbers of synthetic and natural products for biological activity indicative of clinical utility, in-depth evaluation of materials of interest found in DCT screens or reported from other Programs, and development of improved methods for pre-clinical drug evaluation. Principal end products are recommendations to the Associate Director, DTP and Director, DCT of new drugs for development to clinical trial, pre-clinical therapeutic data required to initiate clinical trials, reports on pre-clinical efficacy for inclusion into Investigational New Drug Applications to the U.S. Food and Drug Administration, communication of pertinent findings to clinical cooperative groups and the scientific community via presentations in meetings and symposia and publication in scientific literature.

II. Implementation

Program is implemented through contracts with qualified institutions. As of April 30, 1982, DEB directed 16 contracts funded at approximately $8,000,000 (Table 1). The workscope of each contract is summarized in the Report of the Director, DCT.

III. Organization

Internal reorganization of DEB, abolition of the Screening Section and establishment of three new Sections, was completed. The principal functions of each Section is outlined below.

A. Screening Operations Section

1. Establishment of routine *in vivo* screening protocols.

2. Maintenance of *in vivo* screening capacity.

3. Conduct of initial *in vivo* screens.

4. Reference of confirmed pre-screen active materials to Selected Agents Section.

B. Selected Agents Section

1. Selection of compounds for further evaluation in the DCT panel of *in vivo* tumors and other secondary pre-clinical models.

2. Recommendation of those drugs warranting presentation as candidates for development to clinical trials.

3. Implementation of non-routine testing, and biological and biochemical evaluations on compounds of interest in order to facilitate progressive and timely development.

4. Preparation of reports on the pre-clinical therapeutic efficacy of new drugs for inclusion in the Investigational New Drug Applications (INDA) and clinical brochures.

C. Cell Culture Section

1. Conduct and evaluation of projects aimed at application of human tumor clonogenic assays to screening.

2. Management of *in vitro* cytotoxicity testing.

3. Evaluation of potentially improved cell culture methods for drug evaluation.

IV. Accomplishments

A. In Vivo Screening

1. Selection of Compounds for Development to Clinical Trial

From April 1, 1981 through March 31, 1982, a total of 18,804 materials were screened for the first time in this Program. Of the total, 13,854 were synthetic compounds compared with 13,799 for the previous year. There was a substantial decrease in the number of crude natural products screened, from 8,684 in the previous period to 4,950 during this period, a reflection of the DCT decision to cease screening of crude plant extracts.

As of March 31, 1982, 2,030 materials (excluding drops) had been selected for testing in the DCT Pre-clinical Tumor Panel (TP), 332 during the current period. Of the latter, 274 were selected by the P388 pre-screen, 33 were selected to "by-pass" the pre-screen, and 25 were recommended by various DCT working groups. The Pre-screen Subcommittee of the Drug Evaluation Committee (DEC) reviewed 1,764 materials, 982 with confirmed activity in the P388 pre-screen. Twenty-nine compounds meeting Decision Network (DN) 2 activity criteria in at least one TP model were referred to DEC which recommended 17 to the Pre-Decision Network Committee as potential DN candidates. Eleven compounds were presented to the DCT DN Committee and ten passed DN-2A. These are listed in the current report of the Selected Agents Section, DEB.

3. Recommendations for Modification of In Vivo Screens

Based on tumor panel experience, DEB recommended substantial changes in the Flow of Compounds through NCI Pre-clinical Screens. The plan is consistent with the following theses.

a. No one pre-clinical model is capable of predicting all clinically useful drugs.

b. The initial test (pre-screen) should select, from a large population, a reasonable number of compounds worthy of more intensive study. The pre-screen should measure antitumor selectivity, but be sufficiently sensitive to minimize false negative predictions for activity in subsequent testing.

c. The screening process should be sequential; i.e., successive step should be progressively more discriminating leading to the selection of a few compounds for development to clinical trial.

d. The stepwise process of "tightening" the screen may be accomplished by using more refractory drug resistant experimental tumors and also by increasing the challenge to the compound using an individual tumor. Both approaches are incorporated into the recommended plan.

e. Compounds may be scored and priorities for development set by assigning values to each model consistent with the challenge that it presents. Compounds may be assigned value points by demonstrating desirable effects such as extraordinarily high activity in a single model, activity over a wider spectrum of tumors, ability to reach the target cell from a remote injection site, ability to overcome a more severe tumor challenge or by overcoming acquired resistanct to therapy.

Step 1 (Pre-Screen). Retain mouse leukemia P388. However, the feasibility of developing ancillary pre-screens, particularly in vitro, should be explored because there is no substantial body of data on the potential of the thousands of materials that fail P388 and the current human tumor clonogenic assay is not sufficiently cost effective for large scale use. Test compounds curative when given I.P. to mice with I.P. implanted P388 against S.C. implanted P388 to provide information on ability of the compound to reach the tumor target from a remote injection site. Retain the "by-pass" mechanism, but improve documentation of the reason for the by-pass decision.

Step 2 (Initial Panel Testing for Compounds Emerging from the Pre-Screen and Selected By-Passes). Eliminate active analogs of clinical drugs from the routine screening flow, and develop specific plans for each type aimed at showing clear superiority over parent compound. Retain mouse leukemia L1210 and B16 melanoma and

the human mammary xenograft, MX-1 (subrenal capsule) as presently used. Add I.P. implanted mouse tumor, M5076 to this step. Having originated in the mouse ovary, M5076 is easily transplantable I.P., S.C., and I.V. Regardless of implant site it metastasizes to abdominal sites including ovary, liver, and kideny. Tumor growth and expected life span are highly reproducible. Developmental work with the M5076 tumor was conducted by the Southern Research Institute (CM9-7309), Arthur D. Little, Inc. (CMO-7302), and the Mason Research Institute (CMO-7325).

Step 3 (Secondary Panel Testing). Examine compounds emerging from Step 2 against the appropriate sensitive tumor(s) under more challenging conditions including I.P. treatment of S.C. implanted tumors and I.P. treatment of S.C. implanted MX-1 xenograft and selected drug resistant variants. Where appropriate include I.V.-implanted tumors since activity with I.P. treatment would discriminate compounds warranting testing against metastases. B16 and M5076 are selected because both metastasize readily and both are reasonably sensitive to chemotherapy. The currently used I.V.-LL model is not recommended because of its low yield over the years. Include mouse colon tumor 38 and CD8F1 mouse tumor in this Step. While the low yield and relatively high cost of colon 38 and CD8F1 ma ca indicate that it is not efficient to use them in Step 2, they will be very useful as aids to prioritizing compounds emerging from Step 2.

The human xenografts, CX-1 and LX-1, and the mouse Lewis lung tumor are recommended for deletion from the flow of compounds through NCI screens. The number of DN-2 actives in these models is very low and they emerge infrequently. The tumors are frozen and could be re-established *in vivo* if clinical feedback indicates an extraordinary advantage from their use. During the interim period, the probability of losing a potentially useful drug by not using these models is very low.

B. In Vitro Screening

From April 1, 1981 through March 31, 1982, 12,218 *in vitro* cytotoxicity tests were conducted; 11,417 involving testing of crude and fractionated natural products in the Astrocytoma assay. The remaining tests employed KB or P388 cells to assess the cytotoxicity of both synthetic materials and natural products. In October, 1981 a major portion of the DTP Natural Products Program was terminated and the testing of crude plant extracts was stopped. Consequently, the level of *in vitro* testing from January 1 through March 31, 1982 was 46% of the October 1 through December 31, 1981 level and 30% of the July 1 through June 30, 1981 level. Testing after January 1, 1982 was related to completion of isolation of plant materials already under fraction and fermentation products.

Substantial progress was made towards translation of the human tumor clonogenic assay to drug screening. The pilot project to establish

protocols for screening and test 50 blinded compounds was essentially completed. Thus, during the last year, a working protocol was developed, tumor types most amenable to the assay were identified (breast, colorectal, lung, melanoma, and ovarian) and screening of unknowns was begun. The following tentative conclusions were drawn from the drug tests performed during the pilot phase.

1. Assay discriminates among agents.

2. Assay has reaffirmed the heterogeneous nature of tumors in regard to drug responsiveness.

3. Assay selects most agents regarded as clinically effective and some agents not selected by murine systems.

4. Response rates of clinical agents do not always appear to correlate with clinical experience, especially in the case of melanomas, which appear "super sensitive".

5. Assay misses agents requiring metabolic activation and antimetabolites reversed by medium components.

C. Detailed Drug Evaluation and Model Development

1. Introduction

DEB managed projects designed to provide maximal flexibility for rapid conduct of Program-directed exploratory, developmental, and applied studies pertinent to all pre-clinical therapy related tasks. Tasks are designed to (1) apply fundamental biological principles to the development of new and improved laboratory models; (2) evaluate, in detail, drugs in development to NCI sponsored clinical trial; (3) conduct non-routine studies in response to DCT Program needs; (4) determine host-tumor histocompatibility in appropriate inbred and hybrid hosts; (5) describe biological growth characteristics of primary tumors and metastases; (6) define the operational characteristics of the host-tumor system when used as a screen; (7) determine test-to-test reproducibility and variability; and (8) provide therapeutic trial data relative to clinical predictive value.

In order to maintain a vigorous and progressive drug development program, we have supported in vivo model development projects. Although considerable development work has been supported by NCI through the investigator initiated grant mechanism, few investigators tailor their studies to the special problems associated with large scale drug screening. Because of our past and present support of developmental studies, we were able to fully implement and complete the DCT Tumor Panel.

During the current reporting period, summaries of the pre-clinical therapeutic activity of seven compounds were submitted to the Cancer Therapy Evaluation Program, DCT for inclusion in IND Applications to the U.S. Food and Drug Administration and clinical brochures.

2. Analysis of Tumor Panel Experiment (1976-1982)

Through March 29, 1982, 1,231 compounds had been tested in all eight models (Table 2) comprising the DCT Pre-clinical Tumor Panel (TP). This number was considered sufficient to answer critical questions (Table 3) posed when the TP experiment was conceived in late 1975. The analysis of results was presented to the DCT Board of Scientific Counselors on June 4, 1982 along with recommendations for modifying in vivo screening procedures. Results are summarized in greater detail in the current report of the Selected Agents Section, DEB.

Of all compounds tested in all TP models (including structural congeners of major clinical drugs), 26% met DN-2A criteria for consideration for development to clinical trial in at least one model. Of the structural congeners of major clinical drugs, 90% met DN-2A criteria. Of materials not structurally related to a major clinical drug (non-analogs), 21% met DN-2A criteria.

Of the non-analogs meeting DN-2A criteria, 97% "passed" the P388 pre-screen. Moreover, there was a positive correlation between the degree of activity against P388 and the probability of achieving DN-2A activity level in at least one TP model. Thus, there is no existing evidence suggesting that an alternative to P388 would provide a more effective pre-screen.

Ninety-four percent of the DN-2 actives met the required activity criteria against mouse leukemia L1210, mouse melanoma B16, and/or the human mammary xenograft, MX-1. Three compounds were solely active in the mouse colon 38 model, none in the mouse Lewis lung model, two in the colon (CX-1) xenograft, and two in the lung (LX-1) xenograft.

Of 979 compounds which failed to meet DN-2 activity criteria against mouse leukemia L1210, 119 did meet activity criteria in one or more of the alternative models (Table 3, Goal I).

Human tumor xenograft models selected 26 compounds which failed the five mouse tumor screens (Table 3, Goal II). Twenty-two met DN-2 activity criteria against the mammary xenograft, MX-1, 18 against MX-1 only.

Based on correlations among the limited number of tumors of type (one human and one mouse for breast, colon, and lung) there was no apparent positive correlation between site of tumor origin and drug selection (Table 3, Goal III).

2. The Southern Research Institute (NO1-CM9-7309)

The Southern Research Institute (SoRI) conducted studies related to all of the tasks described in the above narrative. The contractor continued to meet high standards for reliability and has gained an international reputation for studies involving the biology of animal tumors and principles of pre-clinical drug evaluation. Because of a strong commitment to model development, SoRI assisted our Program in developing many of the tumor panel models into systems suitable for mass screening of new agents. They have developed a number of transplantable murine colon and breast tumors with different properties, such as different metastatic potential, growth rates and drug responsiveness, and provided data on the potential of using cell kill as an end point in screening experiments.

3. Mason Research Institute (NO1-CMO-7325)

The subrenal capsule (SRC) assay for screening against human tumor xenografts was developed under this contract. The assay substantially reduced the time and cost of xenograft screening, reduced the backlog of materials to be tested against the three human tumors in the NCI pre-clinical tumor panel, and was the single most important development leading to completion of the tumor panel experiment. The contractor also provided experimental data to aid in the formulation of precise protocols for screening against conventional mouse tumors and xenografts as well. The contractor developed an in vivo model for screening against subcutaneously implanted animal and human tumors, the tumored ear assay. Tumors are implanted S.C. in the pinna of the ear; growth is easily visible; and tumor weights can be determined directly. Two additional projects, directly related to the proposed future thrust of the model development segment of the DEB program are continuing during the final year of this contract. A collaborative pilot study (with Dr. Von Hoff, Cancer Therapy and Research Foundation of South Texas) to compare the drug sensitivities of fresh surgical tumor explants in vivo (SRC assay) and in vitro (clonogenic assay). The Mason Research group has also investigated the stability of the drug sensitivity of human tumors of various histological types to clinically active drugs in the first transplant generation and after having been established in serial passage in athymic mice.

4. Additional Projects for Detailed Drug Evaluation

Two additional contracts devoted to detailed drug evaluation, both with Arthur D. Little, Inc., for "Screening and Detailed Evaluation of Antitumor Drugs and Combined Chemotherapy and Modality Studies" (CMO-7302) and for "Biochemical and Biological Characterization of Antitumor Drugs" (CM1-7397) were monitored by the Selected Agents Section, DEB. Significant findings from these projects are described in the current report of the Selected Agents Section.

D. European Contracts and Collaborations

Contracts with the Insitut Jules Bordet, Brussels, Belgium (CMO-7350) and the Institute of Cancer Research, England (CM4-3736) take advantage of the considerable expertise and competence of highly visible and outstanding European drug development programs. Support by NCI of these projects enhances significantly our ability to obtain drugs from European sources, commercial and non-commercial, and expands, on a collaborative basis, the number of drugs that can be put into clinical trial each year via a Collaborative Agreement with the European Organization for Research in the Treatment of Cancer (EORTC).

1. Institute of Cancer Research (ICR), England (NO1-CM4-3736)

The main tasks of this project are:

a. Validation of human tumor xenografts as models for cancer chemotherapy.

b. The use of xenografts and mouse tumors not used elsewhere in this Program to test new compounds for antitumor activity. The compounds tested are (a) synthesized at the Institute, (b) obtained from other sources in Great Britain or Europe (through the EORTC), or (c) identified from studies of antitumor drug metabolism under this contract.

c. Toxicology required for approval for investigative clinical trial from the Committee on Safety of Medicines, animal and human pharmacology, and initial clinical trials.

d. The design, synthesis, and testing of new drugs on the basis of the biochemical findings related to treatment response or failure.

Since the inception of this contract, the contractor has acquired 1,396 compounds for screening (413 from various Departments of ICR and 983 from external sources) and has screened 1,321 compounds in vivo against one or more mouse tumors or human tumor xenografts. ICR currently maintains 58 human tumors for use in xenograft testing.

2. Institut Jules Bordet (IB), Brussels (NO1-CMO-7350)

During the nine month period July 1, 1981 through March 31, 1982, the total number of screening tests performed was 8,216 L1210 equivalents. Thus, the required level of 11,000 per year was maintained. During the same period, 2,337 synthetic compounds and 23 natural products were tested. Seven of these agents were submitted from the United States; the remainder (99.7%) were from European sources.

Nearly all of the compounds screened under this project are now submitted from European sources. If the rate of new compound testing for this nine month period is maintained, approximately 3,000 compounds per year (estimated 25% of total input) are subjected to pre-screening at the Institut Jules Bordet. The express purpose of this Continental European contract is to encourage European sources to submit materials for NCI screening and to this point, NCI maintains a Liaison Office (Dr. Omar Yoder) in the Institut Jules Bordet. This office serves as an ongoing, day-to-day contact and bridge between Western Europe and the United States and deserves a large share of the credit, along with the DTP Drug Synthesis and Chemistry Branch, for impelling the large input of European compounds to our Program.

V. Other Staff Activities

A. Grant Reviews

Over 100 Grant Applications dealing with various aspects of pre-clinical drug activity except synthesis of new agents and isolation of natural products were reviewed. Three types of evaluation were provided: (1) factual or technical information, such as the status of a particular compound in drug development; (2) Program relevance of each Application and its possible overlap with contract supported activities; and (3) the priority of each Application as high, medium, or low in relation to the objectives of DCT. The information was supplied to the Grants Program Director, Office of Extramural Research and Resources, to the Associate Director, DTP, and to the Director, DCT to aid in the review of Grant Applications, to determine the extent to which grant studies complement or overlap contract projects and to assist DCT in decisions regarding the funding of projects with similar priority scores.

B. Program Management

In addition to duties and responsibilities inherent to functions as Program Managers and Project Officers, DEB staff served on 16 working groups and committees and chaired four committees.

VI. Future Course

Projected major changes in in vivo screening methodology were described earlier in this report. It is anticipated that these changes will permit sufficient reapportionment of funds to develop new improved drug evaluation models. During FY 1983, DEB plans to implement projects for development of human tumor models for correlations in vitro sensitivity to drugs with in vivo response rate; and development of experimental screening procedures for the identification of antimetastatic agents. In addition, DEB is prepared to commit its contract resources, whenever possible and appropriate, to provide test results and information to the anticipated National Cooperative Drug Discovery Groups.

TABLE 1

DEB CONTRACTORS AND FUNDING LEVELS,* FY 1982

1.	In Vivo Screening	$3,699,000
	Arthur D. Little, Inc. (CMO-7346)	400,000
	Battelle Memorial Institute (CMO-7266)	650,000
	Battelle memorial Institute (CMO-7099)	525,000
	IIT Research Institute (CM9-7316)	900,000
	Institute Jules Bordet, Brussels (CMO-7350)	225,000
	Mason Research Institute	999,000
2.	In Vitro Screening	$1,204,000
	University of Arizona (CM1-7497)	346,000
	Mayo Clinic (CMO-7419)	198,000
	University of California at Los Angeles (CMO-7420)	302,000
	Cancer Therapy and Research Foundation South Texas (CMO-7327)	358,000
3.	Detailed Drug Evaluation	$2,558,000
	Arthur D. Little, Inc. (CM1-7397)	142,000
	Arthur D. Little, Inc. (CMO-7302)	421,000
	Southern Research Institute (CM9-7309)	1,995,000
4.	International Drug Development	$ 125,000
	Institute of Cancer Research, England (CM4-3736)	125,000
5.	Model Development	$ 189,000
	Mason Research Institute (CMO-7325)	189,000
6.	Support Services	$ 260,000
	IIT Research Institute (CM9-7213)	260,000
	TOTAL	$8,035,000

*Negotiated or estimated to nearest thousand.

TABLE 2

DCT IN VIVO SCREENING SYSTEMS (1976-1982)

PRE-SCREEN

P388 lymphocytic leukemia

TUMOR PANEL

I. Murine Tumors	II. Human Tumor Xenografts
L1210 lymphoma	
B16 melanocarcinoma	
Colon 38 carcinoma	CX-1 colon tumor
Lewis lung carcinoma	LX-1 lung tumor
CD8F1 mammary carcinoma	MX-1 mammary tumor

TABLE 3

PRE-CLINICAL GOALS OF THE TUMOR PANEL EXPERIMENT

I. To determine whether solid tumors select agents for clinical trial that would be missed by screening with L1210 leukemia alone.

II. To determine whether the human tumor xenografts and murine tumors select the same or different drugs.

III. To determine whether the site of tumor origin is a factor in drug selection.

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CELL CULTURE SECTION

DRUG EVALUATION BRANCH

As part of the Branch reorganization which was implemented during this fiscal year, the Cell Culture Section was established to manage a contract based program for drug screening and development using in vitro methods. The emphasis of this program is currently on application of the human tumor clonogenic assay to drug screening. Additional Section activities include management of contracts for in vitro cytotoxicity testing in support of the Natural Products and Drug Synthesis and Chemistry Branches and ongoing evaluation of new developments in the cell culture field for potential use in the program. Section staff includes a Section Head and one Biologist. In addition to duties as Project Officers for the contracts listed below, Section staff provide representation on the Drug Evaluation Committee and consultation to other Program staff regarding in vitro methods.

I. Application of the Human Tumor Clonogenic Assay to Drug Screening

In FY 1981, substantial progress has been made towards application of the human tumor clonogenic assay to drug screening. A pilot project has been completed during which protocols and procedures for the assay were established and 50 compounds were tested in blinded fashion by each of the four contract laboratories (Cancer Therapy and Research Foundation of South Texas, CMO-7327; Mayo Foundation, CMO-7419; University of Arizona, CMI-7497; and University of California at Los Angeles, CMO-7420). As of March 30, 1982 a total of 1,401 experiments had been performed yielding 5,816 drug assays. Data developed in the pilot study have established the feasibility of applying the assay for drug screening on a moderate scale (approximately 500 compounds per year). A major accomplishment of the pilot has been the identification of tumor types suitable for use in the assay. Certain tumor types have performed relatively well in the system. These include breast, colorectal, lung, melanoma, ovarian, and carcinomas of unknown origin. Lymphomas are not amenable to study using currently available methods. During the pilot project an automated colony counting/data reporting system was developed which provides two way communications via telephone and, in the future, will enable direction of testing of compounds with respect to desired tumor types. A number of proposals for criteria for defining compound activity have been considered. Using the simplistic criterion of 70% or greater cell kill relative to untreated control cultures and a tumor response rate of 10% or greater, nearly all of the established anticancer drugs included in the pilot project were "active" in the assay. Notable exceptions were Cytoxan, DTIC, Procarbazine, and Hexamethylmelamine, all of which are thought to require metabolic activation; and MTX, which appears to be inactive as a result of cellular rescue by culture medium components. Two problem areas were identified in the pilot project: (1) the difficulty associated with preparation of a completely mono-dispersed cell suspension from solid tumors for initiation of clonogenic assays and (2) the large amount of variability associated with measurement of colony formation in both drug treated and control cultures. In the current working protocol, colony counts are performed at the start of the assay such that experiments in which an unsat-

isfactory cell suspension was obtained may be recognized. In addition an internal positive control has been incorporated into each experiment. Four criteria are being proposed to identify valid assays: (1) The initial count derived from the image analysis system must be <24. (2) A net increase in colony count of 36 is required. (3) Coefficients of variation for control groups may not exceed 50%. (4) The internal positive control compound must reduce colony formation to at least the 30% survival level. Data from the pilot project are currently being re-examined after qualification of experiments against criteria 1, 2, and 3. Testing of a second set of 20 "blinded" compounds has been initiated under the new protocol to further define the activity of established agents and to define the activity of negative compounds. Detailed information on this latter point has been considered essential by project statisticians for finalization of activity criteria. Screening of unknown compounds has also been initiated under the new working protocol. The present plan is to utilize the assay to screen approximately 2/3 P388 negative and 1/3 P388 positive compounds at the rate of approximately 500 compounds per year. Further improvements to the assay will be pursued concomitantly with screening activities. Emphasis in developmental studies will be placed on development of methods for preparation of cell suspensions and improvements in quantitation of colony growth.

II. Cell Culture Screening and Bioassay of Natural Product and Selected Synthetic Compounds

At the start of this reporting period, the Astrocytoma assay (which detects tubulin binding activity as well as cytotoxicity), was being employed as an in vitro screen for selecting potential anticancer agents among crude naturally occurring plant, animal, and fermentation products. These materials were simultaneously being screened in the in vivo P388 leukemia assay. Concentration and purification of in vivo actives were then monitored in the Astrocytoma assay, or in the KB or P388 cell culture cytotoxicity assays, as indicated by the activity of the original crude material. These rapid and inexpensive cell culture assays were provided mainly as support for the DCT Natural Products Program. In October 1981, the Board of Scientific Counselors moved to terminate a major portion of the Natural Product Program, including most of the contracts requiring cell culture testing. Since October, the two DEB contractors conducting in vitro testing (Arthur D. Little, Inc. - CM9-7288 and the University of Miami - CM9-7290) have been phasing out operations, along with the Natural Products contractors they support. Table 1 summarizes cytotoxicity testing for the period April 1, 1981 through March 31, 1982.

TABLE 1

NUMBER OF IN VITRO CYTOTOXICITY TESTS CONDUCTED FROM APRIL 1, 1981 THROUGH MARCH 31, 1982*

	SELECTED AGENTS COMPOUNDS (SAC)		OTHER SYNTHETICS		NATURAL PRODUCTS			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
1981								
Second Quarter	17		47		2,275	246	335	2,856
Third Quarter	320		91		3,361	886	120	4,367
Fourth Quarter	174		40		2,258	327	291	2,876
1982								
First Quarter	79		33		954	352	12	1,318
TOTAL	590		211		8,848	1,811	758	11,417

	Second Quarter 1981	Third Quarter 1981	Fourth Quarter 1981	First Quarter 1982	Four-Quarter Period
TOTAL ALL IN VITRO TESTS	2,920	4,778	3,090	1,430	12,218

*Includes tests begun no earlier than April 1, 1981, and reported to the Automated Data Processing (ADP) Contractor by May 7, 1982.

SCREENING OPERATIONS SECTION

DRUG EVALUATION BRANCH

The Screening Operations Section (SOS), Drug Evaluation Branch (DEB) implements and directs contracts for large scale primary screening against tumors in animals, refers to appropriate review groups those materials that meet criteria for activity in initial screens, conducts further tests of materials selected by review groups against a spectrum of animal tumors and human xenografts, establishes protocols for in vivo screening, and reviews and supervises activities of contractors. Results are presented to Division of Cancer Treatment (DCT) staff. Efficacy of compounds in in vivo experimental tumor models is the principal basis for recommending materials for development to clinical trial.

I. Staff Functions

As a consequence of internal DEB reorganization, functions of the former Screening Section were reassigned to the Office of the Chief and three new Sections: the Screening Operations Section, the Selected Agents Section, and the Cell Culture Section. In vitro screening contracts are now managed by the Cell Culture Section. Tumor panel test results as well as detailed drug studies are evaluated by the Selected Agents Section and Office of the Chief. However, the Screening Operations Section staff, either directly or through its Screening Services contractor (IIT Research Institute - contract CM9-7213) and in close collaboration with the Animal Genetics and Production Branch and the Information Technology Branch, has primary responsibility within DEB for the operational aspects of most in vivo screening tasks, for the management of program logisitcs, and for providing management reports of numbers of materials tested, numbers of tests conducted, etc. These are appended to this report (Tables 1-3).

Screening Operations Section staff served as Project Officers, during this report period, on two in vivo screening contracts; Arthur D. Little, Inc. (CMO-7346) and Institut Jules Bordet (CMO-7350), and the University of Miami in vitro screening contract (CM9-7290). These are now monitored by other DEB units.

Currently staff serve as Project Officers for four in vivo screening contracts: Battelle-Columbus Laboratories (CMO-7266 and CM9-7099), IIT Research Institute (CM9-7316), and Mason Research Institute (CM9-7317), which provide an annual test capacity of 110,000 L1210 equivalency tests in the P388 leukemia pre-screen and in eight tumor panel models: five murine tumor test systems (B16 melanoma, CD8F1 mammary adenocarcinoma, colon 38, L1210 leukemia, and Lewis lung carcinoma) and three human xenografts - mammary (MX-1), lung (LX-1) and colon (CX-1). SOS also monitored a screening services contract with IIT Research Institute (CM9-7213) to aid in the management and evaluation of screening data.

Staff collaborated with the other DEB units, the Natural Products Branch (NPB), and the Information Technology Branch (ITB), in the monitoring of screening related tasks performed under contract to these units; and

reviewed the biological effects of materials produced under additional DCT contracts for natural product development.

SOS contracts were closely monitored to assure strict adherence to testing protocols and the goals of the DTP, DCT, and NCI. The staff selected new assays for potential use as screening models, designed and refined screening protocols, evaluated test results, recommended materials for further pre-clinical development and prepared data summaries for presentation to appropriate decision making groups.

II. Significant Accomplishments

A. Determination of Antitumor Activity or New Agents

Transplantable mouse leukemia P388 remained the initial *in vivo* screen for most materials submitted for testing. Active materials from the pre-screen, or those which "by-pass" the pre-screen because of demonstrated alternative biological or biochemical properties were tested against the panel of eight experimental tumor models. Other tumors such as brain, renal, ovarian or bladder were used on a selective basis when appropriate. Materials regarded as potential candidates for clinical trial were examined, prior to large animal toxicology studies, in all of the tumor panel test systems.

Table 1 lists the number of tests conducted from April 1, 1981 through March 31, 1982 with various categories of materials including "regular" synthetic compounds, selected agents (SAC) which are chosen for further study because of activity in initial screening or other special interest, structural congeners of known active compounds (Analogs), and natural products both crude and fractionated. From April 1, 1981 through March 31, 1982, 144,865 *in vivo* tests were conducted (Table 1), a slight reduction from the 146,693 tests done during the previous year. Testing of crude plant and animal products was discontinued in October, 1981, and we anticipate a sharp reduction in testing of natural product fractions. The decreased natural product testing is reflected in Table 1, the fourth quarter of 1981 and the first quarter of 1982.

Testing was initiated and results computer processed for 18,804 materials [13,369 synthetics and 5,435 natural products (Table 2)], as compared with a total of 22,483 during the previous year. The current counts reflect the date of initial testing and not the date of material accession. Table 2 also shows the average lag time between the date a test is initiated and the date that test results are computer processed. Lag time is greatest for selected agents because active materials are tested in multiple models and those undergoing specialized testing require more time for completion. The lower material count for the first quarter, 1982 is a consequence of reporting lag time rather than a decrease in the number of tests initiated.

Table 3 shows the number of tests carried out during this report period in individual tumor models including the DCT Pre-clinical Tumor Panel and ancillary models for discretionary specialized testing. Table 3

also shows the number of xenograft tests using the subcutaneous (H2) and subrenal capsule (G5) sites of tumor implantation. During the current year, routine panel testing included the subrenal capsule assay only. The subcutaneous site was used selectively.

In general, candidate drugs for pre-clinical toxicology are required to have testing completed in all panel systems. For this reason, compounds meeting DN-2 activity criteria received priority for completion in the tumor panel. During this year, 542 materials were completed in the tumor panel. As of March 31, 1982, 2,030 (excluding drops) had been selected for tumor panel testing. Of 332 entering the tumor panel third year, 274 were selected by the P388 pre-screen, 25 were recommended by committees such as analog coordinating groups and 33 were "by-passes".

B. Application and Verification Studies

During this past year, no major changes were made to existing protocols. However, additional toxicity criteria for certain tumor panel models were established. For example, the "Gram Negative Body Weight Change" for the C2G5 xenograft was established at $\geq$ at 8 grams for toxicity, and the "Gram Negative Body Weight Change" for the MBG5 xenograft was established at $\geq$ 6 grams. A study involving all the contract laboratories to determine the doubling time of B16 melanoma as used under current protocols is currently being analyzed. A re-evaluation of acceptable control survival times for P388 and L1210 was completed.

The final draft of the most recent protocols for tumor panel testing is currently under review prior to publication.

III. Related Projects

The Screening Operations Section, with the close cooperation of the Animal Genetics and Production Branch, maintained strict surveillance of all tumor lines and host animals to assure the uniformity of biological characteristics. Frozen tumor banks were maintained at several *in vivo* and *in vitro* screening laboratories as well as a major frozen tumor bank under contract to the Animal Genetics and Production Branch (AG&PB). All studies related to the diagnosis and control of animal disease, and general animal quality were supervised by AG&PB. SOS advised AG&PB of changing animal needs and AG&PB advised SOS of anticipated animal shortages or problems and remedies (e.g., changes of host animals).

SOS, as the major user of the biological automated data processing system, collaborated closely with the Information Technology Branch, to institute or modify programs for the processing of biological data.

IV. Proposed Course

The Screening Operations Section will continue to supervise the testing and preliminary evaluation of materials screened in the DTP Program. This

includes the continuous evaluation of the primary screen and its criteria, logistics, and tumor models. Research and methodology will be carried out at contract laboratories, directed towards the improvement of experimental tumor models and the evaluation of new models for potential usefulness as screens. Finalized protocols for use by all contract laboratories will be prepared.

TABLE 1

NUMBER OF IN VIVO SCREENING TESTS CONDUCTED
FROM APRIL 1, 1981, THROUGH MARCH 31, 1982*

	SELECTED AGENTS COMPOUNDS (SAC)		OTHER SYNTHETICS		NATURAL PRODUCTS			TOTAL
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	
1981								
Second Quarter	12,169	702	15,577	484	8,449	984	2,475	40,840
Third Quarter	10,742	1,486	16,114	616	7,534	2,264	2,211	40,967
Fourth Quarter	9,158	1,081	16,835	565	4,348	526	1,771	34,284
1982								
First Quarter	8,017	886	14,688	786	2,684	401	1,312	28,774
TOTAL	40,086	4,155	63,214	2,451	23,015	4,175	7,769	144,865

*Includes tests begun no earlier than April 1, 1981, and reported to the Automated Data Processing (ADP) Contractor by May 7, 1982.

TABLE 2

SUMMARY OF MATERIALS FIRST TESTED IN ANY SYSTEM FROM
APRIL 1, 1981, THROUGH MARCH 31, 1982*

	SELECTED AGENTS COMPOUNDS (SAC)			SYNTHETICS			NATURAL PRODUCTS				TOTAL ALL
	Synthetic	Analog	Total	Synthetic	Analog	Total	Plants	Animals	Fermen-tations	Total	
1981											
Second Quarter	124	31	155	3,863	81	3,944	1,459	104	270	1,833	5,932
Third Quarter	94	57	151	3,418	92	3,510	1,354	240	180	1,774	5,435
Fourth Quarter	84	45	129	3,314	93	3,407	471	14	354	839	4,375
1982											
First Quarter	30	20	50	2,388	120	2,508	226	190	88	504	3,062
TOTAL	332	153	485	12,983	386	13,369	3,510	548	892	4,950	18,804

Analysis of lag time between the date the tests were initiated at the laboratory (date-on) and the date the test results are processed reveals that the following percentages of the data have been processed in the first, second, and third months beyond the date-on month:

Month Beyond Date-On	SAC		Synthetics		Natural Products	
	Average	Range	Average	Range	Average	Range
First	40%	16% - 72%	64%	39% - 85%	75%	56% - 81%
Second	72%	45% - 99%	97%	91% - 99%	95%	87% - 99%
Third	79%	57% - 99%	99%	96% - 99%	98%	91% - 99%

*Includes compounds for which test results were processed by May 7, 1982.

TABLE 3

NUMBER OF TESTS CONDUCTED AGAINST SELECTED TUMOR SYSTEMS FROM APRIL 1, 1981, THROUGH MARCH 31, 1982*

Tumor Systems	Second Quarter 1981	Third Quarter 1981	Fourth Quarter 1981	First Quarter 1982	TOTAL
L1210 Lymphoid Leukemia (LE)**	2,532	1,844	1,474	1,275	7,125
B16 Melanocarcinoma (B1)**	1,210	1,284	1,103	1,047	4,644
Lewis Lung Carcinoma (LL)**	394	647	682	453	2,176
$CDBF_1$ Mammary Tumor (CD)**	629	673	684	550	2,536
Colon 38 (C8)**	696	739	413	671	2,519
M5076 Ovarian Carcinoma (M5)	109	24	0	44	177
CX-1 Colon Xenograft (C2G5)**	746	621	655	675	2,697
CX-1 Colon Xenograft (C2H2)	32	12	4	0	48
LX-1 Lung Xenograft (LKG5)**	808	699	600	625	2,732
LX-1 Lung Xenograft (LKH2)	22	8	0	0	30
MX-1 Breast Xenograft (MBG5)**	1,156	1,021	622	772	3,571
MX-1 Breast Xenograft (MBH2)	27	10	0	0	37
TOTAL ALL TUMOR SYSTEMS	8,361	7,582	6,237	6,112	28,292

*Includes tests begun no earlier than April 1, 1981, and reported to the Automated Data Processing (ADP) Contractor by May 7, 1982.

**Panel Tumor Systems

SELECTED AGENTS SECTION

DRUG EVALUATION BRANCH

I. Scope

The responsibilities of the Selected Agents Section (SAS) include the following:

1. Selection of compounds for further evaluation in the DCT panel of *in vivo* tumors and other secondary pre-clinical models.

2. Recommendation of those drugs warranting presentation as candidates for development to clinical trials.

3. Implementation of non-routine testing, and biological and biochemical evaluations on compounds of interest in order to facilitate progressive and timely development.

4. Preparation of reports on the pre-clinical therapeutic efficacy of new drugs for inclusion in the Investigational New Drug Applications (INDA) and clinical brochures.

II. Staff

The staff of the Selected Agents Section consists of two senior professionals, one junior professional and one secretary. The staff achieve the objectives of the Section by serving on committees and working groups involved with drug development (see Table 1), by presenting reports to these committees, and by serving as Project Officers for three contracts. The contracts monitored by the Section staff are CMO-7346 entitled "*In Vivo* Screening Program", CMO-7302 entitled "Screening and Detailed Evaluation of Antitumor Drugs and Combined Chemotherapy and Modality Studies", and CM1-7397 entitled "Biochemical and Biological Characterization of Antitumor Drugs". The contractor for each project is Arthur D. Little, Inc. In addition, the staff advise the Branch Chief on the technical performance of segments of multifaceted contracts managed by the Office of the Chief when the tasks conducted relate to functions of the Section. They participate in meetings related to the USA-USSR and USA-FRG Bilateral Agreements, and review Grant Applications (23 in this reporting period) for Program interest and contract overlap.

III. Significant Accomplishments

A. Selection of Compounds for Additional Antitumor Evaluation

The Pre-Screen Subcommittee (PS) of the Drug Evaluation Committee (DEC) is an interdisciplinary working group which is responsible for the review of screening data, chemical structural and other pertinent information for each compound demonstrating confirmed activity in the P388 leukemia "pre-screen". A consensus judgement is reached by the Sub-

committee on whether or not to assign a compound for special testing or for evaluation against the five murine tumors and three human tumor xenografts that constitute the current DCT Tumor Panel. In addition, PS reviews all data obtained from secondary tumor testing, and recommends compounds suitable for further development to DEC.

During this reporting period (April 1, 1981 - March 31, 1982), PS reviewed data for 1,764 compounds of which 982 were new confirmed actives (Table 2). The number of compounds assigned to the tumor panel (TP) was 332; 274 were new compounds selected on P388 activity, 30 "bypassed" the P388 pre-screen on the basis of known biological or biochemical action, 3 were assigned to TP because of unusual structure, and 25 were selected by analog coordinating groups and other DCT committees. During the same period, 29 compounds demonstrating Decision Network (DN) 2 level activity in at least one TP model were referred to DEC. Structural analogs of the 29 drugs identified by a computer search of the files also were referred to DEC to aid the decision-making process.

The DEC evaluates those compounds which meet the criteria for DN-2A, ensures that sufficient testing has been performed to recommend the compound for further development and may recommend additional testing such as evaluation against a resistant tumor. The Committee either recommends the compound for consideration by the Pre-Decision Network Committee (Pre-DN) or may defer a compound if the drug is deemed of insufficient interest.

During the time interval April 1, 1981, to March 31, 1982, DEC reviewed 69 compounds as potential Decision Network candidates. These included 28 compounds which were discussed for the first time, 17 compounds which were re-reviewed after additional testing had been completed or additional information had been gathered, and 24 other compounds with chemical structures closely related to those in the first two categories. Of the total discussed, 17 were referred to the Pre-DN Committee as potential Decision Network candidates (Table 2).

Eleven drugs meeting DN-2A criteria were presented by SAS staff to the DN Committee. Ten passed DN-2A and one, NSC-291520D, was recycled for additional information on its solubility and stability. In the previous year, 16 compounds passed DN-2A on the basis of screening activity. However, this does not represent a large drop in the selection of novel compounds as 8 of the 16 previously selected were analogs of Cis-platin or Doxorubicin. Table 3 lists the ten compounds selected for further development and indicates the basis for their selection. On March 23, 1982, Section staff presented summaries to the DN Committee on 11 previously selected DN-2A drugs. As a result of that review, 10 DN-2A compounds (NSC-146068, 212509, 233853, 256927, 262666, 263164, 266210, 267702, 298223, 327471D) were removed from further development by DCT.

B. Detailed Drug Evaluation

The SAS is responsible for detailed evaluation of drugs which pass DN-2A. Tasks are designed to (1) evaluate the effects of treatment

route and schedule on a drug's antitumor activity, (2) evaluate the activity of the formulated drug, (3) develop preliminary information on the mechanism of action of new agents under development, and (4) conduct non-routine studies in response to DCT Program needs.

1. Fourteen experiments with nine compounds in development to clinical trials and one compound of interest to EORTC were conducted to determine the effects of treatment route and schedule on antitumor activity. Of particular interest were the studies with NSC-284356 (an Ethenopyrrolocylobutisoindole derivative), NSC-286193 (Tiazofurin) and NSC-312887 (Fludarabine phosphate). Daily treatments with NSC-284356 were effective against both the I.P. and S.C. implanted L1210 leukemia whether the drug was administered I.P., I.V. or P.O. Tiazofurin demonstrated good activity against the I.P. implanted L1210 leukemia at several dosage levels following I.P., I.V. or P.O. daily treatment. Like Ara AMP, the 2-fluoro analog, Fludarabine phosphate, appeared to be more effective on a days 1,5, and 9 treatment schedule when the total dose administered was divided into 8 smaller doses given at three hour intervals. However, unlike Ara AMP, Fludarabine phosphate demonstrated good activity when administered daily on days 1-9.

2. Fourteen experiments comparing antitumor activity of bulk drugs and experimental clinical formulations were conducted on 7 agents in development toward clinical trial and one compound in clinical trial. In four cases, either the evaluation of more than one experimental form for an individual drug, or more than one experiment with the same formulation, was required to insure against loss, or significant reduction, of antitumor efficacy during the formulation process.

3. Studies to obtain basic information on the cytotoxic and and biochemical effects of new antitumor agents that aid DCT in deciding whether to develop drugs to clinical trial and in assigning priorities to those drugs under development were conducted under contract CM1-7397 which was awarded to Arthur D. Little, Inc., in September 1981 through the competitive process. To achieve the objectives of the contract, the contractor followed a series of in vitro protocols by which a biological profile of a new agent can be described rapidly. During the past year the contractor either initiated studies, or conducted follow-up studies, on 14 antitumor agents of interest to the Program. Three examples of these studies are summarized below.

   Initial experiments with Macbecin II (NSC-330500), an antibiotic related to Geldanamycin, suggest that it acts as a mitotic spindle poison. Macbecin produced mitotic arrest in P388 cells exposed to 0.27 μM drug for 16 hours (mitotic indices for drug-treated and control cells were 11.3 and 3.4%, respectively). However, the degree of arrest was not as profound as that obtained with 0.11 μM Vincristine (mitotic index = 39.7%). At 10 μM, Macbecin completely blocked both DNA and protein synthesis. The inhibition of protein synthesis was immediate while that of DNA synthesis was delayed by

30 min. With the same drug concentration, there was also a delayed partial inhibition of RNA synthesis.

Studies on NSC-305785, a Diaminoquinazoline derivative active against the murine colon 38 carcinoma, were conducted to determine whether the compound demonstrated biochemical characteristics which would differentiate it from Methotrexate and other antifols in development. NSC-305785 proved to be an 1,000-fold less potent inhibitor of Dihydrofolate reductase (IC_{50} 31 M) than Methotrexate (48 nM) and JB-11 (NSC-249008, IC_{50} 69 nM). Also, although it inhibited the growth of P388 cells in vitro it was 3,000-4,000 fold less potent than MTX and JB-11. As is the case with most antifols, its growth inhibitory properties were reversed by Leucovorin, and it inhibited Deoxyuridine incorporation by P388 cells. Therefore, although it is less potent than MTX, NSC-305783 appears to act as a classical antifol.

No leads as to the mechanism of action of NSC-284356, an Etheno-pyrrolocyclobutisoindole derivative with activity against the CD8F1 mammary carcinoma and the P388 and L1210 leukemias, have been found. Although NSC-284356 was growth inhibitory to P388 cells in vitro with an IC_{50} of 19 M, the drug did not kill P388 cells (< 1 log cell kill) at concentrations of up to 100 M with a 24-hr. exposure. There was no acute inhibition of DNA, RNA or protein synthesis in P388 cells exposed to 1 mM drug, nor were any effects observed on macromolecular synthesis following a 4-hr. exposure of the cells to drug prior to addition of labelled precursors. Also, NSC-284356 had no effect on the mitotic index of P388 cells. Neither L1210 nor KB cells were more sensitive to the agent. Studies are in progress to determine whether the drug requires metabolic activation.

4. Non-routine studies monitored by SAS were conducted by Arthur D. Little, Inc., under contract CMO-7302. The effort of this contract is divided among three principal activities: a) evaluation of congeners and prodrugs, b) detailed drug evaluation and special studies, and c) combination chemotherapy and chemotherapy/radiotherapy studies. Studies are conducted in conventional mice bearing leukemic or solid tumors.

   During the past year the contractor evaluated about 600 congeners and natural products in dose-response studies in various tumor systems (mainly P388, B16 and L1210). Most testing was conducted in direct comparison with a reference or parent compound in order to determine whether newly acquired compounds with related structures showed superior activity. During the past year the proportion of natural products tested on this contract increased to about 17 percent of the total. Some congeners of special interest, including several anthracenediones and fluoroethylnitrosoureas, received more detailed evaluation, such as testing in mice bearing advanced tumors or tumors implanted in various anatomical sites. In the special studies category the contractor determined the influence of dosage fractionation on activity and toxicity of Valinomycin

(NSC-122023) formulated in a lipid emulsion. These studies were decisive in influencing the Decision Network Committee to discontinue development of the agent, as fractionation did not significantly alter the narrow therapeutic index. Tiazofurin (NSC-286193) continued to receive more extensive evaluation. The contractor found that it retained activity in a PALA-resistant subline of Lewis lung, appeared active over a larger dose range than PALA (NSC-224131), and had activity against the I.C. implanted Lewis lung tumor. In combination chemotherapy studies the contractor found that Leucovorin reversed the activity of a Triazine antifol (NSC-127755) in mice bearing the S.C. M5076 ovarian tumor and that Uridine rescued 5-FU toxicity in mice bearing colon 26 tumors. Based on these latter studies a clinical trial of 5-FU with Uridine rescue was planned. The contractor also evaluated WR 2721 (NSC-296961), a radioprotector, in combination with several alkylating agents (Melphalan, Cyclophosphamide and BCNU), but could show no advantage over the use of the alkylating agents alone. During the past year studies involving combinations of drugs and radiation were phased out due to budgetary cutbacks and shifts in Program emphasis. In limited studies the contractor found no evidence that 5-thio-D-glucose (NSC-204984) or Copper hematoporphyrin (NSC-175462), an electron affinic compound which is thought to localize in tumors like Hematoporphyrin, behave as radiosensitizing agents in mice bearing S.C. B16 tumors. This contract, which has just entered its final year, continues to provide the Development Therapeutics Program with a resource for addressing problems which arise during the development of agents to clinical trial. This objective complements the goals of the primary screening contracts whose major focus is to detect activity of promising leads.

C. Summaries of Antitumor Activity for INDAs

Between April 1, 1981 and March 31, 1982, SAS summarized the preclinical therapeutic data for 7 compounds: Homoharringtonine (NSC-141633), Carboplatin (NSC-241240), Dihydro-5-azacytidine (NSC-264880), Teroxirone (NSC-296934), Echinomycin (NSC-526417), N-Methylformamide (NSC-3051) and Tiazofurin (NSC-286193). This represents a substantial increase over the previous year when one summary was prepared. The reports were submitted to the Investigational Drug Branch, CTEP, DCT, for inclusion in the IND applications and clinical brochures.

IV. Status of the Tumor Panel Experiment

The status of TP testing as of March 29, 1982, is summarized in Tables 4 and 5. Two thousand one hundred and sixty-four compounds had been assigned to the TP, but 134 had been dropped prior to completion of testing. Of the 2,030 remaining compounds, 1,231 (60.6%) had completed testing in all eight TP models, 542 between March 30, 1981 and March 29, 1982 (Table 4). However, as many other compounds had completed testing in most systems, the number of compounds with testing completed in each individual system was higher (Table 5). For example, approximately 80% of all compounds had

completed testing in the murine tumor models and 67-71% in the human tumor xenograft systems.

In June 1982, an analysis of TP results was presented to the DCT Board of Scientific Counselors. The analysis was based on 1,085 compounds completed in all models on April 2, 1982. The analysis excluded compounds which had been evaluated against the S.C. implanted human tumor xenografts but not the xenografts implanted under the renal capsule of athymic mice. Also excluded were compounds structurally related to 12 clinical agents (Tables 6-11). Table 6 summarizes, for each test system, the number of compounds meeting DN-2 criteria. To meet DN-2 criteria the compound has demonstrated sufficient activity to warrant consideration for presentation to the DCT Decision Network Committee as a candidate for development to clinical trial. The two most sensitive, models were the I.P. implanted L1210 leukemia and B16 melanoma. The two mammary tumors were moderately sensitive while the colon and lung tumors were relatively refractory to treatment.

Table 7 shows that the seven solid tumors in the panel identified compounds with antitumor activity that were missed by the L1210 leukemia. Of 979 compounds which failed to meet DN-2 activity criteria against L1210, 119 demonstrated DN-2 activity in another TP model. The distribution of this sub-set of active compounds was similar to that of the total compound population observed in Table 6. The total number of DN-2 actives for the seven tumors is greater than 119 because some compounds were active in more than one tumor system.

Table 8 shows that the human tumor xenografts identified DN-2 actives that were not identified by the murine tumors. Of 81 compounds with DN-2 activity in at least one of the three xenograft models, 26 were not active in any of the murine tumor panel models. The majority of the xenograft only actives were effective against the human mammary tumor xenograft.

Analysis of the data obtained with the murine and human colon, lung and mammary tumors indicated that the organ of tumor origin was not a factor in the identification of DN-2 active agents (data not shown).

As illustrated in Table 6, the testing of 1,085 compounds in all 8 TP systems identified 225 drugs demonstrating DN-2 level activity. Two hundred and twelve or 94% were uncovered by testing with just three tumors; the L1210 leukemia, the B16 melanoma and the MX-1 mammary tumor xenograft (Table 9). Lower yields of DN-2 active agents were obtained with all other 3-tumor sub-sets. All 13 compounds missed by L1210, B16 and MX-1 were active against only one of the remaining tumors; six were active against the murine mammary tumor, three against the murine colon tumor, and two each against the human colon and lung tumor xenografts (Table 10). There were no sole actives in the murine lung tumor model.

A positive correlation was found between the quantitative level of activity against the P388 leukemia "pre-screen" and probability of meeting DN-2 activity criteria in at least one of the TP models (Table 11).

Based on the preceeding analysis of the tumor panel, modifications to the flow of compounds through DCT pre-clinical screens were recommended. These recommendations have been summarized in preceeding pages of the Drug Evaluation Branch's Annual Report.

V. Proposed Course of Projects

The Selected Agents Section will continue to select compounds for secondary antitumor evaluation in pre-clinical models and implement non-routine testing in order to facilitate the progressive and timely development of compounds of interest to the clinic. Reports on the pre-clinical therapeutic efficacy of new drugs will be prepared for inclusion in the Investigational New Drug Applications.

TABLE 1

SAS STAFF PARTICIPATION ON DCT DECISION GROUPS, FY 1982

DCT Decision Network Committee
DCT Operating Committee
DTP Pre-Decision Network Committee
DCT Analog Development Committee
DEB Protocol Committee
DEB Drug Evaluation Committee
DEB Pre-Screen Subcommittee*
DCT Anthracycline Coordinating Group
DCT Project to Review Old Drugs

*Chaired by SAS senior staff member.

TABLE 2

STATUS OF ACTIVE COMPOUNDS
April 1, 1980 - March 31, 1981

New confirmed actives	982
Compounds assigned to tumor panel	332
Compounds with DN 2 activity referred to DEC	29
Compounds with DN 2 activity referred to Pre-DN	17
DN 2A candidates recommended to DN	11
Compounds passed DN 2A	10

TABLE 3

COMPOUNDS SELECTED FOR DEVELOPMENT
April 1, 1981 - March 31, 1982
(Decision Network 2A)

NSC No.	Name	Assignment date	Active Systems*
261726	3-deazaguanine, 4H-Imidazo [4,5-c] pyridin-4-one, 6-amino-1,5-dihydro-	81-05-05	s.r.c. MX-1 mammary tumor xenograft
267213	Acetic acid, [[(4-methoxy-phenyl) sulfonyl] hydra-zono]-	81-05=-5	i.p. L1210 leukemia s.r.c. MX-1 mammary tumor xenograft
281272	Ara-5-AC. 1,3,5-Triazin-2-(1H)-one, 4-amino-1- -D-arabinofuranosyl	81-09-22	i.p. L1210 leukemia i.v. Lewis lung carcinoma s.r.c. CX-1 colon, LX-1 lung, and MX-1 mammary tumor xenografts
293015	4H-1-Benzopyran-8-acetic acid, 4-oxo-2-phenyl-, 2-(diethylamino)ethyl ester, hydrochloride	81-06-17	s.c. Colon 38 tumor
303861	(Discreet)	82-02-17	s.r.c. MX-1 mammary tumor xenograft
322921	Phenol, 4-[5-(4-methyl-1-piperazinyl) [2,5'-bi-1H-benzimidazol]-2'-yl]-	81-09-22	i.p. L1210 leukemia
325014	Bactobolin		i.p. L1210 leukemia
328426	Phyllanthoside	81-09-22	i.p. B16 malanoma
330500	Macbecin II	81-09-22	i.p. B16 melanoma
333856D	(Discreet)	82-02-17	i.p. B16 melanoma

*Tumor panel systems in which a compound (non-formulated product) has demonstrated sufficient antitumor activity to warrant consideration for development to clinical trial (DN 2 activity).

TABLE 4

TESTING STATUS OF TUMOR PANEL (TP) COMPOUNDS (MARCH 29, 1982)

Total number of compounds entered into TP	2164
Number dropped prior to completion of testing	134
Number of TP compounds excluding "drops"	2030
Total number completed in all TP test systems	1231
Number completed between March 30, 1981 and March 29, 1982	542

TABLE 5

TESTING STATUS BY TEST SYSTEM FOR 2030 TUMOR PANEL COMPOUNDS
(March 29, 1982)

Test System	Completed Testing No. (%)
Murine Tumors	
B16 melanoma	1618 (80)
$CD8F_1$ mammary carcinoma	1614 (80)
Colon 38	1610 (79)
L1210 leukemia	1650 (81)
Lewis lung carcinoma	1594 (79)
Human Tumor Xenografts (Renal Capsule Assay)	
CX-1 Colon	1437 (71)
LX-1 Lung	1389 (68)
MX-1 Mammary	1356 (67)

TABLE 6

NUMBER OF COMPOUNDS MEETING DN 2 ACTIVITY CRITERIA AGAINST TUMOR PANEL MODELS*

Number of compounds with testing complete in all panel systems on April 2, 1982 = 1085*

Number of compounds with DN 2 activity in at least one system = 225 (20.7%)

Murine Tumor	Number of DN 2 Actives	Human Tumor Xenograft	Number of DN 2 Actives
L1210	106		
Melanoma	106		
Mammary	37	Mammary	71
Colon	15	Colon	9
Lung	5	Lung	16

* Excludes 96 compounds structurally related to 12 clinical compounds: Adriamycin, Actinomycin D, Ara C, Bleomycin, Cis-platin, Cytoxan, 5-FU, Methotrexate, Mitomycin C, Nitrogen mustard, Nitrosoureas, Vincristine. Excludes compounds which have been tested against the s.c. implanted xenografts but not the xenografts implanted under the renal capsule of athymic mice.

TABLE 7

IS THE TUMOR PANEL SELECTING AGENTS FOR CLINICAL TRIAL THAT WOULD HAVE BEEN MISSED BY SCREENING WITH L1210 LEUKEMIA ALONE?

Number of compounds with less than DN 2 activity against L1210 = 979*

Number with DN 2 level activity in some other tumor panel model = 119 (12.1%)

Murine Tumor	Number of DN 2 Actives	Human Tumor Xenograft	Number of DN 2 Actives
Melanoma	78		
Mammary	18	Mammary	45
Lung	1	Lung	10
Colon	5	Colon	9

* 979 of 1085 compounds with testing complete in all tumor panel models.

TABLE 8

ARE THE HUMAN TUMOR XENOGRAFTS AND MURINE TUMORS SELECTING THE SAME OR DIFFERENT DRUGS?

Number of compounds with DN 2 activity in at least one of the xenograft models = 81

Number not demonstrating DN 2 activity in the murine tumor panel models = 26 (32.1%)

	Number of DN 2 Actives In Xenografts Only	
Xenograft	Clinical	Pre-Clinical
Mammary only	2	16
Lung only		2
Colon only		2
Mammary + Colon		1
Mammary + Lung	1	
Mammary + Lung + Colon		2

TABLE 9

YIELD OF DN 2 ACTIVE COMPOUNDS FROM SUB-POPULATIONS OF THE TUMOR PANEL

Tumors	Number of DN 2 Actives	Percent of All DN 2 Actives
All eight	225	100
L1210, B16, MX-1	212	94.2
L1210, B16	184	81.8
L 1210, MX-1	151	67.1
B16, MX-1	149	66.2

TABLE 10

NUMBER OF COMPOUNDS WITH DN 2 ACTIVITY
IN ONLY ONE TUMOR PANEL MODEL

Murine Tumor	Number of DN 2 Actives	Human Tumor Xenograft	Number of DN 2 Actives
Melanoma	55		
L1210	44		
Mammary	6	Mammary	18
Colon	3	Colon	2
Lung	0	Lung	2

TABLE 11

VALUE OF P388 PRE-SCREEN FOR PREDICTING DN 2
LEVEL ACTIVITY IN THE CURRENT DCT TUMOR
PANEL. RELATIONSHIP TO DEGREE OF P388 ACTIVITY

P388 T/C%	No. Tested in TP	No. (%) Active in At Least One TP Model
<120 (Inactive)	118	7 (6)
120 - 129	370	17 (5)
130 - 149	329	47 (14)
150 - 169	123	45 (37)
170 - 199	78	53 (68)
≥ 200	67	56 (84)
	1,085	225 (21)

ANNUAL REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 to September 30, 1982

The Pharmaceutical Resources Branch 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 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 storage, distribution and computerized inventory maintenance of all drug products used in the Clinical Programs.

TABLE 1

RESOURCE CONTRACTS MONITORED BY THE PHARMACEUTICAL RESOURCES BRANCH

Chemical Preparation	7
Radiolabel Synthesis	2
Pharmaceutical Production	5
Pharmaceutical Research and Development	2
Analytical Services	2
Drug Distribution	1
Total Resource Contracts	19

The Branch is also responsible for direct purchase of chemicals and drug products utilizing NIH procurement services.

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

Staff

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

The Branch consists of three Sections:

1. Chemical Resources Section - Head, Dr. Robert R. Engle

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: (a) resynthesis assessment; and (b) pilot plant production of various quantities of bulk substances intended for pharmaceutical manufacture of clinical investigational products. The Section also initiates the acquisition of commercially available chemical substances through NIH procurement contract procedures. This requires the preparation of stringent material specifications to insure that high quality products are obtained.

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.

This Section supervises seven chemical prep lab contracts and two radiolabel synthesis contracts.

2. Clinical Products Section - Head, Mr. Larry M. Kleinman

The Clinical Products Section is responsible for production, procurement and distribution of pharmaceutical dosage forms for clinical trial. The activities of the Section involve multi-contract responsibilities and management of several million dollars in drug acquisition purchases.

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

The Clinical Products Section is responsible for the monitoring of investigational product expiration dates and the recall of products, as necessary, when chemical, biological or physical changes occur.

Investigatonal 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. 82-2141) which is updated periodically. During this reporting period, over 2,000 issues were distributed.

3. Analytical and Product Development Section - Head, Mr. James C. Cradock

This Section is responsible for the quality assessment of chemicals and formulated investigational drugs used in the Division's cancer treatment. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. This Section is responsible for 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 analytical component of this Section is also responsible for chemical evaluation of new investigational dosage forms and for the shelf life surveillance of all formulated investigational products.

A second major function of this Section involves the development of suitable pharmaceutical dosage forms for clinical trial. Since most preparations are intended for intravenous use, studies are undertaken to assess the solubility and stability in a variety of pharmaceutical vehicles. New approaches to enhance solubility are emphasized since few suitable methods are available to the formulator of parenteral products.

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

The Analytical and Product Development Section is responsible for the supervision and managment of six contracts: two analytical contracts; two pharmaceutical research and development contracts; one small-scale production contract; and one contract for characterization and evaluation of proteinaceous substances. The protein substances contract is monitored by the Section, but funded by the Biological Response Modifiers Program.

Goals and Accomplishments

The Branch successfully accomplished its objectves during this reporting period by providing efficient chemical and pharmaceutical services to the Programs of the Division of Cancer Treatment. For example, the extramural contract program under Branch staff supervision provided over 308 kilograms of synthetic chemical substances for subsequent formulation into investigative clinical products. Among the high priority chemical substances provided include: Tiazofurin (NSC-286193), 15 Kg; Methotrexate (NSC-740), 38 Kg; N-Methylformamide (NSC-3051),

10 Kg; WR-2721 (NSC-296961), 32 Kg; Dihydro-5-azacytidine (NSC-264880); and others.

In addition to the delivery of large batch preparations, the Chemical Resources Section assigned 110 panel compounds for resynthesis. The delivery and inventory of panel compounds was coordinated with the Drug Synthesis and Chemistry Branch and an effective monitoring system for these substances now exists. During this reporting period, the Chemical Resources Section also monitored two radiosynthesis contracts with the procurement of 17 radiolabeled substances.

The Analytical and Product Development Section continued to provide superior analytical contract services to the Program. In addition to the evaluation of chemical and formulated products, the analytical contractors assisted the Natural Products Branch with analytical characterization of substances they received. The Drug Synthesis and Chemistry Branch has also been assisted with similar problems of analytical characterizaton and evaluation.

During this reporting period, the Analytical and Product Development Section has initiated the implementation of a program for analytical methods validation. These methods provide measures of the accuracy, precision, specificity and sensitivity of the assay procedure applied for quality control release of new agents. The implementation of this program is a major undertaking involving re-evaluation of all previous procedures and development of new procedures, as needed.

Another accomplishment during this reporting period involved completion of a computerized reference sample file and a bulk drug container inventory for substances intended for pharmaceutical manufacture. This system provides better control of bulk pharmaceutial substances intended for eventual clinical use as required under the Current Good Manufacturing Practices of the Food and Drug Adminstration.

Dosage form development assignments during this reporting period were especially heavy and the formulation problems particularly complex. A number of difficult formulation problems were successfully completed and involved the use of multiple vehicle systems. Some of the products developed for toxicology and subsequent clinical trial included: Menogarol (NSC-269148); N,N-Bis(2-hydroxyethyl-2-nitro-1H-imidazole (NSC-314055); Dow Compound (NSC-253272); Norwich Compound (NSC-305884); Taxol (NSC-125973); and others.

The pharmaceutical production activity has successfully maintained a clinical inventory of high quality investigational products for the Division of Cancer Treatment Program. The contractors under the supervision of the Clinical Products Section produced over 431,000 injectable units and over one million oral units for clinical trial. Several products of continued high interest include: AMSA, AZQ, Methotrexate and Hexamethylmelamine.

A system of "Notification of Expiration" was implemented during this reporting period to recall Phase I drugs not bearing expiration dates on the label. Only those products with shelf life experience have expiration dates on the label. The notification system is necessary for clinical product monitoring and control as required by the Food and Drug Administration.

The Quality Control Committee for Bulk Chemicals and Drugs, which is comprised of senior staff members in the Branch, played a key role in reviewing analytical and manufacturing reports on bulk items and formulated items. This Committee has invested considerable effort in developing product specifications for new investigational materials.

The Branch continues to meet all new challenges with interest and enthusiasm. Our objective is to be prepared to meet all challenges and to maintain a contract capacity to provide efficient and productive pharmaceutical support to the Division of Cancer Treatment Program. The national distribution program for the antiemetic Delta-9-THC continues to operate without major complications. One disappointment has been the inability to synthesize large batch quantities of high quality THC in bulk form. However, a breakthrough in the preparative technology is anticipated within months.

During the past year, the Pharmaceutical Resources Branch contributed to the filing of six new IND filings to the Food and Drug Administration. The drugs were: Homoharringtonine (NSC-141633); Tricyclic Nucleotide (NSC-286193); Dihydro-5-azacytidine (NSC-264880); Echinomycin (NSC-526417); Henkel's Compound (NSC-296934); and CBDCA (NSC-241240).

The increased emphasis on analytical methods validation and the increasing number of poorly soluble and stable active agents have required an increase in contract support in these areas. The additional funding for automated analytical equipment and additional manpower support will hopefully reduce analytical backlogs and expedite the product development process.

In order to comply with all Food and Drug Administration regulations for investigational drugs, a new contract project is being sought for the shelf life surveillance of all clinical products. This contract will be monitored and supervised by the Analytical and Product Development Section and the reports from the contractor will be filed with the FDA as required.

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CHEMICAL RESOURCES SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The function of the Chemical Resources Section is to procure bulk chemicals and drugs for confirmational and other testing, toxicological and pharmacological evaluation, and clinical trials in humans; and to procure radiolabeled materials for pharmacology, clinical pharmacology and related studies. The function of the Section is achieved through the management and supervision of a contract program for the preparation of various quantities of bulk chemicals and drugs and radiolabeled materials, and the acquisition of commercially available chemical substances.

Staff

The staff of the Chemical Resources Section consists of one professional person and a secretary. The professional staff member serves as the project officer on the various contract activities of the Section.

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not readily available in the quality or quantities needed from the original supplier or on the open market.

These laboratories are also used to obtain data for the preparation of the necessary quantities of clinically important chemicals and to develop the most economical means for their preparation. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies and cost data are also provided by these contractors.

The increased emphasis in clinical investigations and the increased effort being devoted to toxicological studies have resulted in the need for larger quantities of the compounds prepared by this program. Also, the decision to evaluate a larger number of compounds demonstrating confirmed activity in the initial screening in the tumor panel has also resulted in the need for larger quantities of the compounds being synthesized for preclinical evaluation. This change in emphasis has been handled by the preparation laboratories without difficulty.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk Chemicals and Drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for five major reasons: (1) clinical investigations; (2) preclinical toxicological and pharmacological evaluation; (3) maintaining a stock of chemicals of clinical and preclinical interest; (4) completion of screening or confirmational testing; and (5) use as intermediates in further synthesis. The compounds so chosen are placed on priority lists based upon their relative importance, with clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from one gram to multikilograms. Factors governing the amounts depend upon: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the patient in the quality of the drugs chosen.

The Chemical Resources Section, through the preparative laboratories, directed the resynthesis of 218 compounds totaling 320 kilograms during this report period. Included in this group were 32 compounds scheduled for or undergoing clinical evaluation totaling 308 kilograms and 110 panel compounds. In addition to the compounds resynthesized, 44 new compounds were also prepared by the preparative laboratories. The Section also purchased six compounds scheduled for or undergoing clinical evaluation totaling more than 274 kilograms.

During this report period, large quantities of N-Methylformamide (NSC-3051) (10 Kg); Methotrexate (NSC-740) (38 Kg); Cycloleucine (NSC-1026) (62 Kg); PALA (NSC-224131) (16 Kg); WR-2721 (NSC-296961) (32 Kg); Tiazofurin (NSC-286193) (15 Kg); PCNU (NSC-95466) (9 Kg); and the SRI Radiosensiziter, SR-2508 (NSC-301467) (5 Kg) were prepared. In addition, the preparation laboratories responded rapidly to the preparation of large amounts of several other high priority materials needed by the Program such as NSC-261036 (Desmethylmisonidazole); NSC-321887 (2-Fluoro-ara-AMP); NSC-283162 (Trimethyl-trimethanolmelamine); NSC-284356 (the Gulf Oil Compound); NSC-241240 (CBDCA); NSC-267213 (the SRI Sulfonylhydrazone); and NSC-139490 (5-Methyltetrahydrohomofolic Acid).

The Section directed seven contract laboratories for the preparation of materials needed by the Program (Table 2).

TABLE 2

CONTRACT LABORATORIES FOR PREPARATION OF CLINICAL AND EXPERIMENTAL COMPOUNDS

Contractor	Investigator	Contract No.
Aerojet Strategic Propulsion Company	Olsen	NO1-CM-17490
Aldrich Chemical Company	Black	NO1-CM-17492
Ash Stevens, Inc.	Markovac	NO1-CM-17488
Monsanto Research Corporation	Ellard	NO1-CM-97255
Pharm-Eco Laboratories, Inc.	Draper	NO1-CM-17487

Contractor	Investigator	Contract No.
Starks Associates, Inc.	Parsons	NO1-CM-17374
Warner-Lambert Company	Pattison	NO1-CM-17491

2. Radiolabeled Materials

The Section coordinates the procurement and distribution of radiolabeled chemicals and drugs for pharmacological and drug distribution studies. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radioactive materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement and/or shipment. All materials are checked for purity prior to shipment and repurified, if necessary, before shipping. A total of 21 radiolabeled materials have been procured from all sources. Of these, 17 were labeled compounds prepared by the two radiosynthesis contractor laboratories including DON (NSC-7365); DHAQ (NSC-279836); AZQ (NSC-182986); Adriamycin (NSC-123127); WR-2721 (NSC-296961); the Isopropyl Pyrrolizine Derivative (NSC-278214); Misonidazole (NSC-261036); and Tiazofurin (NSC-286193). One hundred thirty-four shipments of radioactive substances were made during this report period.

The Section directed two contracts devoted to the procurement of radiolabeled materials (Table 3).

TABLE 3

CONTRACT LABORATORIES FOR
PREPARATION OF RADIOLABELED MATERIALS

Contractor	Investigator	Contract No.
Research Triangle Institute	Kepler	NO1-CM-97313
SRI International	Leaffer	NO1-CM-97256

CLINICAL PRODUCTS SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The responsibilities of the Clinical Products Section include the following:

1. Production, packaging and labeling of investigational dosage forms;
2. Procurement by direct purchase of formulations for clinical use;
3. Shelf life surveillance of all clinical dosage forms;
4. Preparation of FDA-IND attachments 1, 2, 3, 4, 5 and 7 for subsequent submission to the Food and Drug Administration;
5. Preparation of Investigational Drug - Pharmaceutical Data sheets for Clinical Brochures, IND's, etc.;
6. Management of a computerized clinical drug inventory system; and
7. Storage and distribution of clinical dosage forms.

Staff

The staff of the Clinical Products Section consists of two senior professionals and one purchasing agent. The professional staff members are PHS Commissioned Corps Pharmacists and serve as project officers on the various contract activities of the Section.

Production and Packaging of Investigational Drugs

The Section has the responsibility to provide adequate quantities of formulated drugs to the Clinical Programs. The production of investigational dosage forms is mainly performed by contract agreements with qualified pharmaceutical firms.

The formulation contractors produced a combined total of 431,862 parenteral doses and 49,229 bottles of tablets and capsules (1,415,500 oral doses). Production output which is dictated by clinical needs remained constant during this report period. The production contractors have been able to meet the Program's needs for a variety of formulated products. Six contracts were monitored by the Clinical Products Section.

TABLE 4

CONTRACTS MONITORED BY THE CLINICAL PRODUCTS SECTION

Contractor	Investigator	Contract No.
Banner Gelatin Products Corporation	Chaisson	NO1-CM-17402

Contractor	Investigator	Contract No.
Ben Venue Laboratories, Inc.	Wickes	NOl-CM-27508
Elkins-Sinn, Inc.	Reese	NOl-CM-67103
Flow Laboratories, Inc.[1]	Miller	NOl-CM-17398
Philips Roxane Laboratories, Inc.	Boeh	NOl-CM-67053
Yamanouchi Pharmaceutical Co., Ltd.	Kawata	NOl-CM-97307

[1]Responsible for clinical drug storage and distribution and operation of a computerized clinical drug inventory system.

Several investigational products are provided by pharmaceutical companies on a cost-sharing basis. These products are usually provided in return for access to clinical data generated by the studies. The dosage forms are prepared from specifications which are established by mutual agreement between the Branch and the manufacturers. During the period of this report, the following companies supplied a total of 46,144 parenteral units as well as 144,374 tablets and capsules representing 5,682 bottles as part of this joint effort:

Ayerst Laboratories (USA)	NSC-226080	Rapamycin
Dome Laboratories (USA)	NSC-178248	Chlorozotocin
Farmitalia Laboratories (Italy)	NSC-123127	Adriamycin
Hoffmann-La Roche (USA)	NSC-261036 NSC-261037	Desmethylmisonidazole Misonidazole
Janssen Pharmaceuticals (Belgium)	NSC-177023	Levamisole
Lederle Laboratories (USA)	NSC-301739	Mitoxantrone
Pfizer Inc. (USA)	NSC-331615	Levonantradol
Research Industries (USA)	NSC-763	Dimethylsulfoxide
Unimed (USA)	NSC-192965	Spirogermanium
Wellcome Foundation (England)	NSC-220537	C. Parvum

Purchase of Dosage Forms

Another function of the Section involves the direct purchase of formulated products. These items fall into two categories: (1) investigational products; and (2) commercial products. Investigational products are usually manufactured according to NCI specifications and sold to NCI on a cost-reimbursement basis. Commercial products are items available for open purchase. Procurement of all direct purchased dosage forms is handled through the NIH Procurement Branch.

Several procurement mechanisms are used depending on such factors as the type of product, the quantiy required and the priority for clinical need.

A total of 438,834 clinical dosage forms (injectable and oral products) were purchased at a cost of $2,614,339 during this report period. An additional sum of $124,902 was used for the acquisition of bulk chemicals and natural products. All suppliers are required to provide a certificate of analysis on each lot of drug purchased. Bulk chemicals and natural products are assayed by NCI contractors to ascertain whether or not requested specifications are met. The data are reviewed by the Quality Control Committee prior to release for clinical use or formulation, respectively.

The table below summarizes the procurement activities of the Clinical Products Section.

TABLE 5

DRUG ITEMS PROCURED

	Capsules/Tablets	Injectables
Produced by Contractors	1,415,500	431,862
Contributed or Cost-Sharing	144,374	46,144
Purchased Investigational Units	0	299,076
Purchased Commercial Units	101,250	38,508
	1,661,124	815,590

The Clinical Products Section is responsible for the disbursement of funds from Common Account Number (CAN) 2-8322759. This account is used for the acquisition of products intended for clinical trial which include natural products, bulk chemicals and clinical products. The operational budget for FY-1982 is $3,000,000. This figure was lower than past years because of the stoppage of distribution of commercial drugs.

Quality Assessment of Clinical Dosage Forms

Each lot of formulated drug must pass quality assessment criteria prior to release for clinical trials. The Clinical Products Section, in collaboration with the other two Sections in the Branch, maintains a strict assessment program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., while oral formulations are tested for potency, content uniformity, weight variation, hardness, disintegration, dissolution, etc. All products are tested for potency by the formulator with random testing being conducted by an analytical contractor. All new dosage forms and all first production batches of a new formulator are routinely submitted to an analytical contractor to verify the accuracy of the formulator.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of parenteral investigational dosage forms. These studies are designed to simulate the conditions found in the clinical setting. The data generated is assembled and is summarized in the format of an Investigational Drug - Pharmaceutical Data sheet which is incorporated into the Clinical Brochure.

The Clinical Products Section maintains a shelf life surveillance program on all lots of contractor produced investigational dosage forms. Random samples of each lot are stored under specified controlled conditions and are assayed for chemical and/or physical change. The stability schedule has been modified to conform to guidelines proposed by the Food and Drug Administration to establish an expiration date for a product. Three lots of a product will be followed under accelerated conditions (Schedule A). These lots will be followed for a period of four years. In addition, a sampling of each lot of drug produced by a contractor will be retained for two years beyond its expiration date. This will insure that an adequate number of samples are available to repeat any testing required.

Storage and Distribution of Clinical Dosage Forms

The Clinical Products Section administers a contract for the storage and distribution of clinical drugs. During 1981, the contractor prepared 14,582 drug shipments--an average of 58 shipments each day. This represents approximately 87,600 bottles of tablets and capsules and 978,500 vials and ampules for the year. As the implementation of the THC Program has progressed over the last several months, the volume of drugs distributed has been impacted. The average number of orders has increased 20% to approximately 60 each day. The system has functioned very smoothly.

To provide efficient processing of orders and also to provide the necessary inventory control, the computerized Drug Distribution Inventory System has processed each of the 14,582 orders, imputing the required information into the data bases. A total of 520 reports (mostly the routine daily drug inventories) were generated during 1981. Of these, 41 were special one-time queries for such items as recalls due to IND closings and cost determinations for specific drugs or specific investigators. Additionally, a number of improvements to the computer programs were implemented.

Preparation of Investigational Drug - Pharmaceutical Data Sheets

The Clinical Products Section is responsible for the preparation of pharmaceutical data sheets for all investigational dosage forms used in the Program. The data sheets are used in the preparation of Clinical Brochures and are compiled and distributed to health-care practitioners in a book entitled "NCI Investigational Drugs - Pharmaceutical Data". These data sheets provide product descriptions, reconstitution and stability information, and precautions in handling the products. These data sheets are updated as new stability data become available.

FDA-IND Submissions

The Clinical Products Section also prepares Investigational New Drug (IND) Attachments 1, 2, 3, 4, 5 and 7 (labels) for submission to the Investigational

Drug Branch, CTEP, DCT, NCI, for FDA-IND filing. These attachments contain manufacturing, control, packaging and labeling data required for the investigational dosage forms. During this report period, the Section prepared attachments for several original IND's and numerous amended IND's.

Significant Accomplishments

The Clinical Products Section has initiated a "Notification of Expiration" whereby after an appropriate time interval has transpired, initial batches of Phase I drugs are recalled. This system is required because the initial batches do not bear an expiration date since adequate shelf life data are not available at the time of manufacture.

A number of improvements have been made by the production contractors to the existing formulations of several drugs. AZQ (NSC-182986) is currently dissolved in hot ethanol prior to low temperature vacuum drying. Five hours are required to achieve 100% dissolution of AZQ in hot ethanol. The process does not readily lend itself to large-scale production. Solution studies were carried out in which the AZQ was dissolved in dimethylacetamide (which is one of the diluents with which AZQ is packaged). The solution was placed into ampules and set-up on a stability schedule. After one year stored at refrigeration temperatures, the AZQ in dimethylacetamide has not undergone any decomposition. This new approach will be utilized for subsequent batches.

It is always the intent of the production contractors to try to improve formulations so that the best possible product is available for clinical trials.

A new formulation approach was also developed for Homoharringtonine (NSC-141633). The initial approach utilized low temperature vacuum drying from ethanol. The product was then reconstituted with ethanol and further diluted in a suitable infusion solution. The new approach utilized freeze drying from an aqueous solution after formation of a more water soluble salt.

The Clinical Products Section has continued to develop a dialog with pharmacists, physicians and other health-care practitioners participating in clinical oncology programs at the various cancer centers. This dialogue has resulted in a timely exchange of information beneficial to both parties. This information is especially important with drugs undergoing Phase I and Phase II clinical trials.

This activity involved the distribution of over 2,000 NCI Investigational Drug - Pharmaceutical Data books and the handling of 20-25 inquiries per week. During FY-1982, the Section had the book revised. The response from the field has been very enthusiastic.

ANALYTICAL AND PRODUCT DEVELOPMENT SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The analytical chemistry and dosage form development activities are merged into one Section within the Pharmaceutical Resources Branch. Major responsibilities of the Section fall into two distinct categories:

1. Analytical Chemistry

   a. Develop suitable methodology for the analysis and characterization of bulk chemicals and pharmaceutical dosage forms.

   b. Validate the primary method of assay to insure proper application and to establish its stability-indicating capability.

   c. Assess the quality of bulk chemicals and pharmaceutical dosage forms used in the Developmental Therapeutics Program.

   d. Develop specifications to aid in routine quality control of bulk chemicals and for use in procurement actions for these materials.

   e. Serve as a repository for all methods of preparation and analytical characterization of bulk chemicals.

2. Dosage Form Development

   a. Characterize the pharmaceutical properties of new chemical agents, develop dosage forms and evaluate any chemical and/or physical changes under simulated use conditions.

   b. Develop new approaches for enhancing solubility and stability of selected antitumor agents.

Staff

This Section is staffed by one pharmacist, one analytical chemist, a visiting associate and a visiting fellow.

Analytical Chemistry

The development and preparation of drug products for clinical investigation requires the support of extensive analytical testing. Rigorous assessment of identity and purity beginning with the bulk chemical and continuing through to the finished dosage form is essential to insure that these materials are of high quality. When a bulk chemical is received for development, analytical methods are devised by the contract laboratories to confirm the identity as well as to evaluate the purity of the material. Solubility and stability studies are performed to provide information to aid in dosage form development.

After analytical data have been compiled on several lots, bulk chemical specifications are established to aid in the qualtiy control of these materials. These specifications are also used as minimum acceptance criteria for bulk chemicals purchased on the open market on a bid basis.

The pharmaceutical dosage form is similarly examined to establish identity and purity. In addition, content uniformity and weight variation tests may be performed as described in the United States Pharmacopeia XX.

Analytical data compiled on the bulk drug substance and the pharmaceutical dosage forms are submitted to the Food and Drug Administration as a part of IND applications filed by the National Cancer Institute.

The determination of the purity of a new chemical entity is often a complex problem. Consideration must be given to drug stability and the methodology developed must be capable of determining the drug in the presence of decomposition products or impurities. Methods commonly used include: spectroscopy (ultraviolet, visible infrared and nuclear magnetic resonance); chromatography (paper, thin layer, high pressure liquid and gas liquid); elemental analysis; and, when appropriate, optical rotation, refractive index, Karl Fischer water analysis, etc.

During this year, 153 lots of bulk chemicals and clinical formulations were evaluated by the contract laboratories for identity and purity. Also, numerous stability and solubility studies were completed on bulk drugs. In addition to the more routine analytical assignments, additional specific projects are occasionally necessary. Both contractors (Table 6) performed additional studies in response to FDA inquiries regarding analytical data included in IND filings. Responses were submitted to FDA questions regarding Aclacinomycin A, WR-2721 and Desmethylmisonidazole. Work is currently in progress to provide responses to questions regarding the chemistry of Homoharringtonine, 5-Methyltetrahydrohomofolate, CBDCA and Tricyclic Nucleotide.

During the past year, data from the analytical contractors supported six new IND filings with the FDA. These drugs were Homoharringtonine, Tricyclic Nucleotide, Dihydroazacytidine, Echinomycin, Henkel's Compound and CBDCA.

Recently, several changes have been implemented that impact on the analytical chemistry area. Perhaps most significatnt is the implementation of a program for analytical methods validation. At the direction of the project officer, the analytical contractors now perform additional work and prepare a separate report detailing validation of the primary assay method. Validation data are now generated whenever a new assay method is developed. These studies provide measures of the accuracy, precision, specificity and sensitivity of the assay method. The data generated provide evidence to support the suitability of a particular assay procedure for its intended use. Validation data should also aid other contractors (toxicology, formulation, production, etc.) who require the use of assay methods during their respective studies.

The analytical chemist and other members of the Pharmaceutical Resources Branch have worked closely with staff members of the bulk drug repository at Flow Laboratories to develop a computerized inventory system for the control of drugs intended for clinical use. This system is necessary to provide the more rigid

inventory control required by the Current Good Manufacturing Practices (CGMPs) regulations issued by the Food and Drug Administration. The system is now operational. This "GMP Inventory" also maintains inventory records for bulk drug lots designated as Analytical Reference Samples.

During the past year, less emphasis has been placed on the preparation of "Chemical Information Sheets" which summarize the chemical and physical data for each compound. Some of this data are now provided in the expanded "Chemical Information" portion of the Clinical Brochure.

In the future, we plan to provide this "Chemical Information" (assay methods, solubility and stability data, etc.) as part of the Pharmaceutical Resources Branch's annual publication which summarizes pharmaceutical data on investigational drugs provided by NCI. It is hoped that this approach will make the information more widely available to workers in the field.

Analytical services are provided by the two analytical-quality control contract laboratories listed in Table 6. Also, elemental analyses are obtained under a purchase agreement from Micro-Analysis, Inc. of Wilmington, Delaware. These services are essential to insure that high quality pharmaceuticals are provided for NCI sponsored clinical trials and to meet the increasingly detailed and complex Federal regulations applicable to all sponsors of clinical investigations of pharmaceuticals. Also, a small contract has been established with South Mountain Laboratories, Inc. to provide additional sterility, pyrogen and safety test capabilities to the Pharmaceutical Resources Branch. This contract enables PRB to independently conduct sterility and pyrogen tests on contractor produced and purchased materials in the same manner used for chemical assessment of bulk chemicals and pharmaceutical dosage forms.

TABLE 6

CONTRACT LABORATORIES FOR ANALYTICAL CHEMISTRY

Contractor	Investigator	Contract No.
Midwest Research Institute	Cox	NO1-CM-87234
SRI International	Lim	NO1-CM-87183

Dosage Form Development

Dosage form development studies are usually limited to potential antitumor agents that have met Decision Network 2A requirements. However, acquisition of solubility and stability data is frequently desired to aid in the decision making at DN2A. Therefore, some preliminary formulation work is carried out on selected potential DN2A candidates. Formulation work is also carried out on pharmaceutical problems presented by agents used to modify effects of cancer chemotherapy. A collaborative effort on the bioavailability of Delta-9-tetrahydrocannabinol formulations administered orally or intramuscularly in rabbits is in progress.

The first approach to development involves an evaluation of existing physical and chemical data as well as *in vivo* route and regimen studies in rodents.

These results serve to define the scope of the formulation problem. Relatively straightforward development projects are assigned directly to the contract laboratory equipped with production capabilities. The contractor is provided with such information as chemical data, estimated dose and intended route of administration. Consultation with the project officer is carried out regarding formulation approaches. The chemical is subjected to solubility and stability studies in a selected group of physiologically acceptable vehicles. An analytical method (usually HPLC) is devised for detection of the parent compound in the presence of excipients and/or degradation products. The influence of pH, temperature, light, oxygen, packaging components and pharmaceutical additives is assessed on the stability of drug solutions. Pilot batches are then prepared under conditions simulating production scale. Physical and chemical stability of the dosage form is assessed under accelerated and simulated use conditions.

Problem areas in parenteral dosage form development directed to intravenous administration are generally due to inadequate water solubility or instability of the agent of interest. Since biologically suitable solubilization techniques are limited, new approaches are investigated under contract at the University of Kansas and at the University of Kentucky as well as in the Pharmaceutical Resources Branch's formulation laboratory (see Project ZO1 CM 03584-10 PRB).

During this year, the University of Kansas has studied dosage form development problems presented by six different compounds. Additional work was carried out on the isopropyl Anderson Compound (NSC-278214). This compound is exceedingly unstable in aqueous solution ($T_{1/2}$ = 2.5 hours in 10% cremophor). This vehicle was also incorporated into the emulsion at a final concentration of 3-5%. The stability data were then consistent and the formulation is undergoing antitumor evaluation.

The University of Kansas has successfully formulated JB-11 as either a lactate or glucuronic acid salt. These formulations are undergoing antitumor evaluation. The depsipeptide of murine origin, Didemnin B, was also successfully formlated as a solution in 5% ethanol and 5% cremophor. This contractor has also thoroughly examined the solubility and stability aspects of the Hexamethylmelamine analogue, Trimethyltrimethylolmelamine, and has made recommendations regarding a potential formulation approach.

The University of Kentucky has also worked on a number of difficult formulation problems including several anthracycline analogues (Menogarol, Dibenzyldaunorubicin, etc.) and Phyllanthoside. In collaboration with the University of Iowa contract, a satisfactory dosage form of Menogarol was developed using the lactate salt and manufactured for toxicology trials. The University of Kentucky is also in the process of a kinetic study on the stability of Homoharringtonine in aqueous vehicles.

The University of Iowa contract provides both sophisticated product development capability and production capacity for sterile freeze dried dosage forms, small- and large-scale sterile solutions plus tablets and capsules for oral use. The production capacity is usually adequate to meet requirements for large animal toxicity testing or Phase I clinical trial.

As indicated earlier, compounds that appear not to present solubility or stability problems are assigned directly to a formulation contractor with

production capabilities. The University of Iowa has developed formulations for six compounds: Dow Compound (NSC-253272); Sangivamycin (NSC-65346); Norwich Compound (NSC-305884); Menogarol (NSC-269148); N,N-Bis(2-hydroxyethyl-2-nitro-1H-imidazole (NSC-314055); and the Bisbenzimide Derivative (NSC-322921). Initial production batches were manufactured for Dihydro-5-azacytidine, Sangivamycin, 5-Fluoroadenine arabinoside-5-phosphate and Menogarol. The last mentioned compound represents a case of initial development at a formulation research facility and subsequent transfer to a production contractor. Dosage form development and manufacturing data are used in support of IND applications. During the past year, the University of Iowa completed six development and 12 production projects. Most of these products were sterile freeze dried dosage forms, but the contractor has the facilities and experience to prepare a wide variety of dosage forms.

TABLE 7

CONTRACTS FOR DOSAGE FORM DEVELOPMENT

Contractor	Investigator	Contract No.
Iowa, University of	Wurster	NO1-CM-07303
Kansas, University of	Repta	NO1-CM-07304
Kentucky, University of	Hussain	NO1-CM-07381

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03584-10 PRB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Research in the Development of New Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	James C. Cradock	Head	A&PDS	PRB	NCI
Other:	J. Paul Davignon	Chief		PRB	NCI
	Karl P. Flora	Chemist	A&PDS	PRB	NCI
	Babu R. Vishnuvajjala	Visiting Associate	A&PDS	PRB	NCI
	Esmail Tabibi	Visiting Fellow	A&PDS	PRB	NCI

COOPERATING UNITS (if any)

Dr. L. McCarthy, Dept. of Pharmacology, Dartmouth Medical College

LAB/BRANCH
Pharmaceutical Resources Branch

SECTION
Analytical and Product Development Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project describes the formulation activities in the intramural laboratory with drugs of interest to the Division of Cancer Treatment, NCI. Primarily these studies are directed toward evaluation of the stability of certain new antitumor agents and resolution of solubility problems presented by these substances. NSC-284356, 4,8-Ethenopyrrolo(3',4':3,4)cyclobut(1,2-F)-isoindole-1,3,5,7(2H-6H)-tetrone, octahydro-; and NSC-271674, 4-Carboxyphthalato-(1,2-diaminocyclohexane) platinum are representative examples. Formulation studies were not restricted to antineoplastic agents and included formulation studies on the antiemetic Delta-9-tetrahydrocannabinol designed to improve bioavailability of the oral dosage form and provide alternative formulation approaches for administration by intravenous and intramuscular routes. The compatibility of N-Methylformamide, a solvent and potential antitumor agent, with administration devices was also evaluated.

1. Delta-9-tetrahydrocannabinol, NSC-134454 (Flora, Cradock and Vishnuvajjala)

Nausea and vomiting have long been associated with aggressive cancer chemotherapy. Delta-9-tetrahydrocannabinol (Delta-9-THC) has been shown to control the nausea and vomiting in many patients including some whose symptoms were refractory to standard antiemetic therapy. Absorption of Delta-9-THC from the dosage forms currently available from the National Institute on Drug Abuse appears to be erratic and unpredictable. Therefore, studies were undertaken to provide a variety of bioavailable dosage forms of THC.

Current investigations have concentrated on several approaches including: development and bioavailability testing of parenteral dosage forms; development of bioavailable oral formulations; and development of dosage forms for rectal administration.

Collaborative studies of an emulphor containing formulation in the feline emesis model of Borison and McCarthy have been conducted (McCarthy, Dartmouth Medical School). Cisplatin is used to induce emesis in this procedure. A radioimmunoassay kit available from the National Institute on Drug Abuse for the determination of Delta-9-THC in human plasma was adapted in our laboratory for application to cat plasma and used to monitor plasma THC. Numerous courses and doses of Delta-9-THC in 5% emulphor EL620-5% ethanol-90% saline have been administered intramuscularly or intravenously to cats. Animals were also administered Delta-9-THC orally in the NIDA sesame oil containing soft gelatin capsule. A 4 mg/kg dose of I.M. Delta-9-THC in the emulphor formulation completely protected all four cats tested. Oral Delta-9-THC protected only three out of four animals at that dose level. At the 2 mg/kg dose level, intramuscularly administered drug produced a significantly greater antiemetic effect than the same dose given orally. Area under the blood level curves from time of administration to eight hours related inversely to the number of emetic episodes.

A collaborative study was also carried out on THC absorption in the monkey with Dr. Perlin and associates at the Uniformed Services University of the Health Sciences. The results indicate good absorption following I.M. injection and a preliminary report has been presented (The Pharmacologist 23: 142, 1981).

2. N-Methylformamide, NSC-3051 (Vishnuvajjala and Bykadi)

Based on activity versus a xenograft tumor panel, N-Methylformamide (NMF) was selected for further study as a potential candidate for clinical trial. NMF is water miscible in all preparations, therefore, the concentration selected for formulation studies was based on the maximum amount that did not produce hemolysis in a standard in vitro evaluation. Concentrations of NMF at 40% v/v or less in 0.9% sodium chloride were not hemolytic. A rapid and sensitive HPLC method was developed and used to monitor the stability of NMF in a glass ampule. Accelerated degradation studies over a 30 day period demonstrated that NMF is stable at 50° $\pm$ 0.1°C.

NMF is a strong solvent and some concern was expressed regarding the potential of this formulation to extract the additives present in polyvinyl chloride I.V. tubing and bags. The most common additive present in these devices is the plasticizer, diethylhexylphthalate (DEHP), used to make the device flexible.

Since the anticipated mode of administration was intravenous infusion, a study was carried out to determine the amount of DEHP extracted into the infusion fluid from the I.V. tubing. This study will shortly be extended to the plastic I.V. bags.

When I.V. tubing filled with normal saline or various concentrations of NMF in saline was stored for 24 hours to simulate a maximum clinical contact time, only small amounts of DEHP were extracted by normal saline or 20% NMF, 1.8 and 3.8 µg/10 ml, respectively. At 40% NMF, the DEHP extracted was modestly increased (18.7 µg/10 ml), but after 60% or 80% NMF, there was a dramatic increase in the amount of DEHP leached (540 µg/10 ml and 21000 µg/10 ml, respectively. Although the 40% NMF did produce an increase in leached DEHP over the saline control, the amount extracted by a dose equivalent to an LD_{50} for NMF was <100 µg of DEHP. However, a patient undergoing a transfusion of whole blood would receive 10-15 mg of DEHP.

3. 4-Carboxyphthalato-(1,2-diaminocyclohexane) platinum, NSC-271674 (Visnuvajjala and Flora)

NSC-271674 is a platinum analogue with good in vivo activity versus L_{1210} lines sensitive and resistant to Cisplatin. In addition to carrying out conventional formulation studies leading to a stable, soluble I.V. dosage form, analytical methods had to be developed to assess purity in the solid state and monitor the stability of the intact compound in solution. Determination of the purity by conventional chromatographic and spectroscopic methods were not successful due to limitations of solubility and suspected instability in solution. These techniques included a wide variety of HPLC and TLC systems, plus proton C^{13} and Pt^{195} NMR. Solid probe magic angle NMR, a relatively recent development, is presently being studied in collaboration with the NMR Resources Center at Colorado State University. Solids from two different batches of drug were analyzed by the method and appear to be of good quality. The suitability of this method to detect decomposition in freeze dried samples will be evaluated shortly.

A number of the 1,2-diaminocyclohexane substituted platinum complexes are poorly water soluble. The free carboxylic acid group in NSC-271674 was designed to overcome this drawback by enabling the formation of a water soluble salt. Previous work in this laboratory indicated that the drug is unstable in such solution and releases free trimellitic acid (TMA). The rate of release of TMA can be easily followed by HPLC, but a suitable method to detect the intact complex still has not been developed. However, HPLC studies demonstrate that even freshly prepared, cold (0°C) solutions exhibit at least ten peaks. The data suggest that a nucleophilic ligand (i.e., HCO_3^-, H_2O, OH^-, etc.) may displace one of the carbonyl groups of TMA attached to platinum and still not release TMA. The large number of peaks can also be partially explained by the fact that a mixture of isomers of 1,2-diaminocyclohexane was used in the synthesis resulting in an isomeric mixture for NSC-271674 and then the decomposition products. When the decomposition is accelerated, the intensity of the TMA peak increases while the others decrease until the only UV absorption peak is TMA. These results show the chemical heterogeneity of freshly prepared bicarbonate solutions of the drug and suggest that all the species seen in the HPLC contain TMA chemically bound to the platinum. Isolation and identification of these compounds presents a monumental task and probably impractical because of their limited stability.

In light of these observations, it was found necessary to examine other solvents to solubilize the drug. Of all the solvents examined, only DMSO was found to be useful. The drug dissolves in hot DMSO (about 100°, up to 50 mg/ml), remains in solution when brought to room temperature, and does not precipitate on a 100-fold dilution with distilled water. HPLC of such diluted solutions showed only two peaks in addition to that of TMA. The relative intensity of the two peaks parallels the ratio of cis/trans isomers, known to be present in the platinum complex. While the drug slowly hydrolyzes in the diluted aqueous solutions, it was found to be stable in the original DMSO solution even after heating at 100° for 6 hrs. The source of free TMA observed in the solution (6.7%) appears to be the impurity present in the bulk drug. The nature of the compounds responsible for the HPLC peaks is under current investigation.

Since all the preclinical and limited clinical trials of NSC-271674 were carried out in bicarbonate solutions, it was felt necessary to evaluate the efficacy of the drug in a new formulation as a solution in DMSO. Accordingly, as an initial step, solutions of NSC-271674 in DMSO (50 mg/ml, to be diluted with distilled water) in sealed ampules were submitted for in vivo testing versus the L_{1210} tumor line.

4. Benzene-Maleimide Photoadduct (Gulf Compound), NSC-284356 (Vishnuvajjala)

The photoadduct of maleimide with benzene represents a unique compound with antitumor properties. Its insolubility in water and organic solvents presented an interesting formulation problem. A water soluble disodium salt of the compound was prepared as a stable lyophilized product. Solution stability of the drug in reconstituted solutions was studied using HPLC.

The drug was found to be reasonably stable (T_{90} >6 hrs) when the reconstituted solutions were kept <4°, but rapidly degrades at room temperature ($t_{1/2}$ about 4 hrs) to give an inactive compound versus P388 in culture. Kinetic studies were carried out to determine the stability of the drug at various temperatures and buffered solutions. The rate of the reaction was found to increase with increase in temperature as well as increase in the pH of the buffer solution.

The structure of the degradation product was determined by physical methods (NMR, MS, IR, etc.) and confirmed by unambiguous chemical transformations to a known compound. The formulation information and reference sample of the degradation product were provided to a pharmaceutical production contractor for scale-up. A report of this work was presented at the 1982 Annual Meeting of the American Pharmaceutical Association.

In addition to the above mentioned studies, several small, relatively straightforward projects were investigated during the past year. These projects generally involved solubility and/or stability assessments of new chemically related agents.

Publications

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ANNUAL REPORT OF THE TOXICOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 to September 30, 1982

The primary function of the Toxicology Branch is the preclinical toxicologic evaluation of potential oncolytic agents. The agents tested are identified by the Decision Network Committee, Developmental Therapeutics Program on the basis of selection criteria identified in the Linear Array. These toxicology studies are carried out through a Prime Contract/Subcontract network. For contract management purposes the Prime Contract is divided into four tasks. Therefore accomplishments during the past year will be discussed under each of the tasks.

Task I

This task is devoted to protocol studies of new oncolytic agents using a protocol developed over the past year. The purposes of the new toxicology protocol, which has had extensive testing during this year, are to determine a 'safe' starting dose for the initial Phase I clinical trials and to indicate whether or not there are irreversible toxicities that would preclude entry of an agent into clinical trials. The protocol consists of six studies - the mouse single dose and daily times five lethality studies, the dog single dose and daily times five toxicity studies and the mouse single dose and daily times five toxicity studies. The mouse lethality studies are used to develop data from which to extrapolate a 'safe' starting clinical dose. The dog toxicity studies are designed to test the safety of the projected clinical dose in a second species and to predict toxicities that might be encountered in human trials. Two reports are generated from these studies. The first, a Phase I report consists of the data from the mouse lethality studies and the 'in life' portion (all observations up to necropsy) of the dog toxicity studies. This report is submitted to the Food and Drug Administration as Attachment 6a to the Investigational New Drug Application (INDA) and is considered adequate for starting the Phase I clinical trials. A Phase II report which contains all the data from the Phase I report plus the mouse toxicity studies and the histopathologic evaluation of the tissues from the treated dogs is submitted to the FDA ninety days after the INDA has been approved. Phase I and II reports have been submitted to the FDA during this year on the following compounds:

NSC-141633	Homoharringtonine
NSC-264480	5-dihydroazacytidine
NSC-296934	Henkle's compound
NSC-526417	Echinomycin
NSC- 3051	N-methylformamide

Phase I reports on the toxicologic evaluation of NSC-301467, SR-2508 (a radiosensitizer) and NSC-312887, F-ara-AMP are being prepared for filing with the Food and Drug Administration within the next several months.

Prior to this year, only one agent had been tested using the new protocol. It was soluble in a conventional vehicle and toxic at reasonable dose levels.

During this year, however, several of the agents tested have required formulations in unusual vehicles, i.e. cremophor/alcohol, and even then have had limited solubility. For drugs requiring these types of formulation, the new protocol has proved to be inadequate. These relatively insoluble and/or non-toxic agents require volumes larger than the mouse can tolerate as a single bolus. If divided intravenous doses or intraperitoneal doses do not give reproducible results in the mouse, alternative protocols which have been developed with the rat as the rodent species must be utilized. The intravenous route is employed if possible, if not the intraperitoneal is used. NSC-125973, Taxol; NSC-226080, Rapamycin; and NSC-172112, Spirohydantoin mustard, are being evaluated using the rat as the alternate rodent species. Taxol and Rapamycin cannot be administered by the intravenous route to either rodent and, their evaluation therefore has required use of the intraperitoneal route in the rat while Spirohydantoin mustard is being evaluated in the rat using the intravenous route. Three additional agents, NSC-269148; 253272; and 305884 have only recently been assigned to the appropriate subcontractors for protocol studies.

Task II

This task is any portion of the protocol used in Task I or any portion of the 1973 protocol and is used to evaluate agents that have had previous clinical use, preclinical study, etc., but where existing data are not considered adequate for Investigational New Drug Applications. NSC-241240, CBDCA, had been evaluated for emetic potential and renal toxicity in the dog several years earlier. The information lacking to complete the toxicology package for INDA filing was the mouse lethality studies. These studies were accomplished under Task II and the resultant data filed with the Food and Drug Administration.

Studies commissioned earlier to evaluate the mouse as a predictive species for the quantitative and qualitative toxicities of new oncolytic agents have been completed and filed in the NCI/FDA master agreement and where appropriate in the open INDA's. Several facts have emerged in the analysis of the resultant data. The quantitative predictiveness of the mouse for establishing safe starting doses for clinical trials was demonstrated for the nineteen drugs tested. Qualitative predictiveness of the mouse histopathology for human toxicities appeared to correlate as well as that of several other species if the tissues were taken within two days of dosing the animals. Also there appeared to be good correlation between the hematologic values observed in the mouse and the known human hematopoietic toxicity of many of the agents. Clinical chemistry determinations (BUN, SGOT and SGPT) and clinical observations of mice were of extremely limited value. Clinical chemistry data were extremely difficult to interpret since often there were only one or two values obtained at the scheduled sacrifices. In addition, baseline values were too variable to give meaningful data. A pilot study has been developed to assess various methods of eliminating or reducing these deficiencies in the mouse toxicity studies. Pooling of blood samples, sequential sampling, sequential necropsy and use of clinical chemistry parameters other than those presently sampled are being explored.

Task III

This task is devoted to organ specific toxicity testing. No work has been done under this task other than submission of the report dealing with the development of a neurotoxicity screen for vincristine, cis-Pt (II) and maytansine. The toxicity screen as developed appears to be appropriate for testing analogues of these three agents with very few changes in design of the protocol necessary.

Task IV

This task is that portion of the Prime Contract dealing with data handling, subcontractor monitoring as required by the Good Laboratory Practice Regulations, and cost and program management. During this year a new data handling system has been installed, the software written and tested. Subcontractor training on data entering, editing, etc. is currently ongoing. Agents just entering toxicology will be entered into the new computer system. The Toxicology Branch has acquired its own computer and will consequently have direct access to all data on a timely basis.

ANNUAL REPORT OF THE INFORMATION TECHNOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 to September 30, 1982

The Information Technology Branch (ITB) of the Division of Cancer Treatment (DCT) was established in August, 1981. Its primary purpose is to provide modern data management capabilities to other DCT branches, namely the Drug Synthesis and Chemistry Branch, the Natural Products Branch, the Drug Evaluation Branch and the Pharmaceutical Resources Branch. In this connection, ITB is to develop and maintain the NCI Drug Information System (DIS), which acquires all chemical, biological and other data pertaining to materials of interest to DCT. All such data are maintained at a high level of currency in an interactively searchable computer system.

The Information Technology Branch also is required to be aware of contemporary advances in the various aspects of drug development technology both within the Government and elsewhere. It apprises other DCT branches as appropriate of such developments and assists as necessary in their implementation in DCT.

A further activity of ITB concerns the flow of information in other parts of NCI, insofar as the data are of interest to DCT. In this connection, the Branch has been involved in data processing problems with the Biological Response Modifiers and the Cancer Treatment Evaluation Programs.

The ITB is organized in two sections, the Chemical Information Section and the Biological Information Section, and the Office of the Chief. The Chemical Information Section is headed by a senior chemist and consists of a chemist and a part-time data clerk. The Biological Information Section is headed by a senior programmer and contains two junior programmers. The Chief of the ITB is a supervisory chemist. His office employs a chemist and a biologist. Finally, the branch has a secretary assigned to the Branch Chief, making a total of nine full-time and one part-time employees.

Office of the Chief, ITB.

In addition to supervision and coordination of the two Sections of the Branch, this office is particularly concerned with projects which involve other parts of NCI or of the Government. The immediate concerns of, for example, DEB and DS&CB, are the primary responsibility of the ITB Sections and are discussed below. During this reporting period, the Office of the Chief has been active in a number of areas, as are detailed below.

1. Development of the new Drug Information System.

A charge given to the ITB at its formation was to overhaul the various automatic data processing activities previously in use within DCT. It was decided that rather than undertake piecemeal corrective action, as had been past practice,

it was necessary to develop an entirely new "Drug Information System", which would provide online, interactive access to the entire chemistry and biology files of the DCT.

During this reporting period, prior usage statistics were analyzed, the appropriate DCT staff were interviewed and a requirements analysis was completed. An appropriate system design was developed and a contract was established via an Interagency Agreement with the Environmental Protection Agency for implementation of this design. The design calls for four major modules; a chemical acquisition unit, the chemistry search and the biology search modules and a report generator. In the first three months of contract effort, a final design for the chemical acquisition unit has been completed and programming is about to begin. Design of the chemistry search system is essentially complete. This uses much existing software and writing of the necessary additional programs has begun. Design of the biology subsystem is in the preliminary stages. The report generator will allow users to design reports to include any chemical or biological data that are required. A major technical hurdle which must be negotiated is the delivery of high quality chemical structures to such a report via a printer. During this reporting period, effort has been focused upon this problem and this is discussed in Section 5 of the Chemical Information Section report, below.

2. Recording of Committee's minutes.

Much of the data review conducted by DCT staff takes place in committees, and maintenance of the minutes of their meetings has in the past been handled manually. An interactive computer program has been developed which allows the recording of committee decisions as they are made. A summary of the decisions is made available to designated staff as the meetings end. When appropriate concurrence is received, the master database is automatically modified to reflect the decisions. The use is being studied of a projector-computer terminal which can be used for input during meetings and which will also display relevant data for all to see as transactions are completed.

3. Use of Pattern Recognition Techniques.

A study of the potential of pattern recognition in drug development work has been undertaken. A number of experts in this area have been invited to present seminars to DCT and a collaborative effort with the Information Group at Lederle Laboratories has begun. This group is to apply their own "trend vector analysis" procedures to the biological and chemical data for some 500 anthracycline derivatives with a view to prioritizing these compounds for possible further DCT testing.

4. Policy on Release of Data.

The project with Lederle has required the release of considerable data from DCT files and prompted a review of DCT policy with respect to release of information. The general desirability of a stance which allows provision of data to scientists outside NCI indicates that a policy to permit this should be developed. Accordingly, ITB is drafting procedures which will allow release of non-discreet NCI data. This will probably be accomplished upon a case-by-case

basis, as with Lederle, above, and also via the National Technical Information Service, whose charter is to make Government data generally available to the public.

5. Biological Response Modifiers Program.

The Biological Response Modifiers Program (BRMP) has a growing need in the area of data management. During this reporting period, ITB assisted briefly in the design of a file of data relevant to the BRMP effort. The responsibility for data collection remains with the BRMP and ITB will develop an interactive search capability for use with that database.

6. DCT Access to Clinical Data.

The ITB has collaborated with the Cancer Treatment Evaluation Program (CTEP) to establish a means whereby pre-clinical and clinical data can be collaterally evaluated with a view to assessing the relevance of the pre-clinical models that have been used by DCT. So far, a number of preliminary tasks have been completed. These include generation of a list of all Investigational New Drug Applications that have been filed since 1963, a file of all drugs currently in clinical trial and a list of all commercially available drugs. Clinical trials data on some 30 compounds have been collected by CTEP and provided to ITB, and data for a further 60 compounds should be available in the near future.

7. Project to Review Old Drugs.

In a related project, ITB has been invited to assist in the development of a computer system for use in connection with the Project to Review Old Drugs (PROD). This is related to the collaborative effort with CTEP because both pre-clinical and clinical data will be involved. A requirements analysis and a tentative design for the PROD have been completed.

8. Acute Toxicity Data.

Probit programs, originally developed at Southern Research Laboratories, have been modified and installed at DCRT. These permit calculation of toxicity (LD) values from survival data in the NCI database and have already been used to produce a considerable volume of acute toxicity data. An effort has begun within ITB to collect and evaluate these data with a view to merging them into the public Registry of Toxic Effects of Chemical Substances, a database which is maintained by the National Institute of Occupational Safety and Health, as a part of its mission.

9. ITB Reports.

Finally, in an effort to provide general support to the various branches in DCT, ITB has prepared and circulated some 15 technical reports during this reporting period. These cover diverse subjects ranging from the use in drug selection of partition coefficient data to a review of the published reports of cardiotoxicity in anthracyclines.

Biological Information Section, BIS.

The Biological Information Section of the ITB has, as its primary responsibility, the task of maintaining the continuous flow of new biological screening data into the master database and also responding to requests from DCT staff for information from the database for use in decisions regarding continuation or termination of testing. During this reporting period, the Biological Information Section has been active in a number of projects, as is described here.

1. Screening Database.

The master database has been reorganized into three separate files. These are a master archive, a current archive and a file of currently active compounds. All three files are searchable and, in addition to their being stored on magnetic tape in several copies, they have been completely microfilmed.

2. Reports to DEB.

A variety of different reports have been provided to DEB. These include the continuous Operating Committee and Compounds of Interest reports, and also specifically requested reports such as Analog/Parent data for Analog Committees and statistical reports dealing with in vitro and solid tumor data. Data have also been provided upon request to DS&CB for their work with the Hodes model. During the reporting period over 225 queries of the biology database were processed for NCI staff and other requestors. The Pre-screen Subcommittee of the Drug Evaluation Committee requested and received queries on a biweekly basis. This group reviews compounds with biological activity and also reviews requests for direct input to the Tumor Panel. The Pre-screen minutes were also added to the master file and copies distributed to appropriate staff. A new TALLY program was implemented to perform statistical analysis reports for all compounds reviewed by this committee.

3. Reports to NPB.

The Natural Products Branch (NPB), again received data processing support including the monthly Material of Interest Report and the quarterly P388/L1210 Report. In addition, several large queries were also run for the NPB in order that material selection methods might be refined and that contractor activity might be more closely monitored. The NPB also requested and received special Analogue/Parent compound comparison reports for the Bleomycin and Mitomycin classes to aid in compound selection.

4. Other Reports.

New data have been provided to the Chemical Abstracts Service upon a biweekly basis for their updating of the biological database that is used by the online "Interlink" which provides access to both biological and chemical data for selected compounds. A standing "Quantity Not Sufficient" (QNS) report is provided on a biweekly basis to DEB for use in the scheduling of retests of active compounds. This report lists those materials which need to be acquired and alerts staff when they are available. Prior to every Operating Committee

meeting a special report is run for DEB. Manually generated data extracted from these reports are then input to the Operating Committee Report System and/or the Compound of Interest File.

For a number of years, commercial suppliers of chemicals have received Selected Data Summary (SDS) reports concerning the compounds they supplied. It has been known for some time that many recipients experience difficulty interpreting the SDS reports. Accordingly, a new Prescreen Summary Report has been developed and put into use. This is a simple, one-page report which avoids all codes and is generated and mailed automatically every two weeks. All suppliers were offered continued mailings of the SDS in addition to the new report, but only 29 suppliers (out of nearly 1,000) have requested the SDS. The new summary is now standard and its use every two weeks leads to a biweekly savings on the order of $500 in printing and mailing costs. Some major suppliers of chemicals also request copies of testing data on magnetic tape and these are supplied upon request. This work is currently done at government expense but it is planned in the future to require the requestor to defray this cost.

5. Human Tumor Clonogenic Assay Data System.

A major effort for the BIS during this reporting period has been the development of a data system for the Human Tumor Clonogenic Assay. This new in vitro screening project involves four new screeners, each of which uses a Bausch and Lomb FAS II Image Analysis System which has a Data General NOVA-4 minicomputer as its controller and processor. In order that the screening data be transmitted from the screeners to DCRT, necessary telecommunications equipment and protocols were installed and tested. The pathology of the human tumors involved requires the use of definitions from the International Classification of Disease for Oncology and a computer-readable version of these was acquired from DCCP and tailored to fit the needs of BIS and DEB.

6. Electronic Mail.

In view of the growing expense of the voluminous telephone, telex and mail traffic between DEB and its screening contractors, access to an electronic mail system, which uses a telecommunications network, is carrying some 700 DEB transactions per month. Each message is available in hard copy to both the sender and the recipient(s) and, in addition, is filed electronically. The average cost of a message is between $3 and $4. This is thought to be considerably less than the cost, which is difficult to figure, of long distance telephone calls plus cables plus letters. It is planned to expand the use of electronic mail to other DCT contractors and collaborators, particularly those outside the U.S., where even more substantial savings should be realized.

Chemical Information Section, CIS.

The primary responsibility of the Chemical Information Section of the ITB is to provide appropriate data processing support in the area of chemistry, primarily to DS&CB. In this connection, the CIS has, during this reporting period, been active in the following areas.

1. Chemical Acquisition.

During this reporting period, DCT has acquired about 12,000 chemicals for testing. A computer record for each of these has been developed and merged into the chemical database. Supplier and Inventory records, as supplied by DS&CB, have also been merged into the database and the entire file has been available for searching by DCT staff.

2. Chemical Searching.

Substructure searches of the chemical file are carried out on a routine basis by DS&CB to elicit information concerning analogous compounds in the database. This capability has been maintained on the computers of the Chemical Abstracts Service in Columbus, Ohio. During this reporting period, some 300 such searches were carried out.

3. Telecommunications.

Telecommunications between the Blair Building and Columbus, Ohio, necessary for searching of the chemical file as described above, have for some time been troublesome. The number of available lines has frequently proved inadequate and the number of ports at the Columbus computer likewise has been insufficient. As a solution to all these problems, a multiplexed telecommunications line has been installed between the two facilities. This gives DCT six (soon to be eight) lines to the computer, upon an "always open" basis and not only provides essentially continuous access to the database, but has led to savings in telephone costs of about $30,000 per year.

4. Data Security.

Much of the information in the chemistry database is "discreet" and this imposes upon the CIS some responsibility for data security. An external review of CIS practices with respect to data security was conducted during this reporting period. Current performance was concluded by the reviewers to be adequate within the perceived requirements of the DCT, and some guidelines were proposed for data security in the new, highly interactive Drug Information System (DIS), currently under development by the ITB. An important conclusion of this review was that in the DIS, protection could be administered at the level of NSC number, not at the system level, as is the current practice. Thus, users without authority to examine discreet data could search and display other parts of the database, but they would be prevented from seeing - or even becoming aware of the existence of - discreet compounds.

5. Graphics Input and Output.

A major CIS effort during this reporting period concerns the input and output of chemical structures. The ITB perceives the current state-of-the-art to be inadequate and so various efforts have been initiated with a view to improving this situation. In the area of input, a very powerful program has been written to accept the vectors from esthetically satisfactory structures. These vectors are to be used to generate the connection tables that are essential for searching and also to reproduce the structural diagrams which were judged at input to be satisfactory. This program is running at a development level and work has just begun to write a production version. In the area of output, there is a serious need for a high-speed printer which can deliver chemical structures, in addition to large volumes of normal textual material. The modern laser printers which are just appearing on the market have this dual capability, and much effort has been put into a study of these with a view to DCT purchasing one for use as the central high-speed printer in the Blair Building. In the meantime, the older generation Versatec printer is being used heavily to produce the ITB reports which carry chemical structures. Some 40 such reports were produced during the current reporting period.

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ANNUAL REPORT OF THE EXTRAMURAL RESEARCH AND RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 to September 30, 1982

Description

The Extramural Research and Resources Branch is responsible for the direction and support of preclinical research leading to the development of effective anticancer drugs which exhibit minimal toxicity to the host. As part of the Developmental Therapeutics Program, the Branch provides program management for extramural research in the areas of drug design and synthesis, natural products development, experimental therapeutics, comparative pharmacology and mechanism of drug action. This Branch maintains cognizance of the research interests of the National Cancer Institute and periodically readjusts the emphasis it places on certain categories of preclinical drug development as new findings and significant developments occur.

Close liaison is maintained with other operating units in the Division and with various working groups such as the Anthracycline Analogs Committee, the Platinum Analog Coordination Group and the Decision Network Committee. The exchange of information resulting from these interactions ensures that the research efforts of all components of the Division of Cancer Treatment will be efficiently and effectively coordinated with research conducted by extramural investigators for development of new or improved means of treating cancer.

During Fiscal Year 1982, the Branch supported 306 research projects totalling $31.1 million. For more effective allocation of steadily declining fiscal resources for extramural research funds, the Biochemistry and Pharmacology Program issued revised assignment guidelines which sharply delineate its program interests from those which involve more basic or clinical aspects of treatment research. Proposals with objectives which are not of immediate relevance are transferred to other institutes of the National Institutes of Health or to other newly established and more appropriate programs of the National Cancer Institute.

Consistent with programmatic importance, this Branch strives to maintain an overall balance among the different areas of research. Through interchange of information and program priorities from other operating units--selected projects are funded through special exceptions provided by the Executive Committee. Below is an analysis of the number of research projects and the amount of support in each of the program's scientific categories for Fiscal Year 1982.

BIOCHEMISTRY AND PHARMACOLOGY

BY SUB-CATEGORY

FY 1982

(Dollars in Millions)

	FY 82 Number of Grants	FY 82 Total Cost Awarded
SYNTHESIS & CHEMISTRY	131	11.7
NATURAL PRODUCTS	25	1.8
SCREENING & EXPERIMENTAL THERAPEUTICS	34	2.8
COMPARATIVE PHARMACOLOGY	14	1.3
OTHER PRECLINICAL ASPECTS	11	1.0
MECHANISM OF ACTION	86	7.9
PROGRAM PROJECTS	5	4.6
TOTAL	306	31.1

Significant Recent Results

New Tumor Inhibitors

A comparative study of antitumor activity of pyrrole and pyrrolizine derivatives resulted in the synthesis and evaluation of two compounds, the bis(N-cyclohexylcarbamate) and the bis[N-(2-propyl)] derivatives of 2,3-dihydro-5 (3,4-dichlorophenyl)-6,7bis(hydroxymethyl)-1H-pyrrolizine. In experimental therapeutic studies, the drugs showed a high degree of activity against B16 melanocarcinoma, CD8F1 mammary tumor, colon tumor 26, and colon tumor 38. A significant number of "cures" were recorded. Both compounds showed a similar profile of activity; but the isopropyl compound was judged to be more potent than the cyclohexyl compound.

These compounds represent a new class of antitumor agents possessing a broad spectrum of activity that includes activity against the more intractable solid tumor models and against the doxorubicin-resistant P388 model which was shown to be cross-resistant to other agents that act through deoxyribonucleic acid (DNA) intercalation. The mode of action of both compounds appears to involve crosslinking DNA strands with resultant prevention of replication. Efforts to control the reactivity of active metabolites while reducing toxicity has led to the synthesis of a series of pyrroles, pyrrolizines and similar heterocyclic compounds. The isopropyl pyrrolizine compound has been selected for further development for clinical trials.

Studies are also in progress to delineate structure-activity relationships and to determine the precise mode of action of this unique class of compounds. (CA 22935 Anderson)

Natural Product Research

Several leads have emerged as a result of investigator initiated search for effective anticancer agents from plant, microbial and marine origins. Marine organisms have provided a number of highly active compounds against tumor test systems. A cyclic depsipeptide (NSC 325319) obtained from the Caribbean tunicate genus Didemnum has shown high activity against the B16 melanocarcinoma with minimal toxicity to the host. A relatively large proportion of samples obtained from tunicate species have displayed a high degree of cytotoxic activity in the in vitro (L1210) and in vivo (P388) test systems. The depsipeptide compound has been selected for further development toward clinical application. Studies on structural determination of the depsipeptides from Didemnum and from another promising genus, Lissoclinum are continuing. Detection of high activity has prompted a search for active compounds in twenty tunicate genera from four marine provinces in various parts of the world (CA 29821, Ireland).

Biosynthetic Cell Culture

Certain anticancer compounds of high interest to the drug evaluation programs of the National Cancer Institute must be isolated from plants which are rare, or which produce active materials only in very low yields or are available only from geographically remote areas (ex. taxol, bruceantin, homoharringtonine). Because the yields from natural plant sources are limited by one or more of these circumstances, it is expensive to obtain adequate quantity of the compound from natural sources. Initial success in the development of high yield clones of specific plant cells and the development of biosynthetic procedures through plant cell cultures derived from cells of the target species may yield adequate quantities of rare plant material.

Cell culture production methods may be extended to acquire adequate quantities of active analogs since such analogs may exist in Taxus species. Taxol, an alkaloid isolated from species of the plant genus Taxus, is of major interest as a potential chemotherapeutic agent. Activity has been shown against the B16 melanoma and the CX-1 colon and MX-1 mammary tumor test systems. Taxol acts to stabilize microtubules both in vitro and in vivo. Recent experiments indicate that cell replication is prevented because the microtubule cytoskeleton is unable to depolymerize when bound to this compound. Improved procedures gained from plant cell culture research should provide efficient and inexpensive means to increase yields of these relatively rare compounds (CA 32472, Dougall; CA 15714, Horwitz).

Human Colon Cancer Chemotherapy

Human colon cancers are refractory to conventional chemotherapy probably because of the presence of a large non-cycling fraction of cells. In rapidly metastasizing tumors, levels of certain key enzymes vary in concentration and in the degree of activity during cell growth and replication. When compared

with slow growing colon tumors, these levels are of much greater magnitude, but not in a strictly proportional manner. Development of new drugs must be based on the understanding of predictable changes in cell biochemistry at each stage of the cell cycle. Precise measurements of key enzymes across a variety of human cancers should indicate the stages at which a specific anticancer agent or agents must be administered in order to reduce or to augment the effects of these proteins. Studies of rat hepatomas showed a reciprocal relationship between synthetic and catabolic enzymes during cell growth. Recent work with human colon tumor xenografts has revealed similar patterns. A detailed study of levels of seventeen enzymes involved in purine and pyrimidine biosynthesis showed elevated levels while purine degradation enzymes exhibited reduced levels. Single agents targeted for a specific enzyme may fail to inhibit nucleotide synthesis since an alternate enzyme, performing a similar function, may be present in elevated levels to perform its function. Development of multiple agents therapy to overcome this problem will depend on the characterization and precise knowledge of the relative activities of enzymes and their metabolic implications. These concepts should prove helpful in the design of enzyme-targeted chemotherapy in colon tumors (CA 05034, Weber).

Metal Coordination Complex Compounds

Platinum coordination complexes, in combination with various other chemotherapeutic agents have been shown to be effective against a variety of neoplasms such as osteogenic sarcomas, prostate, bladder, lung, uterine and head and neck cancers.

All chemotherapeutic agents which bind to DNA are subject to the constraints of both their own molecular configurations as well as that of the DNA helix. Increased efficacy is achieved whenever different or additional binding sites become available on the DNA molecule. The active cis complex agents inhibit DNA replication by producing interstrand, intrastrand and DNA-protein crosslinks. Recently, it has been demonstrated that the intercalator ethidium bromide, administered in conjunction with cisplatin, permits the latter to bind to sites other than those when the drug is used as a single agent. It is postulated that this intercalator causes the DNA helix to become more tightly coiled and more rigid thus providing new sites for cisplatin attachment.

Recent experiments with the cisplatin agent cis-diammine-dichloro-platinum (II) revealed that it could inhibit the action of a DNA processing enzyme. The locus of action, in this case, was at some distance from the DNA molecule, involving partial unwinding and an alteration in the configuration of the preferential binding site of the enzyme. Based on this rationale, attempts are in progress to synthesize an intercalator which combines cisplatin with an ethidium bromide molecule. This molecule might seek regions of DNA rich in guanine and cytosine, and exhibit less toxicity and greater antitumor activity (CA 15826, Lippard).

Although it is known that cisplatin and related anticancer drugs react by direct binding to DNA rather than by insertion into the helix, the exact mechanisms of this process are still under investigation. The complexes bind preferentially to guanine bases at O-6 position. It is proposed that normal cells possess a mechanism which can repair the O-6 lesions. However, the process that transforms such cells into malignant cells disrupts that mechanism with resultant inability to repair the damage (CA 11349, Rosenberg).

Counteractive Measures Against Toxicity

The clinical use of bleomycin is limited by extensive pulmonary toxicity after repeated dosage. It has been shown that the pulmonary injury is the combined result of a bleomycin-generated toxin, such as the lipid peroxide or the oxygen radical, and faulty repair secondary to bleomycin damage to DNA. Current research is directed toward an understanding of the mechanisms of pulmonary damage and development of identifying biochemical markers which can detect incipient or moderate tissue involvement at an early stage. These markers, if sufficiently sensitive to progressive stages of cell injury, will alert clinicians to modulate therapeutic regimens. The ability of Type II cells to repair bleomycin-induced alveolitis is a major factor in determining whether irreversible lung damage will occur. If specific transferase or glycoprotein is found to be altered in bleomycin treated animal lungs or Type II cells, the serum levels of these markers may reveal early lung injury before fibrosis has occurred. Projected studies will attempt to identify other serum markers that measure activity of cell proliferation following initial damage but preceding fibrosis (CA 20892, Calabresi).

Gene Amplification and Methotrexate Resistance

Acquisition of cellular resistance or immunity to foreign substances is a normal evolutionary event. The role of one of the genetic mechanisms facilitating such resistance--gene amplification, is being studied in an effort to counter cellular resistance to methotrexate (MTX). The gene, which codes for the dihydrofolate reductase (DHFR) enzyme, undergoes repeated duplication, eventually becoming incorporated into a part of the chromosome known as the homogeneously staining region and accounting for approximately five per cent of the DNA present. Current research seeks to identify the mechanism(s) controlling the initial and subsequent duplication of the DHFR gene.

Recent studies utilizing recombinant DNA techniques and DNA sequencing have determined the nucleotide base composition and number of bases in the DHFR gene. In the human, mouse, rat and Syrian and Chinese hamster cell lines, a family of sequences sharing considerable similarities have been identified. Amplification studies of the DHFR gene indicate that enlargement occurs in the same region of the gene in the animal cell lines, and gene structure appears to be similar in all such lines. Following clinical administration of MTX, human tumor cells exhibited a five-fold increase in DHFR genes. Further studies will concentrate on the possible relation between gene amplification and the development of cellular cross-resistance to other anticancer drugs. As the mechanisms of progressive growth of these resistant cells becomes better understood, it should be possible to establish a multidrug protocol as the most effective means of selectively killing the cells at various stages in their development (CA 16318, Schimke).

Bleomycin - Mechanism of Action

Bleomycin, a glycopeptide with high anticancer activity, induces DNA strand scissions by freeing the purine and pyrimidine bases. It probably acts in conjunction with ferrous ions and oxygen molecules to generate free radicals which in turn react with a critical carbon linkage. Current research attempts to determine precise sites of drug binding to DNA in nuclear chromatin. The selected experimental test systems closely approximate in vivo conditions and therefore are more meaningful for an understanding of the biological actions of the parent compound. Intercalating agents often bind to specific nucleotide sequences which can be identified by a variety of techniques. These genetic "probes" reveal new information on the configuration of the gene and on the mechanisms by which certain parts of a DNA molecule are protected from attack.

The use of recombinant DNA techniques permits selective modification of nucleic acid sequences induced by carcinogenic, carcinostatic and mutagenic agents. Such mutant sequences may excite cellular DNA repair mechanisms to correct the damage, thus reducing the effect of agents such as bleomycin, neocarzinostatin and ionizing radiation. Results of these experiments may indicate ways to influence DNA repair mechanisms or to prolong the effects of drugs until neoplastic growth is controlled (CA 17395, Grollman).

Future Plans and Design Strategies

The rational design of selectively effective chemotherapeutic agents must be based on an understanding of the comparative biochemistry, enzymology, molecular biology, pharmacology and toxicology of normal and malignant cells. To achieve selective targeting of proliferating cells it is necessary to gain insight into the regulation of gene expression. This involves a study of the qualitative and quantitative phenotypic and regulatory differences between normal and neoplastic cells.

One of the objectives of this program is to identify key metabolic, enzymatic and isozymic targets for enzyme-pattern-targeted chemotherapy. Knowledge of such key factors would be helpful in development of combination treatment modalities possessing a high degree of selectivity to cancer cells to achieve therapeutic results in man. Research will be directed toward identification of highly purified enzyme preparations, and their role in the regulation of growth.

Another area for future development involves monoclonal antibodies in combination with chemotherapeutic agents which are specific to a neoplastic cell. High activity could be attained by attaching a potent cytotoxin to the monoclonal antibody. The combination of the two would provide a powerful targeted missile for interruption of the proliferative process of the cancer cell. Potent toxins produced by plants, like ricin and abrin, in combination with monoclonal antibodies, should be selective and highly active anticancer devices.

Emphasis and Projections

High priority is accorded to research investigating the mechanisms of membrane transport and its role in drug effectiveness. Transport of antifolates as it relates to the free intracellular antifolate level is clearly a critical element in cytotoxicity and selectivity. New developments in the area of MTX transport will be further explored to reveal information on the role of transport in other drug-cell interactions and to further basic investigation that might be applied in enhanced chemotherapeutic regimens.

Another area of emphasis is the development of new multi-drug regimens through monitoring for pharmacokinetic and biochemical parameters of individual tumor entities to achieve maximum therapeutic response. The resulting therapeutic synergism will make the choice of drug combinations, as well as their optimal scheduling, a more predictable and rational process. Research in this area will lead to improved responses by the eventual tailoring of multi-drug chemotherapy to fit the individual cancer patient.

Newly emerging techniques of molecular biology involving recombinant DNA techniques may be applied to understanding the mechanism of induction of drug resistance or tumor inhibition. Research directed at the coupling of highly cytotoxic or cell cycle effective chemicals, e.g., ricin or concanavalin, to monoclonal antibody preparations and studies of the effect of such combinations on tumors is of high programmatic interest.

Research encompassing multiple aspects of drug design, studies on the biochemical, toxicological and pharmacological mode of action of drugs in experimental systems, and extrapolation of research into laboratory models of human disease can be directly related and applied to treatment of the cancer patient. This type of balanced program project, encompassing several areas of research, is likely to contribute to more effective application of laboratory findings to clinical treatment. Investigator initiated research projects involving isolation, purification and development of new anticancer compounds and synthesis of analogs of natural products receives strong program encouragement.

Effective measures against dose limiting toxicity of several clinical drugs (e.g., methotrexate, adriamycin, platinum compounds) can be achieved through modification of dosage regimens or by use of adjunct rescue factors. Emphasis is placed on the study of the mechanisms of action and counteractive measures for toxicity of new clinically effective drugs through preclinical pharmacologic research.

Other Program Activities

The Extramural Research and Resources Branch provided support for two conferences in Fiscal Year 1982. A conference on the Therapeutic Application of Liposomes was held in San Francisco, California, on November 5-7, 1981. The purpose of the conference was to bring together basic scientists and clinicians to plan and to possibly implement clinical trials with

liposomes and various chemotherapeutic agents. Synthetic lipid vesicles are of considerable interest; however, due to a lack of appropriate methodology, progress toward application of liposomes as drug carriers has been relatively slow. The program included sessions on the state-of-the-art of targeted delivery in experimental systems, specific disease states that are candidates for vesicle technology, and problems associated with liposome technology (CA 31826, Carter).

The Conference on Rational Basis for Chemotherapy was held in Keystone, Colorado, on April 18-23, 1982. The purpose of this conference was to acquaint basic and clinical researchers with the problems and developments that have occurred in their respective communities. Leading basic scientists and clinical practitioners discussed such topics as the use of monoclonal antibodies in tumor treatment, steroid hormone action, mechanism of antimetabolite resistance, combined modality treatment and free-radical mediated tissue injury. This was one of the few conferences held during the past five years dealing with both the basic and the applied aspects of the fields across the broad front of cancer treatment (CA 31746, Fox).

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ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

OCTOBER 1, 1981 to SEPTEMBER 30, 1982

Efforts in the Laboratory of Chemical Pharmacology, DTP, DCT are concerned with conducting research, teaching and training, contract administration, and providing administrative and scientific support to the Developmental Therapeutics Program and the Division of Cancer Treatment.

The primary research activity of the Laboratory of Chemical Pharmacology is the study of new and established antitumor agents. These studies are chiefly concerned with elucidating the pharmacological properties of such agents, and include an evaluation of their disposition and metabolism, their mechanism of action, the mechanisms by which tumor cells become resistant to them, and their potential adverse effects. Studies are conducted not only in laboratory animals, but also in humans, and the test compounds include antitumor agents as well as other foreign compounds used as models for defining specific processes or mechanisms. Of special concern in the disposition studies of new antitumor agents is the tissue distribution of the compound, including its ability to cross the blood-brain barrier; in addition, the lymphatic absorption of the compound, its biotransformation and its rate of elimination are evaluated in parallel with attempts to develop a model for its pharmacokinetic behavior. These studies require in many instances the development of new analytical methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition and metabolic fate is not fully understood. Other studies in the Laboratory of Chemical Pharmacology are concerned with the development of novel approaches and combined modality regimens for tumor therapy, the rational design of new antitumor agents, evaluation of chemotherapeutic agents in various model tumor systems, and characterization of the transport properties of the blood-brain-CSF system. Efforts are also underway to characterize the toxicity of new antitumor agents, and to develop methods of reversing their toxicity.

Studies on the pharmacology and metabolism of several antitumor agents and radiosensitizers, including misonidazole, desmethylmisonidazole, m-AMSA, and riboxamide were carried out during the past year. Other drug-related studies include the physiologic disposition of liposome-entrapped ara-C and melphalan, and the intra-tumor distribution of misonidazole and methotrexate in brain and flank tumors using quantitative autoradiography. Double-label quantitative autoradiographic studies of ^{14}C-misonidazole distribution and blood flow were performed in RT-9 brain and flank tumors. The larger tumors consist of a relatively narrow outer rim of viable anaplastic and pleomorphic cells and a relatively large central region composed of necrotic and cystic regions separated by cords of viable tumor cells. ^{14}C-misonidazole and labeled metabolites distributed rapidly and uniformly in brain and tumor tissue within 30 min; regional tissue blood flow in brain and brain tumor did not influence the 30 min distribution. Studies at 2 and 4 hrs demonstrated a heterogeneous distribution pattern of misonidazole-derived-radioactivity which correlated in part to viable-appearing cells around necrotic foci. At 4 hrs an average tumor high/low activity ratio of 4/1 and

an average tumor-high/brain ratio of 5/1 was measured. The distribution pattern of misonidazole-derived radioactivity in RT-9 flank tumors was quite different from that described above. The highest activity of misonidazole-derived radioactivity was always located within the tumor rim and the lowest activity was always within the central region. Regional tissue blood flow matched the regional distribution of misonidazole-derived radioactivity. The highest rates of blood flow (2-10 ml/100g min) were found around the outer rims of the flank tumors, whereas the lowest rates (<0.5 ml/100g min) were found in the center of these tumors. The low blood flow in central tumor regions resulted in levels of misonidazole-derived radioactivity that were <5% of that in plasma. Since the extraction of misonidazole-derived radioactivity was 30% or more in the center of the RT-9 flank tumors, the delivery and distribution of misonidazole-derived radioactivity was mainly limited by blood flow in these areas.

Clinical studies with misonidazole have indicated that the dose limiting toxicity associated with this drug is neurotoxicity. An approach suggested for decreasing the incidence of neurotoxicity was the development of less lipophilic analogs of misonidazole that were equally effective radiosensitizers but would have diminished ability to cross the lipid blood-brain and blood-neural barrier. In addition, a relationship has been observed between the neurotoxicity and the extent of tissue drug exposure, i.e., the area under the plasma-drug concentration-time curve (AUC). Since desmethylmisonidazole has a lower octanol/water partition coefficient (p = 0.13) than misonidazole (p = 0.43) and was reported to have a shorter plasma elimination half-time than misonidazole, a clinical evaluation of desmethylmisonidazole using an iv preparation was initiated. Plasma and urine desmethylmisonidazole levels were obtained during this study to determine pharmacokinetic parameters and help clarify the relationships between neurotoxicity and pharmacokinetic parameters which have previously been observed for misonidazole. Pharmacokinetic data were obtained in 84 patients who received desmethylmisonidazole doses ranging from 0.9 g/m^2 to 4 g/m^2. The results of this study showed: (a), desmethylmisonidazole had a 6.0 $\pm$ 0.6 hr plasma elimination half-time; (b), desmethylmisonidazole produced a one hour desmethylmisonidazole plasma level of 38 $\pm$ 3 µg/ml per g/m^2 dose; (c), desmethylmisonidazole was distributed in an apparent volume of 45 $\pm$ 2L; and (d), that 60 $\pm$ 7% of the parent compound administered was recovered in the patients urine. Comparison of these data with those previously determined for misonidazole after iv administration shows similarities in the apparent volume of distribution (misonidazole = 37L) and the one hour misonidazole plasma levels (46 µg/ml per g/m^2 dose). Differences were observed in the plasma elimination half-time (misonidazole = 9.9 hr, desmethylmisonidazole = 6.0 hr) and the extent of renal excretion of the parent drug; only 26% of the misonidazole dose was recovered in the patients urine, 10% as misonidazole and 16% as desmethylmisonidazole. A correlation between the incidence of neurotoxicity and the desmethylmisonidazole AUC was observed similar to that found for misonidazole. A comparison of the mean total AUC for those patients who exhibited neurotoxicity after receiving either misonidazole or desmethylmisonidazole indicated that desmethylmisonidazole (AUC = 5150 + 461 µg hr/ml was approximately 52% less than that observed for misonidazole (AUC = 10800 + 900 µg hr/ml). These data suggest that desmethylmisonidazole is approximately 2 times more toxic than misonidazole and are consistent with the formation of a similar metabolite from each compound that is responsible for the observed neurotoxicity. Future studies will attempt to identify toxic metabolites.

The pharmacokinetics of riboxamide were determined in mice at two doses (25 and 250 mg/kg). At the higher dose, an initial plasma concentration of 1.38 mM was determined which rapidly declined with a T 1/2 of 21 min. The terminal half-life was much longer (at least 260 min), resulting in a nearly constant plasma concentration of approximately 0.11 mM. The kinetics of the 25 mg/kg dose were similar; the initial plasma concentration was 0.11 mM which declined with an initial half-life of 11 min and the plasma concentration in the terminal phase was nearly constant at 0.08 mM with a T 1/2 of at least 310 min. The initial plasma concentrations were determined by extrapolating the curve to the y axis. However, at both doses this value is about 1/10th the concentration that would be expected, suggesting that there is a very rapid initial phase with a T 1/2 of about 1 min. The amount of circulating metabolites at either dose was small; five percent or less of the circulating radioactivity was found as the carboxylic acid and no significant amount was found as phosphorylated products. The integral of the plasma concentration was 110 mmoles min/L for the 250 mg/kg dose and 8.3 mmoles min/L for the 25 mg/kg dose, indicating that the drug exposure is directly proportional to the dose over the range of 25-250 mg/kg. The steady state volume of distribution is very large in all cases (about 300 x the total mouse volume), which suggests the drug is bound, sequestered, and/or metabolized.

The regional entry of ^{14}C-methotrexate (MTX) into brain and Walker 256 brain tumor of rats was measured using quantitative autoradiography both with and without intracarotid hyperosmolar mannitol opening of the blood-brain barrier. MTX concentration was heterogenous in tumors (1.8 to 14 μg per gram at 15 min and 0.9 to 9.14 μg per gram at 1 hr), while distant brain concentrations were at or below 0.5 μg per gram (blood-to-brain transfer constant, k = 0.000093 ml/g min). An intracarotid hyperosmolar mannitol infusion increased MTX concentrations in cortex of distant brain (3.3 μg per gram), although white matter did not exceed 0.22 μg per gram. Brain tumor concentrations were again heterogenous (up to 10.8 μg per gram) but did not exceed nonmannitol controls. These data demonstrated that MTX in the Walker 256 brain tumor was not increased by intracarotid mannitol, while MTX in brain distant from tumor was markedly increased.

Studies have continued during the past year on liposomal encapsulation of antitumor agents as a means of altering their toxicity, physiologic disposition and therapeutic effects against experimental lymph node metastases. New studies in this area have focused on the uptake of liposomes by regional lymph nodes and their cellular localization; in addition, attempts to target liposomes to specific sites and to specific cells have been initiated using monoclonal antibodies coupled to the liposome membrane. In one study colloidal dye was injected ip or sc at various locations including the thigh, thorax and footpads of the fore and hind limbs. At various times after injection, animals were sacrificed and various lymph nodes removed and examined microscopically for pattern and intensity of staining. For most injection sites studied, dye was detected only in regional lymph nodes in the immediate vicinity of the injection site. However, in mice injected in the footpad of the hind limb, lymph nodes distant from the injection site were stained in addition to regional lymph nodes. In these mice, the intensity of staining in the various lymph nodes varied with their location relative to the footpad, such that intensity diminished with increasing distance from the injection site in the order popliteal > lumbar > renal > mediastinal > axillary > inguinal > cervical. Although the intensity of staining varied among different lymph nodes, the pattern of distri-

bution was similar. Dye was present mainly in the margins of the nodes and at their junctions with the afferent and efferent lymphatic vessels. However, in the most intensely stained lymph nodes, dye could be observed throughout the entire node. Irrespective of the injection site, intensity of staining was maximal approximately 3 - 4 hr after injection and remained at this level for at least 24 hr. To characterize the cellular localization of liposomes in lymph nodes, liposomes containing the fluorescent phospholipid NPDE were prepared and injected into the footpads of mice. At 3 hr after injection, the animals were sacrificed and lymph nodes removed and examined by fluorescence microscopy. The pattern and intensity of fluorescence observed was similar to that seen in mice injected with colloidal dye. After teasing the lymph nodes to release cells, intracellular fluorescence was observed but was limited and appeared to be confined to macrophages. Quantitation of intracellular fluorescence in the teased lymph nodes using a fluorescence activated cell sorter confirmed that only a small proportion of the total fluorescence present in the lymph nodes (~ 1%) was associated with cells. Studies are presently underway to confirm that macrophages are responsible for cellular uptake of liposomes in lymph nodes using fluorescent antibodies to identify the various cell types present in lymph nodes. In another study, attempts are underway to improve the cellular uptake of liposome entrapped materials into lymph nodes by using liposomes to which monoclonal antibodies (Ab) are coupled. In a preliminary study, I^{125} labelled Ab directed against specific surface antigens on lymphocytes was injected into the footpads of C57B10A mice in which the Ab is expressed and in control C57Bα6 mice in which the Ab is not expressed. At 3 hr after injection, animals were sacrificed and various tissues and lymph nodes removed. In the control mice, less than 2% of the radioactivity was associated with lymph nodes, whereas more than 50% was present in corresponding nodes of C57B10A mice. Thirty percent of this radioactivity was present in popliteal nodes, 12% in lumbar nodes and 7% in renal nodes. Our findings suggest that coupling Abs to the surface of liposomes may provide a means of more precisely targeting drug-containing liposomes to specific sites such as the lymph node; they also open the possibility that Abs raised against experimental tumors and coupled to the liposome membrane may have therapeutic activity against lymph node metastases. Studies are in progress to investigate the distribution of liposomes labelled with ^{14}C-cholesteryl oleate in lymph nodes of monkeys using quantitative autoradiography. At 2 hr after receiving ^{14}C liposomes by ip and subcutaneous (thigh) routes, animals were sacrificed and various lymph nodes removed, frozen and sectioned. Quantitative autoradiography of inguinal, axillary and mediastinal lymph nodes indicated that the major portion of the ^{14}C present in lymph nodes is localized predominantly in the marginal sinuses.

Nonhuman primates bearing diethylnitrosamine-induced hepatocellular carcinomas have been used to compare the plasma clearance, tissue distribution (including uptake by tumor tissue), metabolism and excretion of free and liposome-entrapped ara-C. Animals were dosed iv with free [^{3}H]Ara-C or [^{3}H]Ara-C entrapped in liposomes composed of phosphatidyl choline and labelled with [^{14}]cholesteryl oleate. At 2 hr after dosing, animals were sacrificed and various tissues removed and counted for radioactivity. In addition, plasma and urine were analyzed for unchanged Ara-C and its metabolite Ara-U by HPLC. Clearance of Ara-C equivalents from the plasma was found to be slower for liposome-entrapped Ara-C than for the free drug. The rate of metabolic breakdown of Ara-C, as indicated by the ratio of Ara-C to Ara-U in plasma, was found to be slower in the monkeys receiving the liposome-entrapped drug than in animals given free

Ara-C. Urinary excretion of Ara-C and its metabolite was significantly reduced in animals receiving liposome-entrapped Ara-C (27%) as compared to monkeys given the free drug (40%). Liposome-entrapment also markedly altered the tissue distribution of Ara-C. Tissue concentrations of Ara-C equivalents were higher by 50 and 8 fold respectively in the spleen and liver of animals receiving the liposome-entrapped drug than in corresponding tissues of monkeys given free Ara-C. Tumor concentrations of Ara-C equivalents were significantly higher by 2 fold in animals treated with the liposome-entrapped drug as compared to monkeys receiving the free drug. This finding indicates that a modest increase in the concentrations of an antitumor agent in liver tumors can be obtained by entrapping the agent in liposomes. In animals treated with free [^{3}H]Ara-C, tritium levels in liver tumor and surrounding normal liver tissue were essentially the same. In contrast, in monkeys given liposome entrapped Ara-C, levels of both [^{3}H] and [^{14}C] activity in tumor tissue were 5 fold lower than in surrounding normal tissue. The possibility of exploiting the differential uptake of liposomes between tumor and surrounding normal tissue to detect tumors of the liver and spleen using CT scanning in conjunction with liposome-entrapped radioopaque material is being investigated.

Studies on the effect of liposomal entrapment of melphalan (MPL) on its tissue distribution and activity against lymph node metastasis of the rat 13672 mammary adenocarcinoma were described in last years Annual Report. Studies to define the optimal liposome size and lipid composition for maximal lymph node uptake are in progress. In these studies, liposomes containing MPL are prepared using phosphatidyl-PC and cholesterol, and clarification of the liposome suspension during the course of sonication is assessed by measuring the optical density (OD). When the suspensions were sonicated until the OD reached a plateau (0.035-0.040 OD), the vesicles formed were small (37 nm in mean diameter), and relatively constant rates of entrapment for MPL and the aqueous solution were obtained. Cloudy suspensions (1.8-2.0 OD) prepared by a short term sonication contained large vesicles and higher entrapment rates were obtained for both MPL and the aqueous phase. The tissue distribution ^{14}C-MPL/^{3}H-PC-liposomes prepared by short-term and long-term sonication was compared in rats. Sc injection of small liposomes resulted in a significantly higher and more sustained concentration of MPL equivalents in regional lymph nodes, while large liposomes were cleared from the injection site more slowly and produced only a slight increase in the lymph node concentration of MPL. In an attempt to optimize the lipid composition of MPL-containing liposomes, various neutral, positive and negative liposomes have been prepared. The MPL entrapment rate, the in vitro stability and the lymph node uptake of liposomes composed of PC:cholesterol (2:1) PC: cholesterol:phosphatidic acid (6:3:1); PC:cholesterol: stearylamine (6:3:1) sphingomyelin:cholesterol (2:1), sphingomyelin:cholesterol: phosphatidic acid (6:3:1), and sphingomyelin:cholesterol:stearylamine (6:3:1) is being compared. Tissue distribution studies of these liposomes are not yet completed. However, thus far it appears that the entrapment rate for MPL is significantly higher for the PC:cholesterol:phosphatidic acid liposomes than for the others, and that the in vitro stability is better than, or equivalent to, that of the other liposome preparations tested.

The ability of several proteolytic enzymes, administered concomitantly with MPL-liposomes, to hasten clearance of liposomes from the sc injection site and enhance regional lymph node uptake is being evaluated. Hyaluonidase, collagenase and chondroitinase singly and in combinations, have thus far been tested. Hyaluronidase was most effective in increasing the clearance rate of liposomes

from the sc injection site and produced a 2 fold increase in the uptake of liposomes by the reginal lymph node at 1 hour after dosing; the concentration of hyaluronidase used (100-200 IU/ml) did not produce histologic evidence of tissue damage at the injection site, nor did it appear to alter the integrity of the liposomes themselves. Thus the use of proteolytic enzymes to break down the intersititial matrix and/or basement membrane may be a feasible means of enhancing lymph node uptake of liposomes from the intersititial space. Rats bearing the 13762 adenocarcinoma were given a single sc injection of free or liposome-entrapped MPL 3 days after surgical resection of the primary tumor. Liposome-entrapped MPL produced a greater reduction in the weight of lymph node metastases than did free MPL. Thus, when MPL was entrapped in liposomes, a dose of 0.125 mg/kg reduced the weight of lymph node metastases to about 50% of controls. A dose of 0.5-1.0 mg/kg was required to exert an equivalent effect on lymph node tumor growth when free MPL was administered. These results suggest that the intersitial injection of liposomes containing MPL may be useful for the postoperative treatment of lymph node metastases.

A major thrust of research within the Laboratory is directed toward understanding the mechanism(s) of selective toxicity of antitumor agents. Emphasis is placed on elucidating the importance of metabolic reactions that alter the cytotoxic effects of drugs and on endogenous factors that affect the selective toxicity of antitumor compounds. To study the mechanism of selective toxicity of a compound, one must use in vivo systems and those in vitro systems that can approximate in vivo conditions. We have spent a considerable amount of effort developing such systems. Our first priority is to study the reaction in vivo. When this is not possible we develop methods to study enzyme reactions in the intact cell or intact organ so as to maintain the microenvironment of the biologic process under study as close to the in vivo condition as possible. Research from this Laboratory (see previous annual report) has shown that the isolated perfused rat liver exports uridine at concentrations similar to that found in plasma. Furthermore, plasma appears to be an important source of preformed pyrimidines that can be utilized by cells with an intact salvage pathway and thus could be an important factor determining the effectiveness of certain antimetabolite antitumor agents. We found, for example, when cultured L1210 cells are incubated in various constant concentrations of uridine, that concentrations of uridine equivalent to that found in the plasma substantially inhibited de novo pyrimidine biosynthesis. The salvage pathway was less sensitive than the de novo pathway to intracellular uracil nucleotide concentrations indicating a preferential use of salvage over de novo synthesis. Furthermore, plasma concentrations of uridine reversed the growth inhibitory effects of PALA in cultured L1210 cells. Thus, manipulation of circulating nucleoside concentrations could be an important method of enhancing the selective toxicity of antimetabolites. Studies were extended in the following areas: (a) manipulation of the donor function of the liver to increase or decrease biosynthesis of salvageable pyrimidines; (b), evaluation of the relative dependencies of cells on de novo vs. salvage pathways for pyrimidine biosynthesis; (c), synthesis and evaluation of inhibitors of uridine kinase to block the salvage pathway; and (d), regulation of de novo pyrimidine biosynthesis in isolated hepatocytes and the inter-relationship of this pathway with enzymes of the urea cycle.

Compounds were studied in the isolated perfused rat liver in an attempt to dissociate the nucleoside export function of this organ from its uptake

catabolic function. We demonstrated that the isolated liver efficiently removes uridine from the perfusate and metabolizes the majority of it to products beyond uracil in the catabolic pathway of uridine, while maintaining circulatory levels by constant excretion of the nucleoside. Inhibition of the breakdown of uridine in the isolated liver with a new inhibitor of uridine phosphorylase [(5-benzyl-1,2'-hydroxyethylmethyl)uracil, BZHU] changed the pattern and kinetics of circulating uridine in the perfusate. 20 μM BZHU increased the half life of circulating uridine from 8 to 20 min, while 100 μM BZHU increased the half life to 60 min. Thus, concentrations as low as 20 μM BZHU inhibit the breakdown of uridine and dramatically slow its removal from the perfusate by the liver which cannot efficiently salvage preformed pyrimidines. In terms of circulating levels of uridine, 20 μM BZHU caused a concentration of 4-5 μM uridine (higher than control levels of 1-2 μM) to be maintained in the perfusate. 100 μM BZHU resulted in a linear increase in the perfusate uridine concentration, such that after 3hr it had reached 19 μM uridine which is equivalent to an output rate of 154 nmol/hr/g wet weight maintained over 3hr. Thus, in the presence of this inhibitor, output of uridine by the liver is apparently unaffected, but the liver's ability to catabolize uridine is inhibited, causing a net increase in the concentration in the perfusate. It is therefore possible to manipulate the role of the isolated liver as a donor organ of circulating uridine. Further investigation is required to extend these results from the isolated organ into the intact animal.

We found that NH_4Cl stimulates de novo pyrimidine biosynthesis in isolated hepatocytes. The effect of NH_4Cl on the output of uridine by the perfused liver was studied. When animals were predosed with 200 mg/kg NH_4Cl 30 and 45 min prior to perfusion of the isolated liver, the output of uridine was increased 3-4 times over control levels for the first 10-45 min of perfusion. When the NH_4Cl was mixed into the circulating perfusate, 4 doses of 2 mM NH_4Cl at 25 min intervals had no effect on uridine output over 120 min of the perfusion. It was found that 20 mM NH_4Cl caused a substantial increase (5-10 fold) in the output of uridine, but there was a lag time of 60-75 min after the first addition of NH_4Cl before any change in circulating levels was observed. Thus, it appears that high concentrations of NH_4Cl are required to modify uridine output by the liver and our predosing experiments indicate that the effect is short lived. The lag time required for the increase in circulating uridine concentrations is presumably the time necessary for the combined effects of stimulation of de novo synthesis, accumulation of uracil nucleotides, and 5'-nucleotidase action to yield an increased export of preformed uridine. Glutamine, which also stimulates de novo synthesis in hepatocytes, produced no significant change in the output of uridine by the liver when given to animals at 360 mg/kg, 30 and 45 min prior to perfusion of the isolated liver . Another endogenous modifier of de novo synthesis in isolated hepatocytes is ornithine, which stimulates the urea cycle at the expense of pyrimidine biosynthesis. Both 2 and 10 mM ornithine in the circulating perfusate reduced the output of uridine by the liver during the initial 45 min of the experiment, such that the normal "overshoot" of uridine output was prevented. However, circulating concentrations of 1-2 μM were slowly achieved, then maintained throughout the course of the experiment. Predosing a rat with 200 mg/kg ornithine 30 and 45 min prior to perfusion also inhibited the primary rise in uridine concentration in a manner similar to that achieved when ornithine is added directly to the perfusate.

The role of the liver as a supplier of purines to other tissues for salvage has long been an accepted premise. The results of Pritchard et al. (Am. J. Physiol. 229: 967-972, 1975) indicated that the liver removes hypoxanthine from the plasma and exports adenosine. With the development of new purine analogues as antitumor agents, we initiated studies in the isolated rat liver to determine the role of this organ as a supplier and regulator of purines that could modify the toxicity of these analogues in purine-requiring organs and tumors. Purine bases and nucleosides were quantitated by HPLC analysis and metabolic interconversions of added purines were determined by radiotracer methodology. Hypoxanthine and inosine were rapidly metabolized to uric acid and approximately 2% of each of these tracers was salvaged by the liver and incorporated into liver purines. Adenosine was rapidly deaminated and the resulting inosine metabolized to uric acid. Adenosine was salvaged to a greater extent by the liver with 20% of the total radioactivity being incorporated into liver purines. Adenine was found to be exported by the liver in increasing concentrations until it reached 1 μM and then plateaued. No adenosine was found in the effluent of the perfused liver, even when the liver was exposed to a 10 μM concentration of hypoxanthine. Thus, it appears that the liver exports adenine and not adenosine as had been previously postulated. Experimentation is underway to explain the discrepancy between our results and those of Pritchard et al. and to determine the importance of adenine export by the liver as a modifier of the action of purine antimetabolites.

A method was developed to measure the flux through the de novo pyrimidine pathway in tumors and organs in vivo. This method quantitates the incorporation of stable-labelled isotopes of carbon and nitrogen into nucleosides by GC-MS. It is the first method for making such determinations in vivo and allows, for the first time, an evaluation of the differential effects of inhibitors of de novo pyrimidine synthesis in normal vs. tumorous tissues. The technique quantitates the incorporation of inhaled $^{13}CO_2$ and parenterally administered ^{15}N-glutamine or ^{15}N-NH_4Cl into pyrimidine nucleosides. Also, the technique has enabled us to investigate the utilization of ^{15}N-NH_4Cl and ^{15}N-glutamine by isolated hepatocytes (i.e. the relative role of carbamyl phosphate synthetases I and II in pyrimidine biosynthesis). Initial in vivo studies with standard inhibitors of de novo synthesis revealed that treating animals with 400 mg/kg PALA 24 hrs before measurement of the flux through the pyrimidine biosynthetic pathway resulted in 70-90% inhibition of this pathway in Lewis lung tumors. In L1210 tumors similarly treated, there was no significant change in the precursor incorporation in sc implanted tumors and only a 34% inhibition of the pathway was evident in the ip tumors. In contrast to the lack of inhibition of flux through the pathway after the 24 hr pretreatment, mice with L1210 sc tumors pretreated for 1 hr with 400 mg/kg PALA showed a significant decrease in precursor incorporation of over 90%. Additionally, 48 hrs after a 400 mg/kg dose of PALA, there was no significant inhibition of the pathway in Lewis lung tumors made resistant to PALA or L1210 sc and ip tumors, but there was a 90% inhibition in normal Lewis lung tumors. Mice with L1210 ip tumors were also treated with acivicin (20 mg/kg, 2 hr pretreatment), pyrazofurin (100 mg/kg, 3 hr pretreatment) and 6-azauridine (1g/kg, 1 hr pretreatment), all of which inhibited precursor incorporation into the uracil nucleotide pool of the cells by approximately 90%. The size of the uracil nucleotide pool in the tumors was significantly reduced following PALA pretreatment at 24 and 48 hours, but not after 1 hr pretreatment. All other drug treatments did not significantly reduce the size of the uracil nucleotide pool, and may reflect the shorter time of

pretreatment chosen for these compounds. These studies will be expanded to investigate the time course for onset of inhibition, extent of inhibition, and duration of inhibition of this pathway for new and established antitumor agents and for drug combinations in different tumors. Additionally, this methodology will be used to study the effects of compounds on the flux through the de novo pathway in normal vs. malignant cells in vivo to gain insight into the mechanism(s) of selective toxicity exhibited by antitumor agents.

Studies in tumored mice and in isolated hepatocytes using the precursors ^{15}N-NH_4Cl and ^{15}N-glutamine revealed that ^{15}N enters both the N_1 and N_3 positions of the uracil molecule. The GC-MS technique was further refined to quantitate incorporation into each nitrogen separately. These values were determined for hepatic and tumorous tissues in vivo and in isolated hepatocytes using ^{15}N-NH_4Cl and ^{15}N-glutamine. In experiments in which di-labelled uracil is formed, the following components of the uracil nucleotide pool can be determined: (a) the amount of labelled product produced by the pathway, (b) the amount of non-labelled product produced by the pathway, and (c) the amount of product not produced by the pathway during the time course of the experiment. These calculations are based on probability theory and the necessary mathematical equations were derived and computer programs written to handle the computations. This is a new approach for determining such values and should prove valuable in determining the contribution of the de novo pathway to uridine pools in normal and tumorous tissues in vivo.

The stable-labelled isotope technique was exploited to gain insight into the possible inter-relationship of the urea cycle and the de novo pyrimidine pathway in hepatic tissue. An understanding of the control of pyrimidine biosynthesis in this organ that can supply preformed pyrimidines for use by other organs (including tumors) could enable us to selectively manipulate circulating pyrimidine concentrations. The mammalian liver contains two separate carbamyl phosphate synthetase enzymes (CPS-I and CPS-II). CPS-II is a cytoplasmic enzyme which catalyzes the initial rate limiting reaction of de novo pyrimidine biosynthesis. CPS-I, which catalyses the initial step in the urea cycle, is found almost exclusively in the mitochondria of hepatocytes and is in greater abundance than CPS-II in these cells. The nitrogen donating substrates with the strongest affinity for these two enzymes are glutamine (CPS-II) and ammonia (CPS-I). However, we found that ammonia stimulates de novo pyrimidine biosynthesis in the liver as measured by increased $H^{14}CO_3^-$ incorporation into uracil nucleotide pools ($\sum$uracil). In an effort to elucidate the role of ammonia and glutamine as substrates for de novo biosynthesis of pyrimidines in the liver, we used the GC-MS technique described above to measure the incorporation of ^{15}N from these two precursors into $\sum$uracil in isolated hepatocytes, cultured L1210 cells, and also into L1210 tumors and livers in the intact animal. $^{15}NH_4Cl$ and ^{15}N-glutamine are both substrates for de novo pyrimidine biosynthesis in isolated hepatocytes, cultured L1210 cells, and in mouse livers and sc tumors in vivo. ^{15}N-glutamine forms only mono-labelled uracil molecules in vivo and in L1210 cells in vitro, presumably at the N_3 position by acting directly as a substrate for CPS-II. In isolated hepatocytes however, although mono-label predominates, di-labelled uracil is also present. Thus, ^{15}N-glutamine labels aspartate pools of these cells, a process which requires the formation of NH_3 as an intermediate. Administration of $^{15}NH_4Cl$ results in only mono-labelled uracil molecules in L1210 tumors in vivo, but there is evidence that this label is in either the N_3 or the N_1 position of the uracil moiety indicating labelling of

both aspartate and carbamyl phosphate pools. Although ^{15}N-glutamine is an excellent substrate for in vitro L1210 pyrimidine biosynthesis, high concentrations of $^{15}NH_4Cl$ in these cells had an inhibitory effect and neither substrate stimulated de novo synthesis as measured by $^{14}HCO^-_3$ incorporation. In hepatocytes, increasing $^{15}NH_4Cl$ concentrations increased the incorporation of labelled precursors into ∑uracil. This lack of an inhibitory toxic effect of NH_4Cl in hepatocytes could be due to more efficient conversion of $^{15}NH_4Cl$ to glutamine in the liver. Alternatively, $^{15}NH_4Cl$ is a substrate for CS P-I which could be donated as carbamyl phosphate for utilization by aspartate transcarbamylase and this may not be subject to the feedback control of UTP as is CPS-II. Evidence for the validity of this latter theory is that in the presence of high concentrations of an inhibitor of glutamine synthetase (methionine sulfoximine) the pattern of label incorporation in ∑uracil from $^{15}NH_4Cl$ remains unchanged, indicating no dependence on glutamine formation. $^{15}NH_4Cl$ readily labels aspartate and carbamyl phosphate pools to a high degree in hepatocytes, as is evidenced by the predominance of double labelled uracil molecules. From our data, there is no evidence that either glutamine or NH_4Cl is a better substrate for pyrimidine biosynthesis in the liver. However, their incorporation into ∑uracil may occur by different mechanisms. At concentrations approximating those found in the liver, glutamine appears to be more readily utilized than NH_4Cl. However at equimolar concentrations, NH_4Cl appears to be more stimulatory and is perhaps able to circumvent feedback control mechanisms of pyrimidine de novo synthesis. An understanding of hepatic control mechanisms for pyrimidine biosynthesis, which may be unique to this organ, could be important for pyrimidine antimetabolite chemotherapy.

Thymidylate synthetase and deoxycytidylate deaminase are two critical enzymes in the de novo synthesis of thymine deoxynucleotides. Several important antitumor agents, prominently the fluoropyrimidines and methotrexate, are known to strongly inhibit thymidylate synthetase based on information from in vitro studies using isolated enzymes and from observations of drug effects on DNA synthesis. We developed a method to measure the activities of these two enzymes in intact cells using ^{3}H-5-deoxycytidine and ^{3}H-5-deoxyuridine. The tritium is released into cellular water during the methylation of the 5-position by thymidylate synthetase. Preliminary studies have shown that intracellular thymidylate synthetase inhibition in the intact L1210 cell is essentially complete at $1x10^{-5}$M 5-FU, $2.5x10^{-9}$M FUdR, and $1x10^{-5}$M methotrexate. Studies are underway to examine the time course of inhibition, the rate of reversal of inhibition on removal of the drug, and the relationship of intracellular enzyme inhibition to cell death as measured by soft agar cloning. Using the intact cell enzyme assay we showed that expanding TTP pools with $1x10^{-4}$M thymidine results in a 50% inhibition of the rate of tritium release by thymidylate synthetase; $1x10^{-5}$M thymidine shows no inhibition. Furthermore, the flux through the deoxycytidylate deaminase pathway is an appreciable percentage of the flux through the deoxyuridine kinase pathway. It appears that the deaminase pathway is approximately 50% as active as the kinase in providing the cell with deoxyuridylate.

We completed a study to determine why L1210 growth and clonogenic ability can be arrested in vitro by thymidine but not in vivo. Cytotoxic effects in vitro indicated that three logs of cell kill could be achieved at a thymidine concentration of 1mM and an exposure time of 96 hours, both pharmacokinetically achievable in vivo with minimal toxicity to the host mice.

However, extensive in vivo trials in L1210 bearing mice indicated no cytostatic or cytotoxic effect of thymidine in L1210 leukemia. In other words, a pharmocokinetic basis for the difference between in vitro and in vivo results was not acceptable. It is known that thymidine exerts its cytotoxic effects through a subsequent nucleotide metabolite (TTP), which inhibits the enzyme ribonucleotide reductase. This inhibition leads to depletion of dCTP, a necessary substrate for DNA synthesis. The salvage metabolite deoxycytidine supplied in sufficient quantity can reverse this cytotoxic effect of thymidine. We found that following thymidine treatment of L1210 bearing mice, the pretreatment plasma deoxycytidine concentration (< 1 micro molar) was raised five to ten fold. These concentrations of deoxycytidine are sufficiently high to reverse the cytotoxicity of 1 mM thymidine in vitro and therefore is the probable explanation for the resistance of L1210 to thymidine toxicity in vivo in CDF1 mice. We are now exploring reasons why deoxycytidine paradoxically increases in the plasma in vivo following high dose thymidine. Preliminary data indicate that the half-life of deoxycytidine changes from a normal 8 mins to 50 mins following a tritiated tracer iv dose of deoxycytidine two hrs after a 3600 mg/Kg ip dose of thymidine. These findings indicate that the body clearance of deoxycytidine is inhibited by the presence of 1 mM thymidine. Further HPLC analysis of plasma extracts indicate that one likely cause of the inhibition of deoxycytidine clearance is the competition of deoxyuridine and thymidine for the same catabolic enzymes. Deoxyuridine, a product of the deaminase reaction of deoxycytidine, then creates some product inhibition of the deaminase reaction to slow the catabolism of deoxycytidine. We are now conducting further studies on the flux of both carbon 14 and tritium labeled deoxycytidine into DNA of intestine, bone marrow and spleen. These studies should enable us to determine whether thymidine is selective in its biochemical modulation effects in vivo. Parallel studies are being conducted with the deoxycytidine analogue, deoxyazacytidine in L1210 bearing mice in order to determine the antitumor effect and its modulation by thymidine. Initial data indicate that the toxic dose response curve for deoxyazacytidine is shifted sharply to the left when a high dose of thymidine (3600 mg/Kg ip) is given just prior to an iv dose of deoxyazacytidine. This indicates that thymidine probably competes for the clearance of deoxyazacytidine like it did for deoxycytidine. Furthermore, at equitoxic doses, the combination of thymidine and deoxyazacytidine is no more therapeutic than deoxyazacytidine alone. This result is from only one dose of thymidine and further doses must be explored. Deoxyazacytidine alone is a potent antitumor agent against L1210 tumor, creating a six log cell kill of L1210 tumor in CDF1 mice after a single dose intravenously.

Since deoxycytidine can modify the biological effects of ara-C and thymidine, we examined the salvage of deoxycytidine by cultured L1210 cells. Deoxycytidine is phosphorylated to dCMP which can be further phosphorylated to dCDP and be incorporated into DNA as dCTP, or is deaminated to dUMP, converted to dTMP, and incorporated into DNA as dTTP. Our interest in the relative importance of these 2 possible modes of incorporation of salvaged deoxycytidine began with an examination of the Km values for pyrimidine nucleoside monophosphate kinase. This enzyme phosphorylates CMP as well as dCMP. The Km for CMP for the kinase isolated from a variety of mammalian cells ranges from 10 to 85 μM whereas the Km for dCMP ranges from 0.7 to 2.8 mM. This difference in the Km values suggested that the extent of phosphorylation of dCMP may be limited due to competition for the kinase by CMP. Thus, the deamination of dCMP and subsequent incorporation of salvaged deoxycytidine into DNA thymidine

might be an important anabolic pathway for deoxycytidine. To determine the relative rate of phosphorylation versus deamination of dCMP, L1210 cells were exposed to [2-^{14}C]deoxycytidine and the extent of incorporation of carbon-14 into the thymidine and deoxycytidine bases of DNA (expressed as a ratio thymidine/deoxycytidine) was determined by HPLC analysis. At 3 μM deoxycytidine the thymidine/deoxycytidine ratio was 3.3 (77% of the radioactivity was in the DNA thymidine and 13% in the DNA deoxycytidine) after 2 hr and 5.7 after 24 hr. At 100 μM deoxycytidine, the ratio remained constant (3.0 ± 0.2) for 24 hr. Thus the deamination of dCMP is highly favored and the percentage incorporated into DNA as dTTP increased at the lower deoxycytidine concentrations. Modified or excess natural pyrimidine were added to the culture media to see how these compounds affect deoxycytidine salvage. It was found that the extent of incorporation into DNA thymidine versus DNA deoxycytidine can be altered by the addition of thymidine, ara-C, cytidine, and 3-deazauridine.

3-Deazauridine as its triphosphate is a known inhibitor of CTP synthetase. We studied the effect of 3-deazauridine on de novo biosynthesis of uracil nucleotides in L1210 cells to determine if 3-deazauridine inhibits de novo synthesis, and, if so, is this inhibition due to an increase in UTP pools or due to 3-deazaUTP functioning as a fraudulent feedback allosteric inhibitor of carbamyl phosphate synthetase II. Experiments in which the carbon-14 labelled bicarbonate incorporation into the uracil nucleotides of 3-deazauridine treated cells was examined demonstrated that the size of the uracil nucleotide pool is unchanged at 3-deazauridine concentrations of 10 μM or lower and is decreased at 3-deazauridine concentrations of 50 μM and greater. However, inhibition of de novo synthesis of uracil nucleotides is observed such that when the intracellular concentration of 3-deazaUTP is 1 to 2.5 nmol/10^6 cells, the incorporation of labelled bicarbonate into uracil nucleotides is 25-40% of controls. When the intracellular concentration of 3-deazaUTP is 5 to 6 nmol/10^6 cells, bicarbonate incorporation is only 15-20% of controls. Thus, 3-deazaUTP appears to be acting as a fraudulent feedback inhibitor of de novo pyrimidine biosynthesis.

Previous studies from our laboratory showed that ara-C 5'-methylphosphonate inhibited dCMP kinase but was not a substrate for this enzyme. FUdR-5'-methylphosphonate (FUdR-MeP) was designed and synthesized with the following expected properties: (1) would be an analogue of FdUMP that did not require activation by TdR kinase; (2) would not be susceptible to phosphatase attack; (3) would penetrate cell membranes; (4) would inhibit dTMP synthetase; and, (5) would not be further phosphorylated by dTMP kinase. FUdR-MeP was found to be a competitive-irreversible inhibitor of isolated dTMP synthetase; inhibition required the presence of cofactor; and the affinity constant for FUdR-MeP was several orders of magnitude less than that for FdUMP. Inhibition of flux through dTMP synthetase in intact L1210 cells by FUdR and FUdR-MeP followed the same concentration dependency as observed with the isolated enzyme. The methylphosphonate analogue of dUMP did not inhibit dTMP synthetase and did not inhibit L1210 cell growth. FUdR-MeP inhibited L1210 cell growth at concentrations 1000-fold higher than FUdR; inhibition was reversed by thymidine.

Tissue culture experiments (L1210 growth and clonogenic assays) were done to evaluate the possibility of modifying the dose response curves of several chemotherapeutic drugs using mildly toxic levels of photodynamic injury provid-

ed by light and hematoporphyrin. Interest in this approach was stimulated by reports of modification of membrane transport induced by such photodynamic injury coupled with the reported phenomenon of increased hematoporphyrin localization in some tumors. Hematophoryrin is used clinically to create phototoxicity to surface tumors. It is also used with fiber optics to deliver light to certain cavity-localized tumors but the synergistic effects of chemotherapeutic agents has not been evaluated. A system using prolonged hematoporphyrin incubation and standardized illumination was worked out which allowed reproducible tissue culture experiments to be done to measure the combined toxic effects of photodynamic injury and chemotherapy. Introduction of a paired control experimental design was important in achieving reproducible measurements of the two effects in combination, both of which have steep dose-response curves which are sensitive to minor perturbations in experimental technique. It was found that reproducible enhancement in Melphalan and Actinomycin-D dose response curves could be measured. The effects were not as large as would have been suggested by transport experiments using Actinomycin-D that were reported by other experimenters.

Anthracycline-induced cardiotoxicity is an important problem in the clinical use of these agents. Studies were continued into the biochemical mechanism for this toxicity. Utilizing the isolated rat heart cell system developed in this Laboratory to test the relative cardiotoxicity of anthracycylines, studies were directed at identifying the intracellular sites of action. A protein was isolated from heart tissue that appears to be an intracellular receptor for anthracyclines. Identification of this high molecular weight, high affinity mitochondrial protein may provide insight into the long sought mechanism of cardiotoxicity of this important class of antitumor drugs.

A basic understanding of the movements of drugs and physiological materials between blood, brain, CSF, and systemic tissues is essential for an appreciation of normal CNS and systemic functions as well as the changes in these transport systems caused by various CNS and systemic malignancies. Sound experimental methods must be used to perform scientifically reliable transport studies. Therefore, various improved techniques for measuring and analyzing transport phenomena have been developed. Among these developments are: (1) better methods of determining blood-brain and blood-tissue transfer constants for materials of low to moderate permeability; (2) a quantitative and more relevant assessment of drug delivery and uptake by normal and tumorous tissue in terms of tissue extraction fractions and microvascular permeability-surface area products; and (3) double-label quantitative autoradiographic and co-imaging techniques to facilitate regional tissue analysis and correlation. These various techniques will be used by other research groups who are interested in doing pharmacological, physiological and biochemical studies of the blood-brain-CSF system and are being expanded to include regional analysis of systemic tissues as well. Drug delivery and drug delivery systems have been investigated since both are important for effective chemotherapy. Drug administration systems involving intra-arterial, intravenous and intraperitoneal routes of delivery are being studied. Experimental analysis of intra-arterial administration of drugs provides experimental confirmation of our previous theoretical analysis of this route of drug delivery. For a model drug and model system (the rat) the theoretical maximum exposure (concentration x time) advantage is given by the ratio of cardiac output (300 ml/min) to the infused artery blood flow (10 ml/min) and was calculated as 30. Our experimental data for this system yielded an intra-arterial to

intravenous concentration x time advantage of 21 for a two min experiment. Intra-arterial administration in comparison to intravenous administration of drug may also result in lower drug delivery to other systemic tissues and thus, less systemic toxicity. This, too, was experimentally and theoretically examined with our model drug and biological system. Our theoretical analysis indicated that about 25% less of our model drug would be systemically delivered following an intracarotid infusion than an intravenous one due to brain uptake and metabolism of the drug. Our experimental data indicated that 20-30% less of our model drug had actually been systemically delivered with an intracarotid infusion than with an intravenous infusion. These results show that the maximum advantage in drug delivery to a target organ is set by the ratio of the cardiac output to the flow rate through the infused (target organ) artery and that the reduction in drug delivery to nontarget systemic tissues is set by the rate of metabolism or chemical breakdown within the target tissue. Moreover, further theoretical analysis and other experimental data have indicated that slow systemic metabolism and excretion diminish the advantage of an arterial infusion for increasing drug exposure in the target organ.

A new blood sampling technique for continuously obtaining arterial plasma and/or an ultrafiltrate of plasma from an arteriovenous shunt is being developed. The technique involves a new, specifically-designed filter system that can be inserted into an arteriovenous shunt and permits the continuous withdrawal of plasma and/or plasma ultrafiltrate. Initial testing of the filter system by three different arterial-blood withdrawal techniques demonstrated that the theoretical and experimental arterial plasma concentration-time courses were consistent. These results indicate that the plasma filter system functions well for compounds that are known not to bind to plasma proteins or to slowly enter red blood cells. Further testing with drugs which are known to bind to plasma proteins are planned. This work is currently being done in rabbits and will be applicable for human pharmacokinetic studies.

Previous analysis of blood-brain transfer of various solutes and drugs demonstrates that there is a good correlation between a drug's lipid solubility and blood-to-brain influx for only a limited portion of the lipid solubility range, namely, for olive oil/water partition ratios less than 0.01 or octanol/water partition ratios less than 0.10. Within these ranges of partition ratios, the higher a drug's lipid solubility the more readily it moves across the blood-brain barrier. For drugs with olive oil/water partition ratios greater than 0.1 or octanol/ water ratios greater than 1.0, their velocities of blood-to-brain transfer were limited by the rate of blood flow, that is, the speed of drug delivery to the tissue. Thus, tissue blood flow and not lipid solubility primarily determine tissue delivery of drugs with oil/water or octanol/water partition coefficients above these values.

This laboratory has had a long-term committment to studies of the carcinogenic properties of antitumor agents and other model compounds. The availability of a large breeding and experimental colony of nonhuman primates continues to provide this laboratory and other cooperating units with a unique resource for studies of chemical carcinogenesis as well as other comparative pharmacologic, toxicologic and biochemical studies. Since the inception of this study 20 years ago, 7 spontaneous tumors have been diagnosed in 219 non-treated breeders and vehicle-treated controls, yielding a tumor incidence of 3.2%. Of the 27 substances entered on test during this period, 15 [3-methylcholanthrene, dibenz

(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, N,N'-dimethyl-p-(m-tolylazo)-analine, cyclamate, saccharin, dichlorodiphenyltrichloroethane, low density polyethylene plastic, cigarette smoke condensate, arsenic, Imuran, L-phenylalanine mustard and cyclophosphamide] have not as yet induced tumors. However, several of these compounds have been under evaluation for less than 3 years.

Eight of the 27 substances evaluated have induced malignant neoplasms in non-human primates, producing a tumor incidence ranging from 19-100% of the treated animals. The compounds are: N-nitrosodiethylamine, 1-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B_1, methylazoxymethanol acetate, procarbazine, methylnitrosourea, and urethane. In addition, single cases of malignant tumors have been diagnosed in animals treated with adriamcyin, N-methyl-N'-nitro-N-nitrosoguanidine, sterigmatocystin, and N,N'-dimethyl-p-phenylazoanaline (butter yellow).

DENA is highly predictable as a carcinogen in Old World monkeys, inducing hepatocellular carcinomas when given either orally or by ip injection. In one study, we are using DENA as a model hepatocarcinogen in Old World monkeys to examine the relationship between chronic (milligrams per kilogram) dose, cumulative dose, and latent period for tumor induction. To this end, groups of monkeys are being given bimonthly ip injections of DENA at doses of 0.1, 1, 5, 10, 20, and 40 mg/kg and are observed for the appearance of tumor. In the five groups of monkeys in which tumors have developed, we have found that the latent period increases as the milligram-per-kilogram dose decreases. Thus, the latent period at the 40 mg/kg dose averages 17 months, for the 20 mg/kg dose it is 26 months and at 10 mg/kg it is 37 months. A semilog plot of these 3 mg/kg doses of DENA against the latent periods for tumor induction yields a straight line which intercepts the y-axis at approximately 88 months. This point on the ordinate corresponds to a DENA dose of 0.1 mg/kg. Thus animals in the 0.1 mg/kg group should develop tumors after a latent period of 88 months if the relationship between the milligram-per-kilogram dose and latent period is strictly linear; however, the animals at this dose have only been on study approximately 22 months. Extrapolation from the curve to the ordinate for the 1 mg/kg group yields a latent period of 60 months, although 90% of the animals in this group remain tumor-free after an average of 88 months of observation. The tumors developing in the 8 animals receiving the 5 mg/kg dose required a latent period averaging 69 months, a figure which shows a marked deviation from the value (42 months) expected if the relationship between dose and latent period were linear. Our results thus far indicate that this relationship departs from linearity at lower chronic doses of DENA.

Although the dose rate has a significant effect on the length of the latent period, it does not appear to be of importance in determining the minimum cumulative DENA dose required for tumor induction. The averages for this figure range between 1.4 gm (40 mg/kg) and 3.1 gm (5 mg/kg), with cumulative DENA doses as low as 0.39 gm inducing tumors in individual animals.

Two other nitrosamines have induced primary hepatocellular carcinomas in monkeys. N-nitrosodipropylamine (DPNA) induced liver tumors in all 6 of the rhesus and cynomolgus monkeys given bimonthly ip doses of 40 mg/kg. The average total dose of DPNA was 7.0 gm; the average latent period for tumor development was 28.5 months. 1-nitrosopiperidine (PIP) is also an hepatocarcinogen in macaques. Hepatic cell carcinomas developed in 11 of 12 monkeys receiving

this compound by the oral route, and in 5 out of 11 monkeys treated by the ip route. The average cumulative dose necessary for tumor induction by PIP given orally (1742.5 gm) was higher than for oral DENA (18.0-55.1 gm); similarly, the average cumulative dose of PIP given by the ip route (39.4 gm) exceeded that required for tumor induction by ip DENA (1.7 gm) or ip DPNA (7.0 gm).

Aflatoxin B_1 (AFB_1), a product of a mold (Aspergillus flavus) known to contaminate some human foodstuffs, is carcinogenic in a variety of experimental animals. The carcinogenicity of AFB_1 has been under evaluation in non-human primates for the past 16 years. A total of 47 Old World monkeys, chiefly rhesus and cynomolgus, has received AFB_1 by ip (0.125-0.25 mg/kg) and/or oral (0.1-0.8 mg/kg) routes for 2 months or longer, and 2 are currently alive. Twenty-one of the 45 monkeys necropsied to date developed a total of 27 malignant neoplasms, yielding an overall tumor incidence of 45%. Five of the 21 tumor-bearing monkeys developed hemangioendothelial sarcomas of the liver, 6 developed bile duct or gallbladder adenocarcinomas, and 2 cases of hepatocellular carcinoma were diagnosed. Two monkeys developed osteosarcomas and 6 were found at necropsy to have multiple primary tumors. All of the latter animals possessed adenocarcinoma of the pancreas and/or gallbladder or bile ducts as well as urinary bladder carcinoma, fibrosarcoma and osteosarcoma. The tumors diagnosed in the 21 monkeys developed after latent periods ranging from 49-186 months, and after cumulative AFB_1 doses of 99-1472 mg. Fifteen of the 23 (65%) necropsied monkeys without tumor showed histologic evidence of liver damage, including toxic hepatitis, cirrhosis and hyperplastic liver nodules. Our results indicate that AFB_1 is a potent hepatotoxin and carcinogen in non-human primates, and further support the hypothesis that humans exposed to this substance may be at risk of developing liver cancer.

Cycasin, the active principle in the cycad nut, induces liver and kidney tumors in rats and may be a human carcinogen as well. The carcinogenic potential of cycasin and its aglycone, MAM-acetate, is under investigation in non-human primates. Old World monkeys (rhesus, cynomolgus, and African greens) received cycasin and/or MAM-acetate by po or ip routes for periods up to 12 years. Eighteen monkeys survived > 2 mo after initiation of treatment with cycasin (50-75 mg/kg) or MAM-acetate (1.5-3.0 mg/kg) given po daily 5 days/week, and 11 of the animals have been necropsied. Histopathologic examination of tissue from one of these monkeys revealed hepatocellular carcinoma. A second monkey was noted to have multiple tumors, including hepatocellular carcinoma, intra-hepatic bile duct adenocarcinoma, renal carcinoma and adenomas, and adenomatous polyps of the colon. An adenocarcinoma of the pancreas was diagnosed in a third monkey. Although liver tumors were not observed in the other monkeys, all but one had hepatic lesions such as toxic hepatitis and cirrhosis. A group of 10 monkeys received MAM-acetate by weekly ip injections (3-10 mg/kg). Six of these animals developed tumors after receiving an average of 6.14 gm (range 3.58-9.66 gm) of MAM-acetate for an average of 75 mo (range 50-89 mo). Four of the monkeys developed hepatocellular carcinomas and 2 had multiple primary tumors including hepatocellular carcinomas, renal carcinomas, squamous cell carcinomas of the esophagus and adenocarcinomas of the small intestine. Our results show that MAM-acetate is a carcinogen in monkeys, and add to the evidence that cycasin and its aglycone may be carcinogenic in man.

The carcinogenicity of urethane, with or without whole body irradiation (WBI) was evaluated in rhesus and cynomolgus monkeys. Monkeys received urethane (250 mg/kg) orally, 5 days every week beginning within one month of birth. They

received continuous urethane treatment for 5 years, during which time some monkeys also received 3-10 weekly courses of WBI at 50 rads per course. Urethane administration was discontinued 12-15 years ago and since that time all animals have been held under close observation for development of tumor or other adverse effects of treatment. Thirty of a total of 40 monkeys survived 6 months or longer after the first dose of urethane, and 23 of these animals have been necropsied. A total of 8 malignant tumors were found in 5 (16.7%) of the 30 treated monkeys; in comparison, 7 of a total of 219 (3.2%) control monkeys have developed tumors during this period. One or more primary liver tumors (3 cases of hemangiosarcomas, 1 case of adenocarcinoma of intrahepatic bile ducts, 1 case of hepatocellular carcinoma) were present in 4 monkeys; one of the monkeys with a liver hemangiosarcoma was also found to have an ependymoblastoma, and the fifth monkey developed a pulmonary adenocarcinoma. The animals with tumor had received an average cumulative urethane dose of 260 gm (range 230-339 gm); the latent period for tumor induction averaged 171 months (range 142-229 months). Two of the 5 monkeys developing tumors had received 9 and 10 courses of WBI, respectively. These results indicate that urethane, with or without WBI, is carcinogenic in monkeys; however, the latent period for tumor induction (> 14 years) is long, requiring approximately 50% of the usual lifespan of Old World monkeys in captivity.

Squamous cell carcinoma of the mouth, pharynx, and esophagus developed in 12 of 44 monkeys (27%) receiving oral doses (10-20 mg/kg) of 1-methyl-1-nitrosourea (MNU). Moreover, upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 21 monkeys necropsied to date. All monkeys except one that have received total doses of MNU exceeding 50 grams have developed carcinomas, whereas no malignant tumors have developed in monkeys receiving a cumulative dose less than 50 grams. The average latent period for tumor development was 111 months and ranged between 57-168 months. The order of appearance of the esophageal lesions in our monkeys, as well as the clinical manifestations of the tumors, resembled those seen in humans, and included difficulty in swallowing, frequent vomiting and subsequent weight loss, and sialorrhea. The common complications of esophageal carcinoma in humans (e.g., regurgitation, aspiration, sepsis and hemorrhage) were also noted in our monkeys. In addition, histological examination revealed a morphology similar to that seen in human esophageal carcinomas, despite the highly variable nature of such tumors in both humans and monkeys. Thus, MNU-induced esophageal carcinoma in non-human primates may prove to be a valuable model for the study of the human tumor.

Fifty monkeys have survived for longer than 6 months after receiving treatment with procarbazine by sc, ip and/or oral routes at doses of 5-50 mg/kg. Forty-one monkeys have been necropsied to date, of which 14 (34%) have had malignant neoplasms. Seven monkeys were diagnosed with acute leukemia, all but one of the myelogenous type; the other acute leukemia was undifferentiated. In addition to the leukemias, two cases of hemangiosarcoma, three cases of osteogenic sarcoma, one case of astrocytoma and one case of lymphocytic lymphoma, was found. The neoplasms were diagnosed after procarbazine treatment for an average of 94 months (ranging from 16 to 192 months); the total dose of procarbazine received by the monkeys developing tumors ranged from 2.69-154.4 gm, and averaged 55 gm. Other adverse effects of long-term procarbazine treatment included vomiting, myelosuppression and testicular atrophy with complete aplasia of the germinal epithelium.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03500-18 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluids

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.0	5.5	3.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Drug delivery systems of the brain, brain tumors, plus other tissues and tumors were studied. Single-and double-label and whole-body quantitative autoradiography were the major techniques employed. The rat tumor models examined included the ENU-induced oligodendroglioma, the RT-9 intracerebral and flank gliosarcomas, and the ASV-induced astrocytoma. For most small brain tumors, blood flow and transcapillary influx (the two major components of intravascular drug delivery) were similar to that of normal brain; however for large brain tumors, blood flow was generally reduced but transcapillary influx ranged from normal to greatly increased. Misonidazole delivery in the RT-9 flank tumor was mainly limited by blood flow. Intraperitoneal administration of test solutes yielded good delivery throughout the peritoneal cavity but lesser penetration into the intestine than the abdominal wall and diaphragm. Intra-arterial drug infusions achieve increased exposure of the target organ and decreased exposure of other tissues when drug is rapidly metabolized, blood flow through the infused artery is slow, and infused drug is rapidly broken-down in the target organ. The relative rates of drug transfer across the blood-brain barrier are not proportional to drug lipid solubility when octanol/water ratios exceed 1.0.

Project Description:

Methods Employed:

Animals and animal tumor models

Most of our experimental work is done with in vivo systems and involves the administration of radioactively labelled materials into the vascular compartment, repeated sampling of arterial blood, killing the animals at various times, and obtaining tissue and fluid specimens for radiotracer analysis. Since the organ system of major interest to us is the central nervous system (CNS), we seek experimental processes which cause minimal perturbations of the state of the animal, CNS function and the system we are examining. To accomplish this goal, ways of performing many of these types of experiments on unanesthetized animals have been adopted. Animals which have been studied include the rat, rabbit and monkey.

During the past year, pathophysiological studies have been made on five different brain tumor models. The first is the Walker 256 metastatic carcinoma model of Ushio, Chernik, and Shapiro and is being studied in collaboration with Dr. Shapiro, Memorial Sloan-Kettering Cancer Center. For this model tumor cells are injected into one carotid artery of the Wistar rat, and they subsequently lodge throughout head and neck tissues. After about two weeks, the animal is treated with intravenous cyclophosphamide which eradicates all tumor cells except those in the brain. Weight loss and neurological signs which indicate the presence of CNS tumors appear after an additional 7-14 days.

The second brain tumor model under examination is an ethylnitrosourea induced primary brain tumor in rats. This model was developed by Dr. Jerry M. Rice, Laboratory Comparative Carcinogenesis, DCCP, NCI, and previously reported by him.

The third and fourth brain tumor models are produced in collaboration with Dr. Dennis Groothuis, Evanston Hospital, Northwestern University. The first of these models is generated by injecting an avian sarcoma virus suspension into the cerebral cortex of young rats; this procedure usually produces two or more gliomas per rat. The second of these models uses RG-2 astrocytoma cells grown in tissue culture and implanted into the brains of young rats where they form one or more tumors.

The fith model was developed in collaboration with the Surgical Neurology Branch, NINCDS. This tumor is produced by implanting a suspension of RT-9 tumor cells, originally derived from a nitrosourea induced astrocytoma, in the brains of young adult Fisher rats. After several weeks tumor masses grow within and around the brain.

The "systemic" growth of two of the above described brain tumors have been studied during the past year. The tumor cells used in these studies were derived from the RT-9 and RG-2 systems and subcutaneously implanted in the flanks of rats where they formed relatively large solid tumors.

Experimental Procedures

Animals were prepared for the vascular infusion (blood-to-tissue) studies in the following manner. The procedure consists of inducing anesthesia with halothane or pentobarbital, rapid cannulation of the femoral arteries and veins through a small incision. For those animals (primarily rats) recovering from anesthesia, the wound was covered with xylocaine jelly, the animal's lower quarters wrapped in plaster, the animal secured by its plaster cast to a lead brick, and halothane gas removed, thus allowing the return of consciousness. Most of the animals quickly recover from halothane anesthesia, seem alert and comfortable, and rapidly reach normal awake levels of blood gases and pressure. Labelled materials and drugs can be injected through the venous catheters and blood samples and pressure readings can be easily obtained via the arterial catheters.

Currently we are using quantitative autoradiography and standard isotope spectroscopy to measure regional tissue and blood (plasma) radioactivity for a number of different processes in the intact animal. Cerebral or other tissue blood flow is determined from the distribution of 14C-, 125I-, or 131I-iodoantipyrine, local glucose utilization by the uptake of 14C-2-deoxyglucose, and protein turnover by the accumulation of 14C-leucine. Measurements of capillary and cellular permeability are made with a number of physiological markers such as 14C-α-aminoisobutyric acid (AIB) and 14C-sucrose. We have just completed studies of the distribution of 14C-methotrexate, 14C-thymidine, and 14C-misonidazole in normal and tumor-bearing animals by quantitative autoradiography.

Tissue Analysis

For most of the intravenous administration experiments, the tissue concentrations of various 14C-labelled substances were determined by quantitative autoradiography (QAR). With this technique, "microdissection" of organs and physiological processes can be carried out with a resolution of about 50 to 100 microns. Attempts to expand this methodology to other radioactive isotopes have been successful. Several Auger electron emitting isotopes have been found to produce reasonably sharp radioautographs and useable standards for quantitation.

Double-label quantitative autoradiography has been extensively employed in our section during this year. For double-label studies, one radioisotope is carbon-14 and the other is an Auger electron emitting nuclide of relatively short half-life and higher emission energy than carbon-14, for example, iodine-131. A thin mylar sheet is placed between the tissue sections and the X-ray film. The mylar film absorbs all the 14C-radiation but only a small portion of the more energetic Auger electrons, which subsequently expose the X-ray film. This autoradiographic X-ray image reflects the regional tissue distribution of the Auger electron-emitting isotope alone. After the period of Auger electron exposure is over and sufficient time has elapsed for the Auger electron emitter to decay away (15 half-lives), a new X-ray film without the mylar absorber is placed over the tissue sections and exposed for detection of carbon-14. This second autoradiographic X-ray image reflects the regional tissue distribution of carbon-14 alone. Thus, two single-isotope images are produced on two dif-

ferent X-ray films from double-label autoradiographic experiments and these films can be analyzed separately.

Since only 14C-methylmethacrylate standards are commercially available for QAR, a method had to be devised for making standards for the Auger electron-emitting nuclides used in our autoradiographic studies. Homogenates of brain or other organs are prepared in a tissue grinder. A stock solution containing the radioactive material in sufficiently high concentration to extend beyond the range of radioactivity anticipated in the actual experiments is prepared and mixed with part of the tissue homogenate. A portion of this mixture is serially diluted with tissue homogenate and the resultant series processed for cryosectioning and autoradiographic exposure. The remaining portions of the standard series is processed for either gamma or beta spectroscopy to determine radioactivity per unit mass of standard. A standard series is always placed along with tissue sections into each cassette with X-ray film for autoradiographic exposure.

Quantitation from the autoradiographic images is performed by sequential measurements of optical density within 50 x 50 μ elements of the x-ray film using a computerized high speed scanning microdensitometer. To convert the x-ray film images to tissue radioactivity (nCi/g), the optical densities of the images produced by the standards are measured and a standard curve which relates optical density to tissue radioactivity is generated for each film. The optical density data stored by the computer is converted to radioactivity using the standard curve described above and displayed on a video monitor using an image array processor.

The analysis of autoradiograms produced from sections that include pathologic tissue is particularly difficult in the absence of normal anatomic relationships. One way to overcome this difficulty is to project the image of the same or an adjacent tissue section, which had been fixed and appropriately stained, with a standard microfilm viewer. Selected tissue areas can be identified on the histologic image and defined on the autoradiographic image by using grid overlay matching of the two images and a computer controlled cursor-outlining routine on the video monitor. The mean value and standard deviation of all measurements within the outlined area of the autoradiographic image can be determined by the computer. Thus, specific regional measurements, which are based on anatomic and histologic relationships in the same or an adjacent tissue section, can be made with reasonable accuracy.

Two variations of the above QAR methodology have been devised by our group. One of these variations enables us to do simple solvent extractions on the frozen and dried tissue section and determine the amounts of soluble and bound radioactive nuclide present in the tissue; this has been employed in our studies of the distribution of 14C-thymidine. The other variation allows us to histologically prepare for microscopical examination the very same tissue section which was used for QAR (in the past the histological data was gained from the fixing and staining of the tissue section adjacent to the one taken for QAR); using the same section for autoradiography and histology results in more precise correlations between the morphology and physiology of the tissue.

Blood analysis

A new technique is being developed in collaboration with Drs. Bungay and Dedrick of Biomedical Engineering to continuously obtain arterial plasma and/or an ultrafiltrate of plasma from an arteriovenous shunt. The technique involves a new, specially-designed filter system (Biomedical Engineering) that can be inserted into an arteriovenous shunt and permits the continuous withdrawal of plasma and/or plasma ultrafiltrate. This work is currently being done in rabbits and will be applicable for human pharmacokinetic studies.

Intra-arterial drug administration

We have developed an experimental system for evaluating intra-arterial drug delivery in collaboration with Drs. Harper and Johns, Laboratory Medicinal Chemistry and Biology, DCT. This approach entails the cannulation of the femoral arteries and veins plus the external and common carotid arteries of the rat and the simultaneous administration of two different isotopically labeled forms of the test drug -- one into the common carotid artery and the other into the femoral vein. Samples of arterial blood are subsequently taken from the external carotid and femoral arteries at identical times, and the concentrations of the intra-arterially and intravenously infused drug (distinguishable on the basis of their differing radioactive tags) in these samples and different brain regions are ascertained and compared. If the concentration-time product or exposure is significantly greater for the "intra-arterial" form of the drug than for the "intravenous" form, then the intra-arterial route of delivery for this drug is deemed superior.

Intraperitoneal drug administration

The distribution of drugs following intraperitoneal administration and the transfer of solutes and water during peritoneal dialysis is being studied in an animal (rat) model. Radiolabeled materials are introduced into the peritoneal cavity and/or the vascular system. At various times thereafter, the animals are killed, frozen and whole-body thin (30-50μ) sections are obtained with a large sledge microtome. The radioactivity in the tissue is measured by quantitative autoradiography and the transport of the labeled materials between blood, intraperitoneal fluid, and tissue surrounding the peritoneal cavity is calculated from the resulting autoradiographic data.

Intravenous drug administration

We have devised and employ a special technique for our studies of drug distribution which supplies more specific information on blood tissue exchange than any other method currently available. With this approach, a blood-flow marker plus the test drug are administered intravenously during the course of the experiment and both the rates of blood flow and of drug influx are determined. Very short experimental periods (30 sec- 10 min) are chosen; this strategy allows us to estimate the initial rate of blood-to-tissue transfer for the test drug and minimizes the roles of tissue compartmentation and metabolism in the overall distribution of the drug. Finally, we calculate an extraction fraction and a permeability-surface area product of the tissue microvasculature for each drug from its rate of blood-to-tissue transfer and

the blood flow.

Major Findings:

A. Drug administration systems:

Intra-arterial drug administration

Our experimental analysis of intra-arterial administrations of drugs was undertaken to obtain experimental confirmation of our previous theoretical analysis of this route of drug administration. For a model drug (we chose 2-deoxyglucose or 2-DG) and model system (we used the rat carotid artery), the theoretical maximum exposure (concentration x time) advantage is given by the ratio of cardiac output (300 ml/min) to carotid artery flow (10 ml/min) and was calculated as 30. Our experimental data for the carotid artery yielded a concentration x time advantage for an arterial administration of 21 for a two minute experiment. This agreement between theory and experimental results is excellent since the 2 minute experimental measurement results in an underestimation of the actual value.

Intra-arterial administration in comparison to intravenous administration of drug may also result in lower drug delivery to other systemic tissues and thus, less systemic toxicity. This, too, was experimentally and theoretically examined with our model drug and biological system. Our theoretical analysis indicated that about 25% less of our model drug would be systemically delivered following an intracarotid infusion than an intravenous one due to brain uptake and metabolism of the drug. Our experimental data indicated that 20-30% less of our model drug had actually been systemically delivered with an intracarotid infusion than with an intravenous infusion.

These results show that the maximum advantage in drug delivery to a target organ is set by the ratio of the cardiac output to the flow rate through the infused (target organ) artery and that the reduction in drug delivery to non-target tissue is set by the rate of metabolism or chemical breakdown within the target tissue. Moreover, further theoretical analysis and other experimental data have indicated that slow systemic metabolism and excretion diminish the advantage of an arterial infusion for increasing drug exposure in the target organ.

Intra-peritoneal drug administration

Evans blue dye and 125I-albumin were used as tracers for our study of the intraperitoneal distribution of drugs. They both indicated that all parts of the peritoneum were reached by the administered solution. The abdominal wall and, in particular, its lateral-posterior portion were deeply stained by the dye and contained high concentrations of 125I-activity. Similarily high levels of dye and 125I-activity were found on the diaphragm. Lower concentration of 125I-activity were evident in the intestine. Clear differences in the gradients of 125I-activity from the peritoneal surface into the adjacent tissue were found among the diaphragm, the abdominal wall, and the intestine. The implications of these differences in terms of drug delivery are currently under investigation.

Blood sampling technique

Initial testing of the newly developed arteriovenous shunt - constant withdrawal system was performed in the following manner. The arterial plasma

concentration-time integral (Cxt) of several model compounds was determined by three different techniques: a) multiple arterial blood sampling and plasma measurements; b) constant arterial blood withdrawal and a single plasma measurement; c) constant arterial plasma withdrawal through the newly-developed filter system. In all cases the arterial plasma concentration-time integrals determined by the three methods were essentially identical for the initial set of model compounds. Thus, the theoretical and experimental arterial blood/plasma concentration-time courses were shown to be consistent and indicates that the plasma filter system functions well for compounds that are known not to bind to plasma proteins or to slowly enter red blood cells.

Lipid solubility

Our analysis of blood-brain transfer of labeled solutes demonstrates that there is a good correlation between a drug's lipid solubility and blood-to-brain influx for only a limited portion of the lipid solubility range, namely, for olive oil/water partition ratios less than 0.01 or octanol/water partition ratios less than 0.10. Within these ranges of partition ratios, the higher a drug lipid solubility the more readily it moves across the blood-brain barrier. For drugs with olive oil/water partition ratios greater than 0.1 or octanol/water ratios greater than 1.0, their velocities of blood-to brain transfer were limited by the rate of blood flow, that is, the speed of drug delivery to the tissue. In other words, all of these drugs move across the blood-brain barrier at a similar rate, the blood flow rate. For drugs with olive oil/water partition ratios of 0.01-0.1 or octanol/water partition ratios of 0.1-1.0, their velocities of blood-to-brain transfer are only partly dependent on their lipid solubility. Within these ranges of partition ratios, a drug of higher lipid solubility will pass across the blood-brain barrier at only a slightly higher rate than a drug of lower solubility because drug uptake in this situation depends on both lipid solubility and blood flow.

B. Specific drug studies:

Misonidazole

Double-label quantitative autoradiographic studies of 14C-misonidazole distribution and blood flow were performed in RT-9 brain and flank tumors. The larger tumors consist of a relatively narrow outer rim of viable anaplastic and pleomorphic cells and a relatively large central region composed of necrotic and cystic regions separated by cords of viable tumor cells. 14C-misonidazole and labeled metabolites distributed rapidly and uniformly in brain and tumor tissue within 30 minutes; regional tissue blood flow in brain and brain tumor did not influence the 30 minute distribution. Regional brain tumor blood flow always exceeded 5-10 ml/100g min. Studies at 2 and 4 hours demonstrated a heterogeneous distribution pattern of misonidazole-derived 14C-radioactivity which correlated in part to viable-appearing cells around necrotic foci. At 4 hours an average tumor high/low activity ratio of 4/1 and

an average tumor-high/brain ratio of 5/1 was measured.

The distribution pattern of 14C-misonidazole derived radioactivity in RT-9 flank tumors was quite different from that describe above. The highest activity of ^{14}C-misonidazole derived radioactivity was always located within the tumor rim and the lowest activity was always within the central region. Regional tissue blood flow matched the regional distribution of 14C-misonidazole derived radioactivity. The highest rates of blood flow (2-10 ml/100g min) were found around the outer rims of the flank tumors whereas the lowest rates (<0.5 ml/100g min) were found in the center of these tumors. The low blood flow in central tumor regions resulted in levels of misonidazole 14C-derived radioactivity that were <5% of that in plasma. Since the extraction of 14C-misonidazole derived radioactivity was 30% or more in the center of the RT-9 flank tumors, the delivery and distribution of misonidazole derived 14C-radioactivity was mainly limited by blood flow, and not capillary permeability, in these areas.

Methotrexate

The regional entry of 14C-methotrexate (MTX) into brain and Walker 256 brain tumor of rats was measured using quantitative autoradiography both without and with intracarotid hyperosmolar mannitol opening of the blood-brain barrier. MTX concentration was heterogeneous in tumors (1.8 to 14 µg per gram at 15 minutes and 0.9 to 9.14 µg per gram at 1 hour), while distant brain concentrations were at or below 0.5 µg per gram (blood-to-brain transfer constant, K = 0.000093 ml/g min). MTX diffused from the central regions of the tumor toward the edge, declining exponentially into the brain adjacent to tumor (BAT). Intracarotid mannitol increased MTX concentrations in cortex of distant brain (3.3 µg per gram), although white matter did not exceed 0.22 µg per gram. Brain tumor concentrations were again heterogeneous (up to 10.8 µg per gram) but did not exceed nonmannitol controls. MTX in BAT was as high as that in areas distant from tumor. These data demonstrated that MTX in the Walker 256 brain tumor was not increased by intracarotid mannitol, while MTX in brain distant from tumor was markedly increased.

C. Regional blood flow, transport and metabolism in various tumor models:

Regional Blood Flow

Blood flow was usually reduced in medium and large size brain tumors in comparison with that in small tumors and surrounding brain tissue. This pattern was generally observed in all five of the brain tumor models studied.

For the Walker 256 metastatic tumor, blood flow within small tumors (<1mm in diameter) was indistinguishable from that of surrounding brain tissue; this was true for these small tumors in both gray and white matter. In larger tumors (2 to 5 mm in diameter) blood flow was reduced and inversely related to tumor size. Central tumor regions consistently had the lowest values of flow. Blood flow was less than 10 ml/100g min in the necrotic areas of these tumors, while the histologically viable-appearing regions of the tumors were not associated with blood flow values of less than 20 ml/100g min. Blood flow in the brain around the larger tumor masses was also reduced with values 50 percent

of normal frequently observed.

The ENU-induced, well-differentiated oligodendrogliomas all had similar values of tissue blood flow, which were the same or slightly less than that found for normal white matter. Necrotic centers were not usually observed in this series of tumors despite their relatively large size.

Blood flow in the RT-9 tumors was slightly to moderately lower than surrounding brain tissue; overall tumor values ranged between 25 and 87 ml/ 100g min. In general, flow was inversely related to tumor size. Blood flow varied within each tumor; central tumor regions consistently had lower values of flow than the more peripheral regions. Tumors that grew back along the needle track and expanded over the convexity of the brain demonstrated prominent sarcomatous changes and had slightly lower values of blood flow than intraparenchymal tumors of similar size. This observation suggests that the host tissue vasculature influences the magnitude of blood flow in the tumor.

The ASV-induced group of tumors demonstrated the greatest variation in blood flow between different tumor cytologic/histologic characteristics, and large variations in flow were frequently measured within individual tumors. Blood flow was usually lower within central tumor regions than in peripheral tumor regions; however, the opposite relationship was occasionally observed. Tumors located in cortex or other grey matter structures usually, but not invariably, had lower values of blood flow than surrounding and contralateral brain tissue; whereas, tumors growing in white matter structures usually had higher blood flow than the surrounding "normal" tissue. An increase in tumor size was usually associated with a reduction in blood flow; however one large polymorphic tumor had a mean flow value of 95 ml/100g min. In general, blood flow in brain tissue surrounding the tumor was depressed in comparison to corresponding brain structures in the opposite hemisphere.

In RG-2 intracerebral tumors, a more reproducible pattern of blood flow was observed between different tumors and a more uniform level of flow was measured within individual tumors. The value of blood flow in small tumors (<2 mm diameter) was intermediate between the values for normal grey and white matter, regardless of where the tumor was located. Larger tumors demonstrated slightly lower blood flow values. Values of flow less than 20 ml/100g min in the center of the tumor were not seen in any but the largest of the RG-2 tumors and were associated with the histologic appearance of tumor necrosis.

Regional blood-to-tissue transport

The small neutral amino acid, α-aminoisobutyric acid (AIB), was used as a model of a small, water-soluble drug. The blood-to-tissue transfer constant (K) was measured and used to assess changes in capillary permeability in normal and tumorous tissue. The K of AIB for normal brain tissue is 0.001-0.002 ml/g min. The most striking findings to emerge from these studies was: (1) the marked variability of the transfer constant between different tumor models, in different tumors of the same model and within individual tumors; (2) the apparant lack of correlation between histological features and the transfer constant in many of the tumors; and (3) an apparent correlation of the K in small tumors with the K of the host tissue.

In the Walker 256 metastatic model, small tumors (less than 1 mm in diameter) demonstrated little or no increase in the blood-to-tissue transfer constant of AIB with respect to normal brain, whereas larger tumors were observed to have a 50- to 100-fold increase in K over that of normal brain. In well-differentiated oligodendrogliomas induced by ENU, little or no increase in the transfer constant for AIB was observed in small tumors. Small RT-9 tumors showed a modest 2- to 5-fold increase in the transfer constant for AIB over normal brain, whereas medium-sized tumors showed a 25- to 35-fold increase, and some larger tumor regions demonstrated even greater increases in AIB transfer.

In the ASV-induced tumors, a marked variability in the transfer constant for AIB was found which did not correlate well with tumor size or specific cytologic and histologic characteristics. The range of the transfer constants for AIB extended from 0.002 to more than 0.1 ml/g min in this group of tumors. Some large anaplastic astrocytomas could not be distinguished from the brain background of the ^{14}C-AIB autoradiogram, but very high transfer constant values were measured in several small anaplastic astrocytomas. Sarcomas generally had lower transfer constant values than the gliomas. This difference was also noted between the mesenchymal and astrocytic portions of the mixed glial-sarcomatous tumors.

For the medium and large ENU-induced oligodendrogliomas, relatively normal blood-to-tissue transfer constants were measured for AIB. In contrast, the transfer constants measured in the medium and large RG-2-induced brain tumors were about thirty-fold higher than normal and were fairly similar throughout the tumorous tissue. For the Walker-256, the RT-9, and the ASV tumor models, there was marked regional and subregional variations of the blood-to-tissue transfer constant of AIB for medium and large tumors. No correlations among cell type, tumor size, and transfer constant were evident in the latter three tumor models.

The permeability of capillaries within brain tissue adjacent to tumors with high rates of blood-tissue exchange for AIB was usually increased. The magnitude and extent of this increase appeared to be related to tumor size and the presence of infiltrating tumor cords.

Regional extraction fractions and permeability-surface area products

As indicated under "Method Employed", the extraction fraction or percent of a test drug which crosses the blood vessels and is taken-up by the tissue can be calculated by dividing the blood-to-tissue transfer constant by the rate of blood flow. A regional calculation of extraction can be made in those studies where both transfer and blood flow were measured (namely, those experiments in which double label quantitative autoradiography was employed). The extraction fraction of AIB, our model drug, is about 0.002 or 0.2% for normal brain. Among the various tumor studies in which both blood flow and blood-tissue transfer of AIB were simultaneously measured, the extraction fraction was usually increased. However the percent extraction of AIB virtually never exceeded 10%. This indicates that there are still significant capillary permeability limitations to the free exchange of small water-soluble drugs and compounds such as AIB for even the most "leaky" microvessels in the experi-

mental tumors studied in this series.

As presented in "Method Employed" the transport of drugs and model compounds can also be expressed in terms of a permeability-surface area product of the tissue microvasculature. This quantitative expression of unidirectional transport is calculated from the blood-to tissue transfer constant and the rate of blood flow and is a property or characteristic of the microvasculature. Comparisons of permeability-surface area products are a much more precise and quantitative way to evaluate the transfer of different drugs in different tumor systems as well as in different regions of an individual tumor.

Regional DNA synthesis

The distribution of 14C-thymidine (Tdr) and the incorporation of Tdr-derived radioactivy into a nonextractable (methanol) tissue fraction was studied in the ASV-induced and RG-2 tumors by quantitative autoradiography. The disposition pattern of the nonextractable radioactivity presumably reflects DNA synthesis.

The distribution patterns 14C-Tdr-derived radioactivity were variable. Methanol extraction essentially removed all radioactivity from the brain parenchyma and somewhat reduced the total radioactivity within the individual tumors. In several ASV-induced tumors, the patterns of 14C distritribution before and after methanol extraction were identical with that of surrounding brain tissue. This suggests that these tumors may have relatively impermeable capillaries with respect to Tdr (as was demonstrated for AIB) and that the labeled precursor of DNA does not reach the tumor in appreciable amounts. This observation is consistent with transport studies of Tdr across normal brain capillaries and indicates that transcapillary transport rather than DNA synthetic activity may determine Tdr disposition in some of these tumors.

Regional protein systhesis

The pattern of 14C-leucine incorporation into nonextractable tissue fractions, which presumably reflects protein synthetic activity, has been studied in three tumor models (Walker 256, RT-9, and ASV-induced). In the ASV-induced tumors, a variable pattern of leucine incroporation was observed. Leucine incorporation varied from low to high values within these tumors, seemingly independent of the microscopic appearance and size of the tumor. Some correlation between the nuclear/cytoplasmic ratio or cell density and leucine incorporation appears to exist within individual tumors. Necrotic regions incorporated neglibible amounts of leucine.

Regional glucose utilization

The apparent rate of glucose utilization has been studied in Walker 256 tumors, ENU-induced oligodendrogliomas, and in ASV-induced tumors. In general the apparent rates were quite variable with several notable exceptions. In well-differentiated oligodendrogliomas, the apparent rate of glucose utilization was uniform and similar to that of normal white matter. In the Walker 256 tumors, the apparent rate was considerably higher within histologically viable regions of the tumor than in surrounding brain tissue. In many cases,

very small foci of tumor cells (clumps of 300 to 500 cells) could be clearly differentiated from surrounding brain tissue by their higher rates of glucose utilization. The autoradiographic patterns of larger Walker 256 tumor metastases were grossly irregular, with sharply defined regions of very low and very high rates. The steep, more peripheral gradients of the apparent glucose utilization rate closely followed tumor outlines and demarcated the interface between tumor and surrounding brain tissue. The more centrally located gradients were also steep and corresponded to the interface between histologically viable and nonviable appearing tumor tissue; necrotic tissue always had neglible rates of glucose utilization.

Three animals with 18 ASV-induced tumors were studied by double-label QAR in which both the rates of blood flow and glucose utilization were simultaneously measured. Homogeneous patterns of the apparent rate of glucose utilization were observed in some of these tumors, but more commonly, a variable pattern of glucose utilization was seen within individual tumors. Tumor necrosis and cystic regions were always associated with very low or negligible apparent rates of glucose utilization. A clear correlation between glucose utilization, blood flow, and tumor cytology or tumor classification was not apparent from this limited analysis, suggesting that energy metabolism and blood flow were not coupled in these tumors.

The effect of tumor growth on the metabolism of surrounding brain tissue may have a considerable effect on overall neurologic function. In the Walker 256 tumor-bearing animals, a tumor size-dependent pattern of glucose utilization in adjacent brain tissue was observed. Local cerebral glucose utilization was increased in brain tissue surrounding isolated small foci of tumor, whereas low values were measured in brain tissue surrounding the larger tumors.

Significance to Biomedical Research and the Program of the Institute:

A basic understanding of the movements of drugs and physiological materials between blood, brain, CSF, and systemic tissues is essential for an appreciation of normal CNS and systemic functions as well as the changes in these transport systems caused by various CNS and systemic malignancies. Sound experimental methods must be used to perform scientifically reliable transport studies. Therefore, as part of our work, various "improved" techniques for measuring and analyzing transport phenomena have been developed. Among these developments are: (1) better methods of determining blood-brain and blood-tissue transfer constants for materials of low to moderate permeability; (2) a quantitative and more relevant assessment of drug delivery and uptake by normal and tumorous tissue in terms of tissue extraction fractions and microvascular permeability-surface area products; and (3) double-label quantitative autoradiographic and co-imaging techniques to facilitate regional tissue analysis and correlation. These various techniques will be used by other research groups who are interested in doing pharmacological, physiological and biochemical studies of the blood-brain-CSF system and are being expanded to include regional analysis of systemic tissues as well.

We have investigated drug delivery and drug delivery systems since both are important for effective chemotherapy. The findings provided by our distribution studies of both established and prospective chemotherapeutic agents are

needed to determine the best schedules for delivering drugs to the brain and other tissues. Without this knowledge effective chemotherapy can be easily thwarted by inappropriate drug administration schedules.

We have studied four major routes of drug administration -- intravenous, intra-arterial, intraperitoneal, and intrathecal -- in normal animals. From this work we are characterizing these various routes and their advantages and limitations. This work is important not only to the Division of Cancer Treatment but also to the programs of any groups which are using chemotherapy.

Our quantitative autoradiographic studies of drug transport, blood flow, capillary and cellular permeability, glucose utilization, protein synthesis, and nucleoside distribution in various brain and peripheral tumors provide information on the changes in these pharmacological, physiological, and biochemical parameters which occur in and around tumors. In turn, these findings may suggest better kinds of drugs and strategies for treating tumors and better ways of deliverying therapeutic agents to neoplastic tissue.

Quantitative autoradiography serves as a model for positron emission tomography (PET); therefore, our efforts to develop ways of measuring various biochemical, physiological, and pharmacological parameters in pathological conditions with quantitative autoradiography may be applied to the study of human disease using PET. In turn, PET studies of human tumors should confirm or deny the applicability of these experimental methods to the study of human pathological conditions and enhance or diminish the predictive value of our animal models.

Proposed Course of Project:

The Membrane Transport Section intends to pursue the following research goals for the next year: (1) continue to investigate various drug delivery systems and strategies; in particular, studies of graded hyperosmotic stress are planned to demonstrate whether sub-threshold osmolarity will significantly increase the permeability of tumor microvessels to small water-soluble drugs. Thus, a selective osmotic effect on tumor vessels in the absence of an effect on brain vessels is sought which will increase drug delivery to tumor tissue but not to the brain; (2) gain information about the transport and distribution of specific drugs in normal animals and tissue and in tumor models and neoplastic tissue; for example, quantitative studies with respect to the regional distribution of tumor-specific and non-specific monoclonal antibodies using double-label quantitative autoradiography will be initiated in collaboration with Dr. Bigner of Duke University. In addition, autoradiographic localization and quantitation of liposome-drug complex distribution in primates and possibly rats will be performed in collaboration with Dr. Sieber, LCP and Dr. Weinstein, LTB, DCBD; (3) develop new methods of studying membrane transport, drug distribution, and pathophysiology that will be useful for quantitative autoradiographic studies in animal tumor models as well as for positron emission tomography studies in patients; (4) continue our studies related to the biology of various primary and metastic brain and systemic tumor models; for example, examine the effects of several different tissue sites on the biochemistry and physiology of the tumor cells and the tumor mass itself; (5) study the pathophysiological reactions of CNS tissue to other diseases, radiation, and drug treatment; (6) seek information on the normal mechanisms of amino acid, mono-

saccharide, protein and electrolyte transport between blood, brain and CSF; (7) devise and test a double-membrane model of the blood-brain barrier; and (8) collaborate with other groups in the development of suitable compounds and protocols for PET scanning.

Publications:

1. Blasberg, R.G., Groothuis, D., and Molnar, P.: Application of quantitative autoradiographic measurements in experimental brain tumor models. Sem. Neurol. 1: 203-221, 1981.

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3. Blasberg, R., Johnson, D., and Fenstermacher, J.: Absorption resistance of cerebrospinal fluid after subarachnoid hemorrhage in the monkey; effects of heparin. Neurosurgery 9: 686-691, 1981.

4. Fenstermacher, J. and Davson, H.: The distribution of two model amino acids from cerebrospinal fluid to brain and blood. Am. J. Physiol. 242: F171-F180, 1982.

5. Blasberg, R., Kobayashi, T., Patlak, C., Shinohara, M., Miyaoka, M., Rice, J., and Shapiro, W.: Regional blood flow, capillary permeability and glucose utilization in two brain tumor models: preliminary observations and pharmacokinetic implications. Cancer Treat. Rep. 1982 (in press).

6. Blasberg, R., Gazendam, J., Shapiro, W., Shinohara, M., Patlak, C., and Fenstermacher, J.: Clinical implications of quantitative autoradiographic measurments of regional blood flow, capillary permeability and glucose utilization in a metastatic brain tumor model. Eur. J. Cancer 1982 (in press).

7. Fenstermacher, J.D.: The Comparative Physiology of Blood-brain Exchange. In Nistico, G. and Bolis, L. (Eds.): Progress in Nonmammalian Brain Research. Boca Raton, CRC Press, 1982 (in press).

8. Fenstermacher, J.D.: and Rapoport, S.I.: The Blood-Brain Barrier. In Renkin, E.M. and Michel, C.C. (Eds.): Handbook of Physiology: The Micro-circulation. Bethesda, Am. Physiol. Soc., 1982 (in press).

9. Fenstermacher, J. and Gazendam, J.: Intra-arterial infusions of drug and hyperosmotic solutions as ways of enhancing CNS chemotherapy. Cancer Treat. Rep. 1982 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03502-19 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Drug Kinetics in Experimental Tumors and Normal Tissues of Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.S. Zaharko	Head, Pharmacok. & Pharmacod. Sec.	LCHP	NCI
Other:	S. Creekmore	Clinical Assoc., Pharmacok. & Pharmacod. Sec.	LCHP	NCI
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COOPERATING UNITS (if any)
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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of these experiments is to evaluate the possibility of modifying the cytotoxic response of chemotherapeutic drugs with mildly toxic levels of photodynamic injury caused by hematoporphyrin and light. Tissue culture experiments using both growth and clonogenic assays of L1210 leukemia were used to measure effects. A paired control experimental design was important in the development of techniques for evaluating the combination of these two potentially lethal effects. Prolonged hematophoryrin incubation followed by standarized illumination and subsequent chemotherapeutic treatment inhibits both growth and clonogenic capacity of L1210 cells. Melphalan and Actinomycin-D dose response curves using the paired control experimental technique indicated that the toxicities of these drugs were reproducibly enhanced by prior mild photodynamic injury. However, the effects with Actinomycin-D were not as large as might have been expected from the enhancement of transport of Actinomycin-D by photodynamic injury reported by other laboratories.

Project Description:

Objectives:

1. To explore the modification of chemotherapeutic response with photodynamic injury as a way to further understand drug action.

2. To continue the study of a physiological pharmacokinetic model and its applications in cancer chemotherapy.

Methods and Major Findings:

1. Tissue culture experiments (L1210 growth and clonogenic assays) were done to evaluate the possibility of modifying the dose response curves of several chemotherapeutic drugs using mildly toxic levels of photodynamic injury provided by light and hematoporphyrin. Interest in this approach was stimulated by reports of modification of membrane transport induced by such photodynamic injury coupled with the reported phenomenon of increased hematoporphyrin localization in some tumors. Hematophoryrin is used clinically to create phototoxicity to surface tumors. It is also used with fiber optics to deliver light to certain cavity localized tumors but the synergistic effects of chemotherapeutic agents has not been evaluated.

A system using prolonged hematoporphyrin incubation and standardized illumination was worked out which allowed reproducible tissue culture experiments to be done to measure the combined toxic effects of photodynamic injury and chemotherapy. Introduction of a paired control experimental design was important in achieving reproducible measurements of the two effects in combination, both of which have steep dose-response curves which are sensitive to minor perturbations in experimental technique. It was found that reproducible enhancement in Melphalan and Actinomycin-D dose response curves could be measured. The effects were not as large as would have been suggested by transport experiments using Actinomycin-D that were reported by other experimenters.

2. The continued study of physiological pharmacokinetics this past year involved no additional experimental work because of lack of sufficient space and personnel. However, as part of this continuing project the principle investigator wrote a review chapter for a book on "Folate Antagonists" at the request of the publishers, Academic Press.

Publications:

1. Zaharko, D.S. and Dedrick R.L.: Pharmacokinetics of Methotrexate. In Sirotnak, F.M., Burchall, J.J., Ensminger, W.D., and Montgomery, J.A. (Eds): Folate Antagonists as Therapeutic Agents. New York Academic Press, 1982 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03505-19 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Potential Embryotoxic, Carcinogenic and other Adverse Effects of Anticancer Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. M. Sieber-Fabro	Head, Pharm. & Exp. Therap. Sec.	LCPH	NCI
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COOPERATING UNITS (if any)
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The reproductive toxicity and carcinogenicity of new as well as clinically useful antitumor agents are under investigation. Studies on the embryotoxic effects in mice of hexamethylmelamine, pentamethylmelamine and demethylated analogs have been completed. The effect of long-term, low dose treatment with cyclophosphamide or procarbazine on fertility in male mice and on malformations, survival and tumor incidence in their offspring has been evaluated. The potential carcinogenic activity of various clinically effective antitumor and/or immunosuppressive agents, including procarbazine, N-methylnitrosourea, melphalan, azathioprine, adriamycin and cyclophosphamide, is under evaluation in long-term studies in monkeys. A survey of second malignancies developing in treated cancer patients and on the effects of these agents on the fetus, initiated several years ago, is continuously being updated using data from published reports and from several centers and hospitals.

Project Description:

Objectives:

This project is designed to obtain data on various aspects of the toxicity of antitumor agents, including their embryotoxic, cytogenetic and carcinogenic effects in rodents, monkeys and in the human. Specifically, the objectives are:

To evaluate in mice the adverse effects on reproduction, including embyrotoxic and cytogenetic effects, of various classes of antineoplastic agents. The goal of such studies is to define differences in teratogenic potency among groups of agents with similar structures and antitumor spectra. In addition, the studies are designed to provide information as to the period of embryonic development most sensitive to the effects of the test compounds. The possibility of preventing or reversing induced embryotoxic effects with various chemicals has also been evaluated. In addition, the effect of long-term, low dose treatment with cyclophosphamide or procarbazine on reproduction in male mice is under study. Serial mating studies have been carried out in the animals to assess their fertility after treatment, as well as to determine the effects of drug treatment on the survival, frequency of malformations and tumor incidence in their offspring.

To evaluate in monkeys the carcinogenic potential of various clinically useful antitumor and/or immunosuppressive agents. In addition, the effects of such treatment on other functions, such as the immune system and reproduction, is under examination. A significant proportion of monkeys receiving long-term treatment with procarbazine has developed a malignancy, about half of which have been acute leukemia (see below). The surviving monkeys presently receiving procarbazine are considered to be at high risk of developing acute leukemia, and hematologic and cytogenetic studies are currently being carried out in this group of animals. The goal of these studies in the procarbazine-treated monkeys is to determine whether a "preleukemic" condition exists in chemicaly-induced acute leukemia, and if so, to characterize it.

To monitor the current medical literature and the NCI patient data in order to tabulate and characterize adverse effects of antineoplastic agents in man. Particular emphasis is being given to embryotoxic, mutagenic and carcinogenic effects arising from treatment with clinically effective antitumor agents.

Major Findings:

Evaluation of antitumor agents for reproductive toxicity in mice

The embryolethal and teratogenic effects of hexamethylmelamine, pentamethylmelamine and 4 demethylated analogs were evaluated. The compounds were administered as single ip injections on day 8 of gestation or as multiple injections on days 7, 8, and 9. Hexamethylmelamine was embryolethal when administered on days 7-9 at 100 mg/kg, with an incidence of intrauterine deaths of 30%. It was also teratogenic when administered on this schedule, since 42% of surviving fetuses were malformed. The most common malformations noted were of the axial skeleton and of the lungs. Lower doses of hexamethylmelamine (10, 25 or 50

mg/kg) were not embryotoxic nor were single doses of 10, 25, 50 or 100 mg/kg injected on day 8. In contrast to hexamethylmelamine, pentamethylmelamine and the other demethylated analogs tested were not embryotoxic on either dosage schedule.

The effect of long-term, low dose treatment with cyclophosphamide or procarbazine on reproduction in male mice is under study. Mice were injected with either cyclophosphamide or procarbazine at doses and on schedules which parallel those used in clinical situations and drug treatment continued for 10 weeks, which corresponds to 2 spermatogenic cycles in the mouse. Serial mating studies were carried out in these animals to assess their fertility after treatment, and to determine the effects of drug treatment on the survival, frequency of malformations and tumor incidence in their offspring. The mating studies indicated that prior drug treatment did not adversely affect fertility; in fact, there was some evidence of a rebound phenomenon inasmuch as the treated mice impregnated a higher proportion of females and within a shorter time than did control males. In addition, drug treatment of males did not result in an increased incidence of congenital malformations in their offspring nor has it thus far shortened their survival. Although subgroups of offspring continue to be held under observation for tumor development, results to date indicate that the tumor incidence in these offspring does not differ from that in control offspring. Our results indicate that spermatogenesis in the mouse is considerably less sensitive to cytotoxic agents than is spermatogenesis in nonhuman primates and man.

Evaluation of antitumor agents for carcinogenic activity in monkeys

From our survey of second tumors in treated cancer patients, we have accumulated evidence that Hodgkin's disease patients receiving treatment with the MOPP regimen, one component of which is procarbazine, are at increased risk of developing AML. Although a causal relationship between cyotoxic drug therapy and the appearance of AML in these patients has not yet been established, there is ample evidence that procarbazine is a potent carcinogen in mice and rats. In addition, the carcinogenic potential of procarbazine in 3 species of nonhuman primates has been under study for 16 years. A total of 50 monkeys have survived 6 months or longer after the first dose of drug. Fourteen of the 41 monkeys (34%) necropsied thus far have had malignant neoplasms, seven of which were acute leukemia. The leukemias, chiefly myelogenous, arose in monkeys after latent periods ranging between 16 and 143 months (average 77 months). They developed in monkeys that had ingested an average cumulative procarbazine dose of 45.5 gm (range 2.7 to 103.7). Solid tumors were diagnosed in 7 monkeys on the probarbazine study. Three monkeys developed osteogenic sarcomas, 2 monkeys developed hemangiosarcomas, and single cases of lymphocytic lymphoma and astrocytoma were found. These tumors arose after latent periods of 68-192 months (average 111 months), and after an average cumulative procarbazine dose of 64.6 gm (range 23.8 to 154.4 gm). The 8 surviving monkeys in this study may represent a population at high risk for developing AML. For this reason, they are being utilized in hematologic and cytogenetic studies in order to determine whether a "pre-leukemic" phase is detectable prior to the development of frank drug-induced leukemia. For this purpose, sequential bone marrow samples from all of the monkeys receiving procarbazine are being examined for alterations in cellular morphology and for chromosomal aberrations. Since initiation of

these studies, no specific chromosomal aberrations have been detected in bone marrow cells from the monkeys and no additional cases of acute leukemia have developed. However, a number of the toxic effects of procarbazine seen clinically are also noted in the monkeys, including vomiting and myelosuppression. Its most striking toxic effect, however, is on the reproductive system of the males. The majority of the adult males necropsied to date have had testicular atrophy with complete aplasia of the germinal epithelium.

There is some evidence that patients receiving long-term treatment with melphalan for multiple myeloma or ovarian cancer may also be at increased risk of developing AML. Although a causal relationship between melphalan treatment and the development of AML in these patients has not yet been established, the carcinogenic activity of this agent has been demonstrated in mice and rats. The carcinogenic potential of melphalan in nonhuman primates is therefore under study. Twenty monkeys are currently being treated by the oral route with melphalan (1.2 mg/m^2) daily, 5 days a week. The average cumulative dose administered to these animals is 1.15 gm/m^2 and the dosing interval averages 71 months. Women receiving prophylactic melphalan therapy for ovarian carcinoma would receive in the prescribed 18-month dosing period a total melphalan dose of 660 mg/m^2, a dose lower than that already ingested by these monkeys. None of the monkeys on this study have died and all appear to be healthy.

Kidney transplant recipients and other patients under chronic immunosuppressive therapy with azathioprine appear to be at risk of developing malignancies, primarily lymphomas. Whether this increased risk is related to a direct oncogenic effect of azathioprine or is secondary to a prolonged immunosuppressed state is at present unclear. The carcinogenic potential of azathioprine is being evaluated in two groups of monkeys receiving the drug daily, 5 days every week at doses of 2 and 5 mg/kg, respectively. The 2 mg/kg group is comprised of 14 animals that have thus far received an average cumulative azathioprine dose of 7.4 gm over the course of approximately 48 months. Fourteen animals are receiving azathioprine at 5 mg/kg, and have ingested an average cumulative dose of 9.0 gm over an average dosing interval of 28 months. None of the monkeys on this study have developed a malignancy and all appear to be healthy.

N-methylnitrosourea (MNU) administered by iv injection has been reported by clinicians in the Soviet Union to be effective in Hodgkin's disease and undifferentiated carcinoma of the lung, and the nitrosoureas BCNU, CCNU and methyl-CCNU have been used in this country to treat a variety of human tumors. The latter 3 agents have been implicated in the development of second tumors in 2 patients receiving treatment for CNS neoplasms. Both patients were diagnosed with acute non-lymphocytic leukemia after receiving oral doses of nitrosoureas totalling approximately 1420 and 2700 mg/m^2, respectively. We have evaluated the carinogenic potenital of MNU in three species of nonhuman primates. A total of 44 monkeys have received oral doses of MNU for periods up to 68 months. Twelve of the 21 monkeys (57%) necropsied thus far have had squamous cell carcinoma (SCA) of the mouth, pharynx and/or esophagus; upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 21 monkeys necropsied to date. The tumors developed in monkeys ingesting cumulative MNU doses averaging 130 gm (range 53.2-246.4 gm) over an average of 111 months (range 57-168 months). A minimum of approximately 50 gm of MNU appears necessary for tumor induction by

the oral route. Many parallels were noted between the esophageal SCA observed in the present series of monkeys and human esophageal carcinoma, including the clinical manifestations of the tumor, its complications, its radiographic appearance and its morphology. The MNU-induced lesions of the oropharynx and esophagus of primates may therefore be a valuable model for the study of human esophageal carcinoma.

The potential carcinogenicity of adriamycin was also evaluated. A group of 10 monkeys received an iv dose of drug (12 mg/m^2) once each month for 23-27 months. At the end of the dosing period it was intended to hold the animals under observation for the remainder of their lives. However, approximately 2 months after the last dose of adriamycin 8 of 10 animals developed congestive heart failure. Histopathologic examination of cardiac muscle taken at necropsy revealed lesions characteristic of adriamycin-induced cardiomyopathy in humans, and in some cases these findings were confirmed by electron microscopy. In man, a cumulative adriamycin dose of 550 mg/m^2 has been associated with cardiac toxicity; nonhuman primates appear to be more sensitive to adriamycin-induced cardiomyopathy, as the monkeys in the present study had received an average cumulative dose of 310 mg/m^2 (range 276-336 mg/m^2). One of the 10 monkeys developed acute myeloblastic leukemia after receiving 324 mg/m^2 of adriamycin divided into 27 monthly doses. The 10th monkey in this series is alive and without evidence of illness. It received 25 injections of adriamycin totaling 300 mg/m^2, and received the last dose of adriamycin 50 months ago. This study is being repeated, using 2 groups of 10 monkeys each; the monkeys are receiving monthly iv injections of adriamycin at 2.4 and 4.8 mg/m^2; dosing will be terminated when a cumulative dose of 240 mg/m^2 is attained. Thus far, monkeys receiving adriamycin at 2.4 and 4.8 mg/m^2 have been given cumulative drug doses of 56.4 mg/m^2 and 57.6 mg/m^2, respectively. None of the monkeys have as yet developed signs of congestive heart failure or other indications of ill health.

Cyclophosphamide is widely used as a single agent and in combination regimens for treating human cancer; it is also being used with increasing frequency for treating various non-malignant disorders such as rheumatoid arthritis. The association of transitional cell carcinomas of the urinary bladder and acute myelogenous leukemia with prolonged cyclophosphamide treatment of both malignant and non-malignant disease represents the basis for a recently initiated study on the carcinogenic potential of cyclophosphamide in nonhuman primates. Cyclophosphamide is being administered to a group of 20 monkeys orally, 5 days every week, beginning at 6-7 months of age. The initial dose (3 mg/kg) is increased to 6 mg/kg after 6 months. This study has been underway for an average of only 12 months; during this period an average cumulative cyclophosphamide dose of 2.39 gm has been administered. Three of the monkeys died during an outbreak of measles in the colony last year, but no evidence of malignancy was found upon histopathologic examination of their tissue.

Significance to Biomedical Research:

Cancer chemotherapeutic agents are in increasing use in the treatment of various non-malignant conditions such as psoriasis, chronic glomerulonephritis and rheumatoid arthritis. Advances in chemotherapy have led to improvements in survival in many types of human cancer. These developments have now made it imperative to evaluate the long-term consequence of exposure to antineoplastic

and immunosuppressive agents. For example, there is an increasingly large population of successfully treated (possibly cured) Hodgkins disease patients, and considerable concern has been generated as to their fertility and the effects of the chemotherapy they received on their offspring; subfertility and infertility appears to be a frequent complication of therapy in males. The present study is an attempt to provide much-needed experimental data pertaining to the potential adverse effects on the offspring of males exposed to antitumor agents prior to mating. The problem of second malignant neoplasms developing in successfully treated cancer patients also appears to be increasing in magnitude. Studies on the carcinogenicity of antineoplastic and immunosuppressive agents in nonhuman primates will aid in identifying those agents with the greatest potential for producing neoplasms in man, and will provide information on the cumulative dose and latent period required for tumor development.

Publications:

1. Adamson, R.H. and Sieber, S.M.: Studies on the Oncogenicity of Procarbazine and Other Compounds in Nonhuman Primates. In Advances in Malignant Lymphomas: Etiology, Immunology, Pathology and Treatment, Chapter 15. New York, Academic Press, 1982, pp. 239-257.

2. Adamson, R.H. and Sieber, S.M.: Chemical Carcinogenesis in Nonhuman Primates. In Proceedings of a Workshop on Non-Human Primates in Cancer Research, 1982 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03506-19 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Pharmacology and Disposition of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.5	0.5	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The pharmacokinetics of desmethylmisonidazole (DESMISO, NSC-261036) was studied in 84 patients and the results compared with a previous clinical study of misonidazole (MISO, NSC-261037) to determine relationships between neurotoxicity and pharmacokinetic parameters. The dose of DESMISO ranged from 0.9 g/meter squared to 4 g/meter squared. DESMISO has a plasma elimination half-life of 6.0 + 0.6 hr and an apparent distribution volume of 45 + 2L. A one hour DESMISO plasma level of 38 + 3 microgram/ml per g/meter squared dose was observed and 60 + 7% of the parent compound was recovered in patients' urine. A correlation between the incidence of neurotoxicity and the DESMISO plasma x concentration curve was observed similar to that found for MISO; DESMISO is approximately twice as toxic as MISO when compared in this manner. The disposition of riboxamide (NSC-286193) was studied in mice at doses of 25 and 250 mg/kg. At 250 mg/kg, an initial plasma concentration of 1.38 mM was found which declined with an initial T 1/2 of 21 minutes and exhibited a terminal T 1/2 of 260 minutes. The amount of circulating metabolites was less than 5% of the circulating radioactivity. The steady state volume of distribution is more than 300 x the total mouse volume indicating that the drug is bound, sequestered, and/or metabolized.

Project Description

Objectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To study the clinical pharmacology of new agents in phase I clinical trials.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design new anticancer drugs by rational approaches.

Major Findings:

Disposition of Riboxamide (NSC-286193) in Mice

The pharmacokinetics of riboxamide were determined at two doses. An i.v. bolus of ^{3}H-riboxamide was given at 250 mg/kg (0.96 mmoles/kg) and 25 mg/kg (0.096 mmoles/kg) to pentabarbital anaesthetized CDF_1 mice. Blood samples were taken from the carotid artery starting at 5 minutes and continuing for 6 hours. Plasma and RBC's were separated and analyzed separately. Metabolism to the carboxylic acid derivative and phosphorylated products was determined by paper chromatography of plasma samples. At the higher dose, the initial plasma concentration was 1.38 mM; this rapidly declined with a T 1/2 of 21 minutes. The terminal half-life was much longer (at least 260 minutes), resulting in a nearly constant plasma concentration of approximately 0.11 mM. The kinetics of the 25 mg/kg dose were quite similar. The initial plasma concentration was 0.11 mM which declined with an initial half-life of 11 minutes. The plasma concentration in the terminal phase was nearly constant at 0.08 mM with a T 1/2 of at least 310 minutes. The initial plasma concentrations were determined by extrapolating the curve to the y axis. However, at both doses, this value is about 1/10th the concentration that would be expected. This suggests that there is a very rapid phase with a T 1/2 of about 1 minute that occurs before the first sample is taken at 5 minutes. The amount of circulating metabolites at either dose was small; five percent or less of the circulating radioactivity was found as the carboxylic acid and no significant amount was found as phosphorylated products. The integral of the plasma concentration was 110 mmoles/1xmin for the 250 mg/kg dose and 8.3 mmoles/1xmin for the 25 mg/kg dose. This indicates that the drug exposure is directly proportional to the dose over the range of 25-250 mg/kg. The steady state volume of distribution is very large in all cases (about 300 x the total mouse volume), which suggests the drug is bound, sequestered, and/or metabolized. In another study, a mouse was given a dose of 250 mg/kg intraperitoneally and blood samples were taken for 8 hours. Absorption was slow; the peak plasma concentration of 0.88 mM was attained 45 minutes after injection. The plasma levels slowly declined during the course of the experiment; at 8 hours after injection, the plasma concentration had only fallen to 0.4 mM. Studies are in progress to compare plasma concentrations

of riboxamide with chemotherapeutic effects in tumored animals.

Pharmacokinetics of Desmethylmisonidazole (NSC-261036) in Man

Clinical studies with misonidazole (MISO) have indicated that the dose limiting toxicity associated with this drug is neurotoxicity. An approach suggested for decreasing the incidence of neurotoxicity was the development of less lipophilic analogs of MISO that were equally effective radiosensitizers but would have diminished ability to cross the lipid blood-brain and blood-neural barrier. In addition, a relationship has been observed between the neurotoxicity and the extent of tissue drug exposure, i.e., the area under the plasma drug concentration time curve (AUC). Since desmethylmisonidazole (DESMISO) has a lower octanol/water partition coefficient (p = 0.13) than MISO (p = 0.43) and was reported to have a shorter plasma elimination half-time than MISO, RTOG initiated clinical evaluation of DESMISO using an i.v. preparation. Plasma and urine DESMISO levels were obtained during this study to determine pharmacokinetic parameters and help clarify the relationships between neurotoxicity and pharmacokinetic parameters which have previously been observed for MISO.

Pharmacokinetic data were obtained in 84 patients who received DESMISO doses ranging from 0.9 g/m^2 to 4 g/m^2. The results of this study showed: (a), DESMISO had a 6.0 ± 0.6 hr SE (n = 68) plasma elimination half-time; (b), DESMISO produced a one hour DESMISO plasma level of 38 ± 3 μg/ml SE (n = 84) per g/m^2 dose; (c), DESMISO was distributed in an apparent volume of 45 ± 2 SE L (n = 74); and (d), that 60 ± 7% SE (n = 68) of the parent compound administered was recovered in the patients urine. Comparison of these data with those previously determined for MISO after i.v. administration shows similarities in the apparent volume of distribution (MISO = 37L) and the one hour MISO plasma levels (46 μg/ml per g/m^2 dose). Differences were observed in the plasma elimination half-time (MISO = 9.9 hr, DESMISO = 6.0 hr) and the extent of renal excretion of the parent drug. Only 26% of the MISO dose was recovered in the patients urine, 10% as MISO and 16% as DESMISO. A correlation between the incidence of neurotoxicity and the DESMISO AUC was observed similar to that found for MISO. A comparison of the mean total AUC for those patients who exhibited neurotoxicity after receiving either MISO or DESMISO indicated that DESMISO (AUC = 5150 ± 461 μg hr/ml SE, n = 25) was approximately 52% less than that observed for MISO (AUC = 10800 ± 900 μg hr/ml SE, n = 12). These data suggest that DESMISO is approximately 2 times more toxic than MISO. The data are consistent with the formation of a similar metabolite from each compound that is responsible for the observed neurotoxicity. Future studies will attempt to identify toxic metabolites.

Publications:

1. VonHoff, D.D., Soares, N., Gormley, P., and Poplack, D.G.: Pharmacokinetics of ICRF-187 in the cerebrospinal fluid of subhuman primates. Cancer Treat. Rep. 64: 734-735, 1980.

2. Gormley, P.E., Gangi, D., Wood, J.H., and Poplack, D.G.: Pharmacokinetic study of CNS penetration of cis-diamminedichloroplatinum(II). Cancer Chemother. Pharmacol. 5: 257-260, 1981.

3. Gormley, P.E., Shoemaker, D.D., Rozencweig, M., Ayers, O.C., and Cysyk, R.L.: Absorption of m-AMSA and its biliary metabolites from the rat small intestine. Eur. J. Cancer Clin. Oncol. 17: 1143-1146, 1981.

4. Schwade, J.G., Makuch, R.W., Strong, J.M., and Glatstein, E.: Dose response curve for predicting misonidazole - induced peripheral neuropathy. Cancer Treat. Rep. 1982 (in press).

5. Coleman, C.N., Wasserman, T.H., Phillips, T.L., Strong, J.M., Urtasun, R.C., Schwade, J.G., Johnson, R.J., Gunar, M.B., and Zagurs, G.: Initial pharmacology and toxicology of intravenous desmethylmisonidazole. Int. J. Radiat. Oncol. Biol. Phys. 1982 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03509-19 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3.0	1.5	1.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Twenty-seven substances, including antitumor agents, contaminants of human food-stuffs, rodent carcinogens, pesticides, and artificial sweeteners have been or are being evaluated in four species of non-human primates for their potential carcinogenicity and other long-term toxic effects. Sixteen of these substances have not as yet demonstrated carcinogenic activity, although some have been on test for less than 3 years. Eight of the compounds are carcinogenic in non-human primates, producing tumors in 19-100% of the treated animals. 1-methyl-1-nitrosourea induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors possessing clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with procarbazine resulted in an increased incidence of malignancies, one-half of which were leukemias. The effects of 6 of the 8 compounds (DENA, DPNA, 1-nitrosopiperidine, aflatoxin B-1, MAM-acetate and urethane) were manifested primarily as hepatocarcinogenicity. Single cases of malignant tumors have been diagnosed in animals treated with adriamycin, butter yellow, sterigmatocystin, and N-methyl-N'-nitro-N-nitrosoguanidine.

Project Description

Objectives:

To evaluate the carcinogenic potential and long-term adverse effects of clinically useful antineoplastic and immunosuppressive agents.

To obtain data on which to base a comparison of the response of non-human primates and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and non-human primates in predicting for man.

To develop model tumor systems in primates for evaluating the potential usefulness of new anticancer agents active against rodent tumors before these agents are administered to cancer patients.

To determine whether the non-human primate resembles the rodent in that it is more suscpetible to chemical carcinogenesis as a neonate than as an adult.

To evaluate the possibility of preventing or reversing chemical carcinogenesis in non-human primates with nucleophiles or immunostimulation.

To use normal and tumor-bearing non-human primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To carry out comparative biochemical and immunological studies using normal animals, animals with preneoplastic lesions, and animals with malignant neoplasms.

To develop methods for detecting preneoplastic changes and accomplishing the early diagnosis of tumors.

Methods Employed:

Compounds Under Investigation:

Twenty-seven substances are currently under investigation or have been investigated, including: 3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, ethyl carbamate, N,N'-dimethyl-p-phenylazo-aniline, N,N'-dimethyl-p-(m-tolylazo)-analine, methylnitrosourea, aflatoxin B_1, methylazoxymethanol acetate, cyclamate, saccharin, dichlorodiphenyltrichloroethane, N-methyl-N'-nitro-N-nitrosoguanidine, low density polyethylene plastic, cigarette tobacco smoke condensate, 1-nitrosopiperidine, N-nitrosodiethylamine, N-nitrosodipropylamine, arsenic, sterigmatocystin, Imuran, adriamycin, procarbazine, L-phenylalanine mustard and cyclophosphamide.

The compounds are administered subcutaneously, intravenously, intraperitoneally, or orally. For oral administration to newborn monkeys, the compound is added to the Similac formula at the time of feeding; when the monkeys are six months old, carcinogens given orally are incorporated into a vitamin mixture which is

given to monkeys as a vitamin sandwich on a half slice of bread. An alternate way of giving doses orally is to incorporate the compound into a prune or to intubate. The dose level chosen is dependent on the chemical under evaluation. Antineoplastic and immunosuppressive agents are administered at doses likely to be encountered in a clinical situation; other substances, such as environmental contaminants, are given at levels 10-40 fold higher than the estimated human exposure level. The remainder of the chemicals tested are administered at maximally tolerated doses which, on the basis of weight gain, blood chemistry and hematology findings, and clinical observations, appear to be devoid of acute toxicity.

Animals

The present colony, consisting of 494 animals, is comprised of four species: Macaca mulatta (rhesus), Macaca fasicularis (cynomolgus), Cercopithecus aethiops (African green) and Galago crassicaudatus (bushbabies). Fifty-four of these monkeys are adult breeders which until recently supplied the newborns for experimental studies. The majority of the animals are housed in an isolated facility which contains only animals committed to this study, and with the exception of the breeding colony, most animals are housed in individual cages. The administration of test compounds is usually initiated within 24 hours of birth and continues until a tumor is diagnosed or until a predetermined exposure period has been completed. A minimum of 30 animals is usually allotted to each treatment group, since in a sample of this size it is possible to detect a tumor incidence of 10% within 95% confidence limits.

A variety of clinical, biochemical and hematological parameters are monitored weekly or monthly, not only to evaluate the general health status of each animal, but also for the early detection of tumors. Surgical procedures are performed under phenycylidine hydrochloride, Ketamine or sodium pentobarbital anesthesia. All animals which die or are sacrificed are carefully necropsied and the tissues subjected to histopathologic examination.

Major Findings:

Compounds Carcinogenic in Non-Human Primates

Eight of the 27 substances evaluated have induced malignant neoplasms in non-human primates, producing a tumor incidence ranging from 19-100% of the treated animals. The compounds are: N-nitrosodiethylamine, 1-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B_1, methylazoxymethanol acetate, procarbazine, methylnitrosourea, and urethane. In addition, single cases of malignant tumors have been diagnosed in animals treated with adriamcyin, N-methyl-N'-nitro-N-nitrosoguanidine, sterigmatocystin, and N,N'-dimethyl-p-phenylazoanaline (butter yellow).

Nitrosoamine Carcinogenesis

DENA is highly predictable as a carcinogen in Old World monkeys, inducing hepatocellular carcinomas when given either orally or by ip injection. In one study, we are using DENA as a model hepatocarcinogen in Old World monkeys to examine the relationship between chronic (milligrams per kilogram) dose,

cumulative dose, and latent period for tumor induction. To this end, groups of monkeys are being given bimonthly ip injections of DENA at doses of 0.1, 1, 5, 10, 20, and 40 mg/kg and are observed for the appearance of tumor. In the five groups of monkeys in which tumors have developed, we have found that the latent period increases as the milligram-per-kilogram dose decreases. Thus, the latent period at the 40 mg/kg dose averages 17 months, for the 20 mg/kg dose it is 26 months and at 10 mg/kg it is 37 months. A semilog plot of these 3 mg/kg doses of DENA against the latent periods for tumor induction yields a straight line which intercepts the y-axis at approximately 88 months. This point on the ordinate corresponds to a DENA dose of 0.1 mg/kg. Thus animals in the 0.1 mg/kg group should develop tumors after a latent period of 88 months if the relationship between the milligram-per-kilogram dose and latent period is strictly linear; however, the animals at this dose have only been on study approximately 22 months. Extrapolation from the curve to the ordinate for the 1 mg/kg group yields a latent period of 60 months, although 90% of the animals in this group remain tumor-free after an average of 88 months of observation. The tumors developing in the 8 animals receiving the 5 mg/kg dose required a latent period averaging 69 months, a figure which shows a marked deviation from the value (42 months) expected if the relationship between dose and latent period were linear. Our results thus far indicate that this relationship departs from linearity at lower chronic doses of DENA.

Although the dose rate has a significant effect on the length of the latent period, it does not appear to be of importance in determining the minimum cumulative DENA dose required for tumor induction. The averages for this figure range between 1.4 gm (40 mg/kg) and 3.1 gm (5 mg/kg), with cumulative DENA doses as low as 0.39 gm inducing tumors in individual animals.

DENA is also carcinogenic in the more primative primate Galago crassicaudatus. All 10 treated animals have developed tumors after bimonthly ip injections of DENA at doses of 10-30 mg/kg. In contrast to the DENA-induced primary hepatocellular carcinomas in Old World monkeys, all 10 of the bushbabies developed mucoepidermoid carcinomas of the nasal cavity. In 2 of these 10 animals, carcinoma of the liver was also present, and in both cases metastases to the lungs or to intestinal lymph nodes was noted. The average total dose of DENA given the bushbabies was 0.747 gm, and ranged from 0.295-1.485 gm. It is considerably lower than that required to induce tumors in Old World monkeys and reflects the lower body weight of the bushbabies. The average latent period for tumor induction in this species (23 months) is comparable to that in Old World monkeys at the 20 mg/kg dose. No obvious reason exists for the marked difference in the site of DENA-induced tumors noted between Old World monkeys and the bushbabies. It may be related to differences in the metabolism or distribution of DENA, and this possibility will be investigated.

Two other nitrosamines have induced primary hepatocellular carcinomas in monkeys. N-nitrosodipropylamine (DPNA) induced liver tumors in all 6 of the rhesus and cynomolgus monkeys given bimonthly ip doses of 40 mg/kg. The average total dose of DPNA was 7.0 gm; the average latent period for tumor development was 28.5 months. 1-nitrosopiperidine (PIP) is also an hepatocarcinogen in macaques. Hepatic cell carcinomas developed in 11 of 12 monkeys receiving this compound by the oral route, and in 5 out of 11 monkeys treated by the ip route. The average cumulative dose necessary for tumor induction by PIP given

orally (1742.5 gm) was higher than for oral DENA (18.0-55.1 gm); similarly, the average cumulative dose of PIP given by the ip route (39.4 gm) exceeded that required for tumor induction by ip DENA (1.7 gm) or ip DPNA (7.0 gm).

A pediatric laparoscope is being employed in monkeys receiving treatment with DENA and other hepatocarcinogens. Laparoscopy is a rapid, relatively non-invasive procedure; it will make possible the earlier detection of tumors and will provide us with a more precise figure for latent period. It will also enable us to follow the growth of these tumors and their response to chemo- and immuno-therapy.

Carcinogenic Activity of Aflatoxin B_1

Aflatoxin B_1 (AFB_1), a product of a mold (Aspergillus flavus) known to contaminate some human foodstuffs, is carcinogenic in a variety of experimental animals. The carcinogenicity of AFB_1 has been under evaluation in non-human primates for the past 16 years. A total of 47 Old World monkeys, chiefly rhesus and cynomolgus, has received AFB_1 by ip (0.125-0.25 mg/kg) and/or oral (0.1-0.8 mg/kg) routes for 2 months or longer, and 2 are currently alive. Twenty-one of the 45 monkeys necropsied to date developed a total of 27 malignant neoplasms, yielding an overall tumor incidence of 45%. Five of the 21 tumor-bearing monkeys developed hemangioendothelial sarcomas of the liver, 6 developed bile duct or gallbladder adenocarcinomas, and 2 cases of hepatocellular carcinoma were diagnosed. Two monkeys developed osteosarcomas and 6 were found at necropsy to have multiple primary tumors. All of the latter animals possessed adenocarcinoma of the pancreas and/or gallbladder or bile ducts as well as urinary bladder carcinoma, fibrosarcoma and osteosarcoma. The tumors diagnosed in the 21 monkeys developed after latent periods ranging from 49-186 months, and after cumulative AFB_1 doses of 99-1472 mg. Fifteen of the 23 (65%) necropsied monkeys without tumor showed histologic evidence of liver damage, including toxic hepatitis, cirrhosis and hyperplastic liver nodules. Our results indicate that AFB_1 is a potent hepatotoxin and carcinogen in non-human primates, and further support the hypothesis that humans exposed to this substance may be at risk of developing liver cancer.

Carcinogenic Activity of Cycasin

Cycasin, the active principle in the cycad nut, induces liver and kidney tumors in rats and may be a human carcinogen as well. The carcinogenic potential of cycasin and its aglycone, MAM-acetate, is under investigation in non-human primates. Old World monkeys (rhesus, cynomolgus, and African greens) received cycasin and/or MAM-acetate by po or ip routes for periods up to 12 years. Eighteen monkeys survived > 2 mo after initiation of treatment with cycasin (50-75 mg/kg) or MAM-acetate (1.5-3.0 mg/kg) given po daily 5 days/week, and 11 of the animals have been necropsied. Histopathologic examination of tissue from one of these monkeys revealed hepatocellular carcinoma. A second monkeys was noted to have multiple tumors, including hepatocellular carcinoma, intra-hepatic bile duct adenocarcinoma, renal carcinoma and adenomas, and adenomatous polyps of the colon. An adenocarcinoma of the pancreas was diagnosed in a third monkey. Although liver tumors were not observed in the other monkeys, all but one had hepatic lesions such as toxic hepatitis and cirrhosis. A group of 10 monkeys received MAM-acetate by weekly ip injections (3-10 mg/kg). Six of

these animals developed tumors after receiving an average of 6.14 gm (range 3.58-9.66 gm) of MAM-acetate for an average of 75 mo (range 50-89 mo). Four of the monkeys developed hepatocellular carcinomas and 2 had multiple primary tumors including hepatocellular carcinomas, renal carcinomas, squamous cell carcinomas of the esophagus and adenocarcinomas of the small intestine. Our results show that MAM-acetate is a carcinogen in monkeys, and add to the evidence that cycasin and its aglycone may be carcinogenic in man.

Carcinogenic Activity of Urethane

The carcinogenicity of urethane, with or without whole body irradiation (WBI) was evaluated in rhesus and cynomolgus monkeys. Monkeys received urethane (250 mg/kg) orally, 5 days every week beginning within one month of birth. They received continuous urethane treatment for 5 years, during which time some monkeys also received 3-10 weekly courses of WBI at 50 rads per course. Urethane administration was discontinued 12-15 years ago and since that time all animals have been held under close observation for development of tumor or other adverse effects of treatment. Thirty of a total of 40 monkeys survived 6 months or longer after the first dose of urethane, and 23 of these animals have been necropsied. A total of 8 malignant tumors were found in 5 (16.7%) of the 30 treated monkeys; in comparison, 7 of a total of 219 (3.2%) control monkeys have developed tumors during this period. One or more primary liver tumors (3 cases of hemangiosarcomas, 1 case of adenocarcinoma of intrahepatic bile ducts, 1 case of hepatocellular carcinoma) were present in 4 monkeys; one of the monkeys with a liver hemangiosarcoma was also found to have an ependymoblastoma, and the fifth monkey developed a pulmonary adenocarcinoma. The animals with tumor had received an average cumulative urethane dose of 260 gm (range 230-339 gm); the latent period for tumor induction averaged 171 months (range 142-229 months). Two of the 5 monkeys developing tumors had received 9 and 10 courses of WBI, respectively. These results indicate that urethane, with or without WBI, is carcinogenic in monkeys; however, the latent period for tumor induction (> 14 years) is long, requiring approximately 50% of the usual lifespan of Old World monkeys in captivity.

Carcinogenic Activity of Methylnitrosourea

Squamous cell carcinoma of the mouth, pharynx, and esophagus developed in 12 of 44 monkeys (27%) receiving oral doses (10-20 mg/kg) of 1-methyl-1-nitrosourea (MNU). Moreover, upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 21 monkeys necropsied to date. All monkeys except one that have received total doses of MNU exceeding 50 grams have developed carcinomas, whereas no malignant tumors have developed in monkeys receiving a cumulative dose less than 50 grams. The average latent period for tumor development was 111 months and ranged between 57-168 months. The order of appearance of the esophageal lesions in our monkeys, as well as the clinical manifestations of the tumors, resembled those seen in humans, and included difficulty in swallowing, frequent vomiting and subsequent weight loss, and sialorrhea. The common complications of esophageal carcinoma in humans (e.g., regurgitation, aspiration, sepsis and hemorrhage) were also noted in our monkeys. In addition, histological examination revealed a morphology similar to that seen in human esophageal carcinomas, despite the highly variable nature of such tumors in both humans and

monkeys. Thus, MNU-induced esophageal carcinoma in non-human primates may prove to be a valuable model for the study of the human tumor.

Carcinogenic Activity of Procarbazine

Fifty monkeys have survived for longer than 6 months after receiving treatment with procarbazine by sc, ip and/or oral routes at doses of 5-50 mg/kg. Forty-one monkeys have been necropsied to date, of which 14 (34%) have had malignant neoplasms. Seven monkeys were diagnosed with acute leukemia, all but one of the myelogenous type; the other acute leukemia was undifferentiated. In addition to the leukemias, two cases of hemangiosarcoma, three cases of osteogenic sarcoma, one case of astrocytoma and one case of lymphocytic lymphoma, was found. The neoplasms were diagnosed after procarbazine treatment for an average of 94 months (ranging from 16 to 192 months); the total dose of procarbazine received by the monkeys developing tumors ranged from 2.69-154.4 gm, and averaged 55 gm. Other adverse effects of long-term procarbazine treatment included vomiting, myelosuppression and testicular atrophy with complete aplasia of the germinal epithelium.

Potential Carcinogenicity of Adriamycin

The carcinogenic potential of adriamycin is less clear-cut than that of the preceeding compounds. A group of 10 monkeys received monthly iv doses of adriamycin at 12 mg/m^2. Eight of 10 animals developed congestive heart failure before the end of the anticipated dosing period, and at cumulative adriamycin doses (averaging 310 mg/m^2) well below that considered to be the safe upper limit (550 mg/m^2) in man. The animals died or were sacrificed in moribund condition. One of the 10 monkeys developed acute myeloblastic leukemia after receiving a cumulative adriamycin dose of 324 mg/m^2, and the 10th monkey in this group is alive and without evidence of illness. Old World monkeys appear to be more sensitive than humans to the cardiotoxic effects of adriamycin, and this may apply to its carcinogenic effects as well. Although the single case of leukemia noted in this study does not establish that adriamycin is a leukemogen, it does suggest that this may be yet another adverse effect of adriamycin therapy. This study is being repeated, using lower doses of adriamycin in an additional twenty monkeys.

Potential Carcinogenicity of N-methyl-N'-nitro-N-nitrosoquanidine (MNNG)

MNNG is being administered by the oral route (1.0 mg/kg, daily 5 days every week). A group of 21 monkeys has received this compound for periods of up to 8 years; thus far, 2 animals have died of causes unrelated to treatment with MNNG. The remaining 19 animals appear to be in good health and without signs of toxicity. However 3 additional monkeys were given MNNG as a colon implant; 2 of these monkeys have been necropsied and one monkey was diagnosed with a well-differentiated adenocarcinoma at the rectosigmoid junction. The latter monkey had received a total MNNG dose of 8.65 gm; it was administered in gelatin cubes containing 5.3-42.7 mg MNNG which were inserted into the colon twice every week.

Potential Carcinogenicity of N,N-dimethyl-p-phenylazoanaline (butter yellow)

A total of 32 animals received oral doses of butter yellow, and 24 are alive 18-20 years after the first dose of the compound. Four of the 8 dead animals survived less than 1 year after initiation of treatment; among the 4 necropsied animals that survived longer than one year, one case with a highly invasive liposarcoma was found. Whether this tumor arose as a consequence of treatment with butter yellow or is a spontaneous tumor developing in an aged (20 year) animal is uncertain; this judgement must await further observation of the 24 surviving monkeys on this study.

Potential Carcinogenicity of Sterigmatocystin

Sterigmatocystin has been under test for approximately 6 years. It is being administered po, 1 day/week at 1 mg/kg (15 monkeys) and 2 mg/kg (15 monkeys). Thus far, only one monkey in the 2-mg/kg group has been necropsied, and histopathologic examination of tissue from this animal revealed no evidence of tumor development, although severe toxic hepatitis with hyperplastic nodules was noted. One animal in the 1.0 mg/kg group has been necropsied, and histopathologic examination of its tissue revealed primary hepatocellular carcinoma. This animal had received a total of 1.13 gm of sterigmatocystin during the course of 79 months. Laparoscopic examinations of the livers of the remaining 28 monkeys are in progress.

Compounds Not Carcinogenic in Non-Human Primates

Since the inception of this study 20 years ago, 7 spontaneous tumors have been diagnosed in 219 non-treated breeders and vehicle-treated controls, yielding a tumor incidence of 3.2%. Of the 27 substances entered on test during this period, 15 [3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, N,N'-dimethyl-p-(m-tolylazo)-analine, cyclamate, saccharin, dichlorodiphenyltrichloroethane, low density polyethylene plastic, cigarette smoke condensate, arsenic, Imuran, L-phenylalanine mustard and cyclophosphamide] have not as yet induced tumors. However, several of these compounds have been under evaluation for less than 3 years.

Cyclamate has been under test for the past 11 1/2 years. Two groups of monkeys have received this compound orally, 5 days every week, at 100 and 500 mg/kg, respectively. The 100 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 2.3 gm/day/70 kg man, and is equivalent to drinking about 6 diet drinks per day. The 500 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 11.6 gm/day/70 kg man, and is equivalent to drinking about 30 diet drinks per day. Two of 12 monkeys at the low dose, and 2 of 11 monkeys at the high dose have been necropsied, but no evidence of a malignant neoplasm was found.

Two groups of 10 monkeys each have been receiving oral doses of saccharin (25 mg/kg), 5 days every week. This dose corresponds, on an equivalent surface area basis, to a daily intake of 5 cans of diet soda by a 70 kg man. One group of monkeys has been receiving saccharin for an average of 134 months (range 120-124 months), and the second group of 10 monkeys began saccharin treatment

approximately 4 years ago. Since the inception of the study, none of the monkeys have died, and there is no evidence of toxicity in any of the treated animals.

Similarly, long-term administration of DDT has not resulted in the development of tumors in our non-human primates. A total of 24 animals has received DDT by the oral route (20 mg/kg) daily, 5 days every week in a study that has been underway for the past 146 months. Administration of DDT is discontinued after a dosing interval of 130 months is completed. Although 6 of the monkeys have died thus far, none were found to have developed tumor. The apparent cause of death in 5 of these animals was DDT-induced CNS toxicity, as they experienced severe tremors and convulsions immediately prior to death. The 18 surviving monkeys appear to be in good health.

The carcinogenic potential of arsenic has been under evaluation for approximately seven years. A total of 20 monkeys has received sodium arsenate po (0.1 mg/kg) 5 days/week, and three monkeys in the group have died. The cause of death in the monkeys was unrelated to arsenic treatment, and the surviving monkeys are well and without signs of toxicity. Nine monkeys have received lung implants containing tobacco smoke condensate in a beeswax matrix; all are well and without evidence of toxicity approximately nine years after implantation of the material.

Significance to Biomedical Research:

The present colony has been in continued existence for 21 years; it supports the largest study of chemical carcinogenesis in non-human primates undertaken in this country, and as such it represents a national resource. In addition to providing data on the carcinogenicity of a variety of chemicals, including antitumor and immunosuppressive agents in clinical use, it has also made it possible for us to acquire information in other important areas of primatology. Such information includes the spontaneous tumor incidence in various species of non-human primates, their lifespan in captivity, their reproductive characteristics, the organization and management required to hand-rear 40-50 neonates per year in a nursery, and parameters of growth and development in simian primates. Normal animals of all ages as well as tumor-bearing animals are used in a variety of pharmacologic studies and chemotherapeutic trials. The availability of monkeys receiving chronic treatment with chemicals has made it possible to identify some consequences of long term chemical exposures (other than tumor development) not previously recognized. Tumor-bearing monkeys have been employed in attempts to develop biological markers, such as α-fetoprotein, and other diagnostic tests for detecting premalignant lesions and early tumors. More recently, tumor bearing monkeys have been used to develop new contrast media for computerized tomography of liver and spleen, and to compare the distribution of free and liposome-entrapped ara-C in tumor tissue and in adjacent normal tissue. Our accumulated experience with specific carcinogens has also enabled us to initiate studies on the prevention or reversal of chemically induced tumors. This project is of great significance to biomedical research in general, and continues to serve well the objectives of the Program.

Publications:

1. Adamson, R.H., and Sieber, S.M.: Chemically-induced leukemia in humans. Environ. Health Perspect. 39: 93-103, 1981.

2. Adamson, R.H., and Sieber, S.M.: Chemical Carcinogenesis Studies in Non-human Primates. In: Proceedings of a Symposium on Organ and Species Specificity in Chemical Carcinogenesis. 1982 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06108-13 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies on the Mechanism of Action and Mechanism of Resistance of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	2.5	2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Methodology was developed to study the activity of deoxycytidylate deaminase and thymidylate synthetase in intact cells and to study the flux through the de novo pyrimidine pathway in intact cells and in various tissues in vivo. The dynamics of thymidylate synthetase inhibition by MTX and fluoropyrimidines and the effects of altered nucleotide pools were investigated in intact L1210 cells. The role of 3-deazauridine as a fraudulent allosteric regulator of carbamyl phosphate synthetase II was demonstrated. Several compounds to inhibit uridine kinase were synthesized and evaluated. FUdR-5'-methylphosphonate, an analogue of the activated form of FUdR, was synthesized and evaluated. Acivicin, PALA, pyrazofurin, and 6-azauridine were evaluated for inhibition of de novo pyrimidine biosynthesis in normal and tumorous tissues in vivo using precursor molecules (glutamine, ammonium, bicarbonate) labeled with stable isotopes of carbon and nitrogen and quantitation by GC/MS. The salvage of CdR by L1210 cells and incorporation into cytosine and thymine bases of DNA was studied. The activation of mAMSA by microsomal and non-microsomal enzymes and the role of activation in cytotoxicity was investigated.

Project Description:

Objectives:

A knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To rationally design new anticancer agents.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. Various enzymes are isolated and purified, and the effect of various antitumor agents as inhibitors of these enzymes are studied in addition to effects on DNA, RNA and various proteins. Molecular models are also used in drug design.

Major Findings:

The Role of Microsomally Activated Intermediates in the Cytotoxicity of m-AMSA

Liver microsomes actively metabolize m-AMSA to several highly reactive species which are then conjugated with glutathione and excreted into the bile (see previous annual report). These diimine quinones and quinone imines will covalently bind to a variety of macromolecules and might possibly bind to DNA. Since tumors are known to have small, but measurable, amounts of cytoplasmic and nuclear microsomal activity, we investigated the possibility that microsomal activation was required for m-AMSA lethality. Cells were grown under a variety of conditions designed to inhibit microsomal activity. The cells were made anaerobic under a N_2 atmosphere, were exposed to carbon monoxide gas, and were treated with metyrapone. m-AMSA toxicity, measured by soft agar cloning, was unaffected by any of these treatments. m-AMSA was totally devoid of toxicity when the treated cells were cooled to 4°C prior to and during treatment. DNA single strand breaks were not detected in cells cooled to 4°C prior to m-AMSA treatment. Protection from DNA single strand breaks was not provided by any of the other treatments. L1210 cells were treated with ^{14}C-m-AMSA and the DNA purified and washed. No radiolabel was associated with the washed DNA. Microsomal activation does not appear to play a significant role in the m-AMSA cytotoxicity seen in L1210 cells.

The Role of Non-Microsomal Oxidative Enzymes in the Activation and Cytotoxicity of m-AMSA

Clinical observation has shown that m-AMSA, while having only moderate or minimal activity in most tumors, is particularly effective against acute myeloblastic leukemia. The mechanism for this tumor selectivity is unknown,

but clearly the selectivity lies in the biology of the AML cells. Since m-AMSA is activated to ADQI or AQI through oxidation, the concentration of myeloperoxidase found in some AML cells could provide the mechanism for selective toxicity. Studies were undertaken to determine whether peroxidase could produce toxic intermediates from parent m-AMSA. Experiments showed that, in the presence of H_2O_2, horseradish peroxidase would very rapidly convert m-AMSA into a new chemical species which was highly reactive and which interacted almost immediately with glutathione. The enzyme was also capable of converting o-AMSA into a reactive species. When tested in a soft agar cloning system, the peroxidase treated m-AMSA showed toxicity to L1210 cells comparable to that of parent drug while o-AMSA, which is intrinsically minimally toxic, showed equi-toxicity with m-AMSA after peroxidase treatment. Work is underway using myeloperoxidase positive HL-60 cells to assess the importance of peroxidase activation in the antitumor activity of m-AMSA.

Inhibition of Aldehyde Oxidase by m-AMSA

Aldehyde oxidase is a hepatic flavoprotein enzyme, similar to xanthine oxidase, with an ill-defined physiological role. It is an enzyme that metabolizes MTX to 7-hydroxyMTX and it may be significant in the patient who receives high dose MTX therapy. m-AMSA is a potent inhibitor of aldehyde oxidase with a $K_i = 5x10^{-7}M$ and exhibits mixed competitive-noncompetitive inhibition. m-AMSA is a molecule composed of a 9-aminoacridine base with an aromatic side arm attached through the amino group. Interestingly, 9-aminoacridine is a purely competitive inhibitor of the enzyme while the side-arm shows non-competitive inhibition. Both AQDI and AQI inhibit the enzyme but not more so than parent m-AMSA. All experiments to date have utilized 1-methylnicotinamide chloride as a substrate. Further experiments using 2,4-dichlorophenol-indophenol are planned to determine at which point in the electron-transport chain the enzyme inhibition occurs. Additional *in vivo* work is planned on the effect of m-AMSA on the pharmacokinetics of MTX in the rabbit.

The Dynamics of Thymidylate Synthetase Inhibition by Methotrexate and Various Fluoropyrimidines in Intact L1210 Cells

Thymidylate synthetase is a critical enzyme in the *de novo* synthesis of thymidine. Several important antitumor agents, prominently the fluoropyrimidines and methotrexate, are known to strongly inhibit the function of this enzyme. Existing information on the inhibition of thymidylate synthetase by these drugs has come from *in vitro* studies using isolated, purified enzyme or from observations of drug effects on DNA synthesis. We have utilized ^3H-5-deoxyuridine as a probe to measure the activity of thymidylate synthetase in the intact cell, leaving unaltered all the complex and intertwined metabolic pathways that characterize cellular nucleotide anabolism. Thymidylate synthetase replaces the 5-position hydrogen of dUMP with a methyl group and in the process releases the 5-position hydrogen into cellular water. Since enzymatic release of the 5-hydrogen is unique to thymidylate synthetase, the release of tritium from ^3H-5-deoxyuridine is a direct measure of enzyme activity. Preliminary studies have shown intracellular thymidylate synthetase inhibition in the L1210 cell to be essentially complete when media concentrations reach $1x10^{-5}M$ 5-FU, $2.5x10^{-9}M$ FUdR, and $1x10^{-5}M$ MTX. Studies are underway to examine the time course of inhibition,

the rate of reversal of inhibition on removal of drug, and the relationship of intracellular enzyme inhibition to cell death as measured by soft agar cloning.

The Altered Dynamics of Pyrimidine Pathways in the Presence of Altered Nucleotide Pool Sizes

The measurement of the release of tritium from ^{3}H-5-deoxyuridine permits the activity of thymidylate synthetase to be measured in the intact cell. The substrate for thymidylate synthetase is dUMP, and one of the sources of dUMP is the deamination of deoxycytidine monophosphate by deoxycytidylate deaminase. If the tritium release from ^{3}H-5-deoxycytidine is measured, the relative activity of deoxycytidylate deaminase can be ascertained. Using both of these molecular probes, we have begun a study of the regulation of pyrimidine anabolic pathways in L1210. Early results show that expanding TTP pools by growing cells in media with a TdR concentration of $1x10^{-4}$M produces a 50% inhibition in the rate of tritium release by thymidylate synthetase; a TdR concentrations of $1x10^{-5}$M shows no inhibition. Further, the flux of nucleotides through the dCMP deaminase pathway is an appreciable percentage of the flux through the deoxyuridine kinase pathway. It appears that the deaminase pathway is 50% as active as the kinase pathway in providing the cell with dUMP.

Incorporation of Deoxycytidine into Pyrimidine Bases of DNA: Effect of Endogenous and Modified Pyrimidines

Since deoxycytidine (CdR) can modify the biological effects of 2 clinically used pyrimidines, ara-C and thymidine (TdR), we examined the salvage of CdR by cultured L1210 cells. Intracellularly, CdR is phosphorylated to dCMP which can be further phosphorylated to dCDP and be incorporated into DNA as dCTP, or be deaminated to dUMP, converted to dTMP, and incorporated into DNA as dTTP. Our interest in the relative importance of these 2 possible modes of incorporation of salvaged CdR began with an examination of the Km values for pyrimidine nucleoside monophosphate kinase. This enzyme phosphorylates CMP as well as dCMP. The Km for CMP for the kinase isolated from a variety of mammalian cells ranges from 10 to 85 μM whereas the Km for dCMP ranges from 0.7 to 2.8 mM. This difference in the Km values suggested that the extent of phosphorylation of dCMP may be limited due to competition for the kinase by CMP. Thus, the deamination of dCMP and subsequent incorporation of salvaged CdR into DNA TdR might be an important anabolic pathway for CdR.

To determine the relative rate of phosphorylation versus deamination of dCMP, L1210 cells were exposed to [2-^{14}C]CdR and the extent of incorporation of carbon-14 into the TdR and CdR bases of DNA (expressed as a ratio TdR/CdR) was determined by HPLC analysis. At 3 μM CdR the TdR/CdR ratio was 3.3 (77% of the radioactivity was in the DNA TdR and 13% in the DNA CdR) after 2 hr and 5.7 after 24 hr. At 100 μM CdR, the ratio remained constant (3.0 ± 0.2) for 24 hr. Thus the deamination of dCMP is highly favored and the percentage incorporated into DNA as dTTP increased at the lower CdR concentrations.

Modified or excess natural pyrimidine were added to the culture media to see how these compounds affect CdR salvage. All experiments were incubated for 4 hr which gave a control DNA TdR/CdR ratio of 3.9. Tetrahydrouridine up to 1 mM had no effect on the ratio or on the specific activity of the DNA CdR or DNA TdR. 3-Deazauridine at 0.1 and 1 mM reduced the ratio to 1.4 and increased the specific activity of the DNA CdR 4-fold and of the DNA TdR 1.5-fold. At 0.1 and 1 mM cytidine the ratio increased to 7.0 and 8.0, and the specific activity of the DNA CdR decreased 40-75%. At 0.1 and 1 mM media TdR, the specific activity of the DNA TdR was reduced to <10% of controls while the specific activity of the DNA CdR increased 4-6 fold. Ara-C at 0.1 μM reduced the ratio to 2.0 by doubling the specific activity of the DNA CdR. At 1 to 100 μM ara-C, the ratio was further reduced to 1.4 with decreases in the specific activity of both DNA CdR and DNA TdR at 10 to 100 μM ara-C. Thus, the extent of incorporation into DNA TdR versus DNA CdR can be altered by the addition of TdR, ara-C, cytidine, and 3-deazauridine.

Effect of PALA on De Novo Pyrimidine Biosynthesis in Cultured L1210 and Lewis Lung Carcinoma Cells

Since PALA is curative against Lewis lung carcinoma in mice, but is ineffective against L1210, we examined the ability of PALA to inhibit de novo pyrimidine biosynthesis in cultured L1210 and Lewis lung carcinoma cells. De novo pyrimidine biosynthesis was measured by comparing carbon-14 labelled bicarbonate incorporation into uracil nucleosides in control and PALA-treated cells. After a 4 hr incubation of Lewis lung carcinoma cells with 100 μM PALA, the uracil nucleotide pool decreased 50% and the incorporation of $[^{14}C]HCO_3$ into uracil nucleotides was only 5% of controls. In contrast, no change in the size of the uracil nucleotide pool in L1210 cells was observed at 100 or 200 μM PALA. At 1 mM PALA, a 50% decrease in the uracil nucleotide pool was observed. In addition, 100 μM PALA caused no change in bicarbonate incorporation into the uracil nucleotides of L1210 cells compared to controls. At 200 μM PALA, bicarbonate incorporation was 80% of controls, and at 1 mM PALA bicarbonate incorporation was 20% of controls. Thus, 10-fold more PALA was required to inhibit de novo pyrimidine biosynthesis in L1210 cells as compared to Lewis lung carcinoma cells.

Effect of 3-Deazauridine on De Novo Pyrimidine Biosynthesis

3-Deazauridine as its triphosphate is a known inhibitor of CTP synthetase. We studied the effect of 3-deazauridine on de novo pyrimidine biosynthesis of uracil nucleotides in L1210 cells to determine if 3-deazauridine inhibits de novo synthesis, and, if so, is this inhibition due to an increase in UTP pools or due to 3-deazaUTP functioning as a fraudulent feedback allosteric inhibitor of carbamyl phosphate synthetase II. Experiments in which the carbon-14 labelled bicarbonate incorporation into the uracil nucleotides of 3-deazauridine treated cells was examined demonstrated that the size of the uracil nucleotide pool is unchanged at 3-deazauridine concentrations of 10 μM or lower and is decreased at 3-deazauridine concentrations of 50 μM and greater. However, inhibition of de novo synthesis of uracil nucleotides is observed such that when the intracellular concentration of 3-deazaUTP is 1 to 2.5 nmol/10^6 cells, the incorporation of labelled bicarbonate into

uracil nucleotides is 25-40% of controls. When the intracellular concentration of 3-deazaUTP is 5 to 6 nmol/10^6 cells, bicarbonate incorporation is only 15-20% of controls. Thus, 3-deazaUTP appears to be acting as a fraudulent feedback inhibitor of de novo pyrimidine biosynthesis.

Design, Synthesis, and Testing of Inhibitors of Uridine/Cytidine Kinase

Previous studies in our laboratory have indicated that for an inhibitor of de novo pyrimidine biosynthesis to be clinically effective, the salvage of pyrimidines by uridine/cytidine kinase must also be inhibited. We have undertaken a new project to develop a clinically useful inhibitor of uridine/cytidine kinase. In our preliminary studies we have found that uridine 5'-(β-D-glucopyranosyl) monophosphate does not inhibit the phosphorylation of uridine by uridine/cytidine kinase isolated from mouse L1210 cells. However, 5'-amino-5'-deoxyuridine is a competitive inhibitor with a K_i = 300 μM. The K_m for uridine as a substrate for the enzyme was measured to be 71 μM. A series of pyrimidines with modifications at the 5' position and elsewhere in the pyrimidine base have been designed and will be synthesized and tested in the forthcoming year.

Synthesis and Biologic Properties of FUdR 5'-Methylphosphonate

Previous studies from our laboratory showed that ara-C 5'-methylphosphonate inhibited dCMP kinase but was not a substrate for this enzyme. FUdR-5'-methylphosphonate (FUdR-MeP) was designed and synthesized with the following expected properties: (1) would be an analogue of FdUMP that did not require activation by TdR kinase; (2) would not be susceptible to phosphatase attack; (3) would penetrate cell membranes; (4) would inhibit dTMP synthetase; and, (5) would not be further phosphorylated by dTMP kinase. FUdR-MeP was found to be a competitive-irreversible inhibitor of isolated dTMP synthetase; inhibition required the presence of cofactor; the affinity constant for FUdR-MeP was several orders of magnitude less than that for FdUMP. Inhibition of flux through dTMP synthetase in intact L1210 cells by FUdR and FUdR-MeP followed the same concentration dependency as observed with the isolated enzyme. The methylphosphonate analogue of dUMP did not inhibit dTMP synthetase and did not inhibit L1210 cell growth. FUdR-MeP inhibited L1210 cell growth at concentrations 1000-fold higher than FUdR; inhibition was reversed by thymidine.

Measurement of the Flux Through the De Novo Pyrimidine Biosynthetic Pathway of Mouse Tumors In Vivo and the Inhibitory Effects of Certain Pyrimidine Antimetabolites

A gas chromatographic-mass spectrometric (GC/MS) technique (see Project Z01-CM-06148-03) was used to study the effects of certain antimetabolites on the flux through the de novo pyrimidine pathway in L1210, Lewis lung, and PALA-resistant Lewis lung tumors in vivo.

Treating animals with 400 mg/kg PALA 24 hours before measurement of the flux through the pyrimidine biosynthetic pathway showed 70-90% inhibition of this pathway in Lewis lung tumors. In L1210 tumors similarly treated, there was no significant change in the precursor incorporation in s.c. implanted tumors and

only a 34% inhibition of the pathway was evident in the i.p. tumors. In contrast to the lack of inhibition of flux through the pathway after the 24 hour pretreatment, mice with L1210 s.c. tumors pretreated for 1 hour with 400 mg/kg PALA showed a significant decrease in precursor incorporation of over 90%. Additionally, 48 hours after a 400 mg/kg dose of PALA, there was no significant inhibition of the pathway in Lewis lung tumors made resistant to PALA or L1210 s.c. and i.p. tumors, but there was a 90% inhibition in normal Lewis lung tumors. Mice with L1210 i.p. tumors were also treated with acivicin (20 mg/kg 2 hour pretreatment), pyrazofurin (100 mg/kg 3 hour pretreatment) and 6-azauridine (1g/kg 1 hour pretreatment), all of which inhibited precursor incorporation into the uracil nucleotide pool of the cells by approximately 90%. The size of the uracil nucleotide pool in the tumors was significantly reduced following PALA pretreatment at 24 and 48 hours, but not after 1 hour pretreatment. Thus, our data indicate that changes in the size of this pool do not necessarily reflect the activity of the de novo pathway at any given time. For example, the uracil nucleotide pools of both L1210 i.p. and L1210 s.c. tumors were significantly reduced 24 hours after a single dose of 400 mg/kg PALA indicating an inhibition of de novo synthesis but at 24 hours there was a recovery of de novo biosynthesis as indicated by the lack of inhibition of precursor incorporation. All other drug treatments did not significantly reduce the size of the uracil nucleotide pools, and may reflect the shorter time of pretreatment chosen for these compounds. These studies will be expanded to investigate optimum times of incorporation and relative recoveries of the pathway in different tumors. Additionally, this methodology will be used to study the effects of compounds on the flux through the de novo pathway in normal vs. malignant cells in vivo to gain insight into the mechanism(s) of selective toxicity exhibited by antitumor agents.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06134-07 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Role of the Lymphatic System in the Absorption and Distribution of Antitumor Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of the lymphatic system in the absorption and distribution of free and liposome-entrapped antitumor agents (adriamycin, ara-C and melphalan) administered to rats and to tumor-bearing monkeys by ip, iv or sc routes is under investigation. Liposomal encapsulation markedly altered many pharmacological properties, such as tissue distribution, metabolism and excretion, of the drugs tested; entrapment in liposomes also produced a significant increase in their lymphatic absorption and lymph node uptake. The antitumor effect of melphalan against lymph node metastasis of mammary adenocarcinoma 13762 was enhanced by liposomal encapsulation. Studies in progress are aimed at optimizing liposome characteristics (e.g., size, charge and lipid composition) for maximal lymph node uptake and retention and for maximal chemotherapeutic activity, and attempts are underway to target liposomes to specific sites, such as the lymph nodes, using monoclonal antibodies coupled to the liposome membrane.

Project Description:

Objectives:

The objective of this project is to obtain information on the role of the lymphatic system in the absorption and distribution of clinically useful antitumor agents following their administration by subcutaneous, intravenous or intraperitoneal routes. The development of methods for producing a preferential and selective uptake of antitumor agents by lymphatic vessels and lymph nodes is a major goal in this work. Efforts are currently focused on evaluating liposomal encapsulation of drugs as a means of attaining this goal. These studies are designed to determine the relative rate and extent to which free and liposome-entrapped antitumor agents are removed from the peritoneal cavity and interstitial space by lymphatic versus blood routes. In addition, the effect of liposomal entrapment of antitumor agents on other pharmacologic properties, such as tissue disposition, toxicity and antitumor activity, is being assessed. Reproducible model systems in rodents for tumor metastasis via lymphatic channels to regional lymph nodes are being developed and evaluated so that it will be possible to assess the therapeutic advantage of treating such tumors with antitumor agents targeted by liposomal encapsulation to regional lymph nodes.

Major Findings:

The effect of liposome encapsulation on toxicity of the anticancer drug adriamycin (ADR) was studied using large volume ip administration. Concentrations of 5, 20, and 75 μg/ml of free ADR, ADR encapsulated within lipid vesicles or ADR in the presence of empty lipid vesicles were tested. The most prominent toxic effect was peritonitis, characterized by accumulation of peritoneal fluid, abdominal adhesions, and histologic evidence of inflammation. Two days after treatment, peritonitis was marginally less with encapsulated than with free ADR. Fourteen days after treatment, peritonitis was generally more severe in the encapsulated 20 μg/ml group than in the free ADR group. At 75 μg/ml the effects of encapsulation on local toxicity were mixed, although the systemic effects were less severe with encapsulation. A generalized hyperlipidemia (day 14) and elevated LDH levels (day 2) produced by the highest dose of free ADR group were not observed in rats receiving encapsulated ADR. It is concluded that at the concentrations tested, encapsulation appears to offer minimal advantage in reducing local toxicity, but that it may be of benefit in reducing the incidence and severity of systemic toxicity resulting from large volume ip administration of ADR.

The distribution and cellular localization of liposomes in lymph nodes is currently under investigation in mice. Colloidal dye was injected ip or sc at various locations including the thigh, thorax and footpads of the fore and hind limbs. At various times after injection, animals were sacrificed and various lymph nodes removed and examined microscopically for pattern and intensity of staining. For most injection sites studied, dye was detected only in regional lymph nodes in the immediate vicinity of the injection site. However, in mice injected in the footpad of the hind limb, lymph nodes distant from the injection site were stained in addition to regional lymph nodes. In these mice, the intensity of staining in the various lymph nodes varied with their location

relative to the footpad, such that intensity diminished with increasing distance from the injection site in the order popliteal > lumbar > renal > mediastinal > axillary > inguinal > cervical. Although the intensity of staining varied among different lymph nodes, the pattern of distribution was similar. Dye was present mainly in the margins of the nodes and at their junctions with the afferent and efferent lymphatic vessels. However, in the most intensely stained lymph nodes, dye could be observed throughout the entire node. Irrespective of the injection site, intensity of staining was maximal approximately 3 - 4 hr after injection and remained at this level for at least 24 hr.

To characterize the cellular localization of liposomes in lymph nodes, liposomes containing the fluorescent phospholipid NPDE were prepared and injected into the footpads of mice. At 3 hr after injection, the animals were sacrificed and lymph nodes removed and examined by fluorescence microscopy. The pattern and intensity of fluorescence observed was similar to that seen in mice injected with colloidal dye. After teasing the lymph nodes to release cells, intracellular fluorescence was observed but was limited and appeared to be confined to macrophages. Quantitation of intracellular fluorescence in the teased lymph nodes using a fluorescence activated cell sorter confirmed that only a small proportion of the total fluorescence present in the lymph nodes (~ 1%) was associated with cells. Studies are presently underway to confirm that macrophages are responsible for cellular uptake of liposomes in lymph nodes using fluorescent antibodies to identify the various cell types present in lymph nodes.

We are attempting to improve the cellular uptake of liposome entrapped materials into lymph nodes by using liposomes to which monoclonal antibodies (Ab) are coupled. In a preliminary study, I^{125} labelled Ab directed against specific surface antigens on lymphocytes was injected into the footpads of C57B10A mice in which the Ab is expressed and in control C57B&6 mice in which the Ab is not expressed. At 3 hr after injection, animals were sacrificed and various tissues and lymph nodes removed. In the control mice, less than 2% of the radioactivity was associated with lymph nodes, whereas more than 50% was present in corresponding nodes of C57B10A mice. Thirty percent of this radioactivity was present in popliteal nodes, 12% in lumbar nodes and 7% in renal nodes. Our findings suggest that coupling Abs to the surface of liposomes may provide a means of more precisely targeting drug-containing liposomes to specific sites such as the lymph node; they also open the possibility that Abs raised against experimental tumors and coupled to the liposome membrane may have therapeutic activity against lymph node metastases.

Studies are in progress to investigate the distribution of liposomes labelled with ^{14}C-cholesteryl oleate in lymph nodes of monkeys using quantitative autoradiography. At 2 hr after receiving ^{14}C liposomes by ip and subcutaneous (thigh) routes, animals were sacrificed and various lymph nodes removed, frozen and sectioned. Quantitative autoradiography of inguinal, axillary and mediastinal lymph nodes indicated that the major portion of the ^{14}C present in lymph nodes is localized predominantly in the marginal sinuses.

Nonhuman primates bearing diethylnitrosamine-induced hepatocellular carcinomas have been used to compare the plasma clearance, tissue distribution (including uptake by tumor tissue), metabolism and excretion of free and liposome-entrapped

ara-C. Animals were dosed iv with free [^{3}H]Ara-C or [^{3}H]Ara-C entrapped in liposomes composed of phosphatidyl choline and labelled with [^{14}]cholesteryl oleate. At 2 hr after dosing, animals were sacrificed and various tissues removed and counted for radioactivity. In addition, plasma and urine were analyzed for unchanged Ara-C and its metabolite Ara-U by HPLC. Clearance of Ara-C equivalents from the plasma was found to be slower for liposome-entrapped Ara-C than for the free drug. The rate of metabolic breakdown of Ara-C, as indicated by the ratio of Ara-C to Ara-U in plasma, was found to be slower in the monkeys receiving the liposome-entrapped drug than in animals given free Ara-C. Urinary excretion of Ara-C and its metabolite was significantly reduced in animals receiving liposome-entrapped Ara-C (27%) as compared to monkeys given the free drug (40%). Liposome-entrapment also markedly altered the tissue distribution of Ara-C. Tissue concentrations of Ara-C equivalents were higher by 50 and 8 fold respectively in the spleen and liver of animals receiving the liposome-entrapped drug than in corresponding tissues of monkeys given free Ara-C. Tumor concentrations of Ara-C equivalents were significantly higher by 2 fold in animals treated with the liposome-entrapped drug as compared to monkeys receiving the free drug. This finding indicates that a modest increase in the concentrations of an antitumor agent in liver tumors can be obtained by entrapping the agent in liposomes. In animals treated with free [^{3}H]Ara-C, tritium levels in liver tumor and surrounding normal liver tissue were essentially the same. In contrast, in monkeys given liposome entrapped Ara-C, levels of both [^{3}H] and [^{14}C] activity in tumor tissue were 5 fold lower than in surrounding normal tissue. The possibility of exploiting the differential uptake of liposomes between tumor and surrounding normal tissue to detect tumors of the liver and spleen using CT scanning in conjunction with liposome-entrapped radioopaque material is being investigated.

In order to assess the therapeutic effects of liposome-encapsulated antitumor agents against lymphogenous metastases, it has been necessary to establish a model for lymph node metastasis which is reproducible and quantitative. Two rat tumors, Walker 256 carcinoma and 13762 mammary adenocarcinoma, have been developed for this purpose. Tumor cell suspensions are inoculated subcutaneously in the hind leg of rats, and at various intervals after inoculation the tumor-bearing leg is amputated. For both tumors, metastasis takes place by lymphatics to regional lymph nodes and by hematogenous spread to lungs. For the mammary adenocarcinoma 13762, the time interval between inoculation and amputation has been found to be a critical determinant in the extent of lymph node involvement with tumor, as determined by serial measurement of lymph node weight or volume, and in the survival time of the animals. Amputation prior to 5 days after tumor inoculation reduces the incidence of blood-born lung metastases but also renders the development of lymph node metastasis less reproducible. Amputation on day 9 or 10 after tumor inoculation results in regional lymph node metastases in 100% of the animals at risk, and the weight of the lymph node has been found to reflect the degree of tumor involvement; however, the animals ultimately die of lung metastases.

The effect of liposomal entrapment of melphalan (MPL) on its tissue distribution and activity against lymph node metastasis of the 13762 adenocarcinoma was evaluated in rats. Neutral liposomes containing MPL and the fluorescent marker carboxyfluorescein (CF) were prepared by sonication using phosphatidyl-choline (PC) and cholesterol (molar ratio, 2:1), along with tracer amounts of ^{14}C-MPL

and ^{3}H-PC. Unlike CF, MPL appears to associate with the lipid phase of liposomes; entrapped MPL was not hydrolyzed and was gradually released from liposomes during incubation at 37°C, with a half-life of 3 hours. Free ^{14}C-MPL injected sc into the thigh of rats was rapidly cleared from the injection site, with only 0.6% of the ^{14}C activity remaining after 2 hours. The concentration of MPL in the ipsilateral inguinal lymph nodes showed a transient increase over that in contralateral nodes at 0.5 hour, although by 1 hour MPL concentrations were similar in ipsi- and contra-lateral nodes. After sc injection of ^{14}C-MPL/^{3}H-PC-liposomes, about 4% of the ^{14}C activity was present at the injection site at 2 hours post treatment. The concentration of PC equivalents in ipsilateral lymph nodes was at least 50 times higher than in plasma, lung or contralateral lymph nodes at all time intervals examined. Similarly, liposomal entrapment of MPL enhanced its uptake by ipsilateral lymph nodes; this increase in lymph node MPL concentration was sustained for at least 24 hours after dosing, at which time ipsilateral nodes contained 20 fold and 10 fold higher MPL levels than were present in plasma and contralateral nodes, respectively. Studies to define the optimal liposome size and lipid composition for maximal lymph node uptake are in progress. In these studies, liposomes containing MPL are prepared using phosphatidyl-PC and cholesterol, and clarification of the liposome suspension during the course of sonication is assessed by measuring the optical density (OD). When the suspensions were sonicated until the OD reached a plateau (OD, 0.035-0.040), the vesicles formed were small (37 nm in mean diameter), and relatively constant rates of entrapment for MPL and the aqueous solution were obtained. Cloudy suspensions (OD, 1.8-2.0) prepared by a short term sonication contained large vesicles and higher entrapment rates were obtained for both MPL and the aqueous phase. The tissue distribution ^{14}C-MPL/^{3}H-PC-liposomes prepared by short-term and long-term sonication was compared in rats. Sc injection of small liposomes resulted in a significantly higher and more sustained concentration of MPL equivalents in regional lymph nodes, while large liposomes were cleared from the injection site more slowly and produced only a slight increase in the lymph node concentration of MPL. In an attempt to optimize the lipid composition of MPL-containing liposomes, various neutral, positive and negative liposomes have been prepared. The MPL entrapment rate, the in vitro stability and the lymph node uptake of liposomes composed of PC:cholesterol (2:1) PC:cholesterol:phosphatidic acid (6:3:1); PC:cholesterol:stearylamine (6:3:1) sphingomyelin:cholesterol (2:1), sphingomyelin:cholesterol:phosphatidic acid (6:3:1), and sphingomyelin:cholesterol:stearylamine (6:3:1) is being compared. Tissue distribution studies of these liposomes are not yet completed. However, thus far it appears that the entrapment rate for MPL is significantly higher for the PC:cholesterol:phosphatidic acid liposomes than for the others, and that the in vitro stability is better than, or equivalent to, that of the other liposome preparations tested.

The ability of several proteolytic enzymes, administered concomitantly with MPL-liposomes, to hasten clearance of liposomes from the sc injection site and enhance regional lymph node uptake is being evaluated. Hyaluonidase, collagenase and chondroitinase singly and in combinations, have thus far been tested. Hyaluronidase was most effective in increasing the clearance rate of liposomes from the sc injection site and produced a 2 fold increase in the uptake of liposomes by the reginal lymph node at 1 hour after dosing; the concentration of hyaluronidase used (100-200 IU/ml) did not produce histologic evidence of

tissue damage at the injection site, nor did it appear to alter the integrity of the liposomes themselves. Thus the use of proteolytic enzymes to break down the intersititial matrix and/or basement membrane may be a feasible means of enhancing lymph node uptake of liposomes from the intersititial space. Rats bearing the 13762 adenocarcinoma were given a single sc injection of free or liposome-entrapped MPL 3 days after surgical resection of the primary tumor. Liposome-entrapped MPL produced a greater reduction in the weight of lymph node metastases than did free MPL. Thus, when MPL was entrapped in liposomes, a dose of 0.125 mg/kg reduced the weight of lymph node metastases to about 50% of controls. A dose of 0.5-1.0 mg/kg was required to exert an equivalent effect on lymph node tumor growth when free MPL was administered. These results suggest that the intersitial injection of liposomes containing MPL may be useful for the postoperative treatment of lymph node metastases.

Significance to Biomedical Research and the Program of the Institute:

Liposomal encapsulation is a novel drug delivery system; this project represents an attempt to improve the selectivity of antitumor agents by targeting them, through liposomal encapsulation, to specific sites. Tumor metastasis is a major clinical problem in treating cancer. Lymphatic channels are frequently the route by which tumors metastasize, with micrometastases lodging in regional and even distant lymph nodes. It would therefore be of potential therapeutic advantage in the treatment of early metastases to selectively concentrate antitumor agents in lymph channels and lymph nodes. The results of our studies suggest that liposomal encapsulation is a feasible method for targeting antitumor agents to these sites.

Proposed Course:

To continue to pursue the goals listed under "Objectives" above. Particular emphasis will continue to be given to optimizing liposomal characteristics such as size and lipid composition for maximal uptake by lymphatics and lymph nodes and to targeting liposomes to specific sites with the use of monoclonal Abs. Efforts will also continue to be directed toward developing new experimental models for lymphatic metastasis, improving existing model systems, and utilizing these models for evaluating the therapeutic effects of liposome-entrapped antitumor agents.

Publications:

1. Parker, R.J., Sieber, S.M., and Weinstein, J.N.: The effect of liposome encapsulation of a fluorescent dye on its uptake by the lymphatics of the rat. Pharmacology 23: 128-136, 1981.

2. Khato, J., Priester, E.R., and Sieber, S.M.: Enhanced lymph node uptake of melphalan following liposomal entrapment and effects on lymph node metastasis in rats. Cancer Treat. Rep. 66: 517-527, 1982.

3. Parker, R.J., Priester, E.R., and Sieber, S.M.: Comparison of lymphatic uptake, metabolism, excretion and biodistribution of free and liposome entrapped [^{14}C]cytosine-β-D-arabinofuranoside following ip administration to rats. Drug Metab. Dispos. 10: 40-46, 1982.

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5. Litterst, C.L., Sieber, S.M., Copley, M., and Parker R.J.: Toxicity of free and liposome-entrapped adriamycin following large volume, short term intraperitoneal exposure in the rat. Toxicol. Appl. Pharmacol. 1982 (in press).

6. Parker, R.J., Priester, E.R., and Sieber, S.M.: Effect of route of administration and liposome entrapment on the metabolism and disposition of adriamycin in the rat. Drug Metab. Dispos. 1982 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06142-05 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of thymidine and other nucleosides as modifiers of antineoplastic drug action.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.80	0.5	1.30

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The purpose of this work is to study and further understand biochemical modulation of cancer chemotherapeutic agents in an in vivo situation. Carbon 14 and tritium labeled deoxycytidine are used as tracers of pyrimidine salvage. Normal mice and L1210 bearing mice provide small intestine, bone marrow and spleen in which the selectivity of thymidine action on biochemical pathways is measured. HPLC techniques are used to measure nucleosides and nucleotides in these tissues and plasma. Extractions of lipids, RNA, DNA and protein, plus cesium sulfate gradients will permit analysis of incorporation of tracers into various tissue components. These data will provide the means to determine kinetic rate constants for metabolism, transport and incorporation of various metabolites and the effects of thymidine on those rate constants. Deoxyazacytidine is being used as the antitumor agent in parallel studies on antitumor action in mice. Data are being collected on the degree of L1210 tumor cell kill and host toxicity following administration of this compound in combination with thymidine. The HPLC and tracer data provide clues on the optimal modes of administration of the combination of thymidine and deoxyazacytidine.

Project Description:

Objectives:

1. To study and further understand the biochemical modulation effects of thymidine in various tissues of intact mice.

2. To determine rate constants for metabolism, transport and incorporation of tracer deoxycytidine into intestinal tissue, bone marrow and spleen of normal and L1210 bearing mice.

3. To determine whether the dose, time and mode of administration of thymidine are important in affecting the above rate constants.

4. To further understand the antitumor action of the deoxycytidine analogue, S-Triazin-2 (1H)-one, 4-amino-1-(2-deoxy-β-D-erythro-pento-furanosyl) NSC #127716 and the modulating effects of thymidine on this analogue.

Methods and Major Findings:

1. We completed the study to determine why L1210 growth and clonogenic ability can be arrested in vitro by thymidine but not in vivo. Cytotoxic effects in vitro indicated that three logs of cell kill could be achieved at thymidine concentration of (1mM) and exposure time of (96 hours), both pharmacokinetically achievable in vivo with minimal toxicity to the host mice. However, extensive in vivo trials in L1210 bearing mice indicated no cytostatic or cytotoxic effect of thymidine in L1210 leukemia. In other words, a pharmocokinetic basis for the difference between in vitro and in vivo results was not acceptable.

It is known that thymidine exerts its cytoxic effects through a subsequent nucleotide metabolite (TTP), which inhibits the enzyme ribonucleotide reductase. This inhibition leads to depletion of dCTP, a necessary substrate for DNA synthesis. The salvage metabolite deoxycytidine supplied in sufficient quantity can reverse this cytotoxic effect of thymidine. We found that following thymidine treatment of L1210 bearing mice, the pretreatment plasma deoxycytidine concentration (< 1 micro molar) was raised five to ten fold. These concentrations of deoxycytidine are sufficiently high to reverse the cytotoxicity of 1 mM thymidine in vitro and therefore is the probable explanation for the resistance of L1210 to thymidine toxicity in vivo in CDF1 mice.

These data have been presented at the AACR meeting and are to appear shortly in JNCI.

2. We are now exploring reasons why deoxycytidine (dC) paradoxically increases in the plasma in vivo following high dose thymidine. Preliminary data indicates that the half-life of dC changes from a normal 8 mins to 50 mins following a tritiated tracer i.v. dose of dC two hours after a 3600 mg/Kg i.p. dose of thymidine. These findings indicate that the body clearance of dC is inhibited by the presence of mM thymidine. Further HPLC analysis of plasma extracts indicate that one likely cause of the inhibition of dC clearance is

the competition of deoxyuridine and thymidine for the same catabolic enzymes. Deoxyuridine, a product of the deaminase reaction of dC, then creates some product inhibiton of the deaminase reaction to slow the catabolism of deoxycytidine. HPLC analysis of tissue extracts for nucleotides indicate that the mononucleotides, at least, are altered by both the presence of L1210 and by prior administration of thymidine.

We are now conducting further studies on the flux of both carbon 14 and tritium labeled deoxycytidine into DNA of intestine, bone marrow and spleen. These studies should enable us to determine whether thymidine is selective in its biochemical modulation effects in vivo.

3. Parallel studies are being conducted with the deoxycytidine analogue, deoxyazacytidine in L1210 bearing mice in order to determine the antitumor effect and its modulation by thymidine. Initial data indicate that at a high dose of thymidine 3600 mg/Kg i.p. just prior to an i.v. dose of deoxyazacytidine (5dAC), the toxic dose response curve for 5dAC is shifted sharply to the left. This indicates that thymidine also probably competes for the clearance of 5dAC like it did for dC. Furthermore, at equitoxic doses, the combination of thymidine and 5dAC is no more therapeutic than 5dAC alone. This result is from only one dose of thymidine and further doses must be explored. 5dAC alone is a potent antitumor agent against L1210 tumor. It is in our hands capable of creating a six log cell kill of L1210 tumor in CDF1 mice after a single dose intravenously.

Publications:

1. Zaharko, D.S. and Ramonas, L.M.: Comparison of in vitro and in vivo effects of thymidine on L1210 leukemia in mice. JNCI 68: 875-880, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06148-03 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Endogenous Modifiers of Drug Action

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Other:	Jean M. Karle	Staff Fellow	LCHP	NCI
	Patricia A. Monks	Visiting Associate	LCHP	NCI
	John M. Strong	Sr. Staff Fellow	LCHP	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Chemical Pharmacology, DTP, DCT, NCI

SECTION
Drug Metabolism Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5	2.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were continued on the role of the liver as a source and modulator of circulating purines and pyrimidines. Compounds were studied in the isolated perfused rat liver in an attempt to dissociate the nucleoside export function of the liver from the uptake catabolic function. A potent inhibitor of pyrimidine nucleoside phosphorylase was found to substantially increase circulating concentrations of uridine. A GC/MS technique was developed to measure the flux through the de novo pyrimidine pathway in isolated hepatocytes and cultured cells in vitro and in normal and tumorous tissues in vivo by quantitating the incorporation of stable labelled precursors into the uracil nucleotide pool. The formation of di-labelled uracil molecules was exploited to quantitate the total flux through the de novo pathway which includes both labelled and non-labelled products. The inter-relationship of the urea cycle and the de novo pyrimidine pathway in isolated hepatocytes was investigated using isotopically labelled ammonia, glutamine, and bicarbonate. Circulating concentrations of uridine were found to inhibit de novo pyrimidine biosynthesis indicating that salvage of circulating pyrimidines is favored over de novo synthesis.

Project Description:

The activity of certain antimetabolite antitumor agents is the result of their action as enzyme inhibitors. The cytotoxic and antitumor effects of these agents are known to be influenced by administration of the product of the inhibited reaction or by compounds that can circumvent the inhibited reaction. For example, inhibition of the de novo pyrimidine biosynthetic pathway can be overcome by administration of preformed pyrimidines that can be utilized via salvage pathways. The overall objective of this project is to determine if antitumor agents alter the availability (via the serum) of endogenous compounds that have the potential of influencing the toxic effects of the agent at the tumor site.

Specifically:

1. To determine if inhibitors of the de novo pyrimidine and purine biosynthetic pathways alter circulating levels of preformed nucleosides that have the potential of reversing the cytotoxic effects of these agents at the tumor site.

2. To determine the mechanism of regulation of serum levels of pyrimidine and purines and if these levels can be altered to influence drug effect.

3. To determine if it is necessary to inhibit de novo synthesis in normal donor organ(s) simultaneously with inhibition in tumor cells in order to achieve an antitumor effect.

4. To determine if drug-induced fluctuations in serum levels of endogenous nucleosides can be used to rationally design scheduling and combinations of drugs for maximum chemotherapeutic advantage.

Methods Employed:

Usual biologic, pharmacologic and biochemical techniques. Among these are analytical techniques for quantitating endogenous compounds; isolated organ perfusion techniques; tissue culture techniques; tumor transplantation and harvesting of cells; enzyme isolation; techniques for measuring the flux through biosynthetic pathways.

Major Findings:

Effect of Endogenous Modifiers of Hepatic De Novo Pyrimidine Biosynthesis on the Output of Uridine by the Isolated Perfused Rat Liver

Research from this laboratory (see previous annual report) 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. Reducing circulating uridine concentrations may enhance the efficacy of pyrimidine antimetabolites which inhibit enzymes of the de novo pathway prior to the formation of uridine monophosphate; e.g., PALA, acivicin and 6-azauridine. Increasing donor organ production of uridine may

allow for rescue of normal tissues following antimetabolite treatment, where administration of the nucleoside itself for this purpose could be ineffective due to its short half life in the blood. We demonstrated that NH_4Cl stimulates de novo pyrimidine biosynthesis in isolated hepatocytes. The effect of NH_4Cl on the output of uridine by the perfused liver was studied. When animals were predosed with 200 mg/kg NH_4Cl 30 and 45 minutes prior to perfusion of the isolated liver, the output of uridine was increased 3-4 times over control levels for the first 10-45 minutes of perfusion. When the NH_4Cl was mixed into the circulating perfusate, 4 doses of 2 mM NH_4Cl at 25 minute intervals had no effect on uridine output over 120 minutes of the perfusion. It was found that 20 mM NH_4Cl caused a substantial increase (5-10 fold) in the output of uridine, but there was a lag time of 60-75 min after the first addition of NH_4Cl before any change in circulating levels was observed. Thus, it appears that high concentrations of NH_4Cl are required to modify uridine output by the liver and our predosing experiments indicate that the effect is short lived. The lag time required for the increase in circulating uridine concentrations is presumably the time necessary for the combined effects of stimulation of de novo synthesis, accumulation of uracil nucleotides, and 5'-nucleotidase action to yield an increased export of preformed uridine.

Glutamine, which also stimulates de novo synthesis in hepatocytes, produced no significant change in the output of uridine by the liver when given to animals at 360 mg/kg, 30 and 45 minutes prior to perfusion of the isolated liver .

Another endogenous modifier of de novo synthesis in isolated hepatocytes is ornithine, which stimulates the urea cycle at the expense of pyrimidine biosynthesis. Both 2 and 10 mM ornithine in the circulating perfusate reduced the output of uridine by the liver during the initial 45 min of the experiment, such that the normal "overshoot" of uridine output was prevented. However, circulating concentrations of 1-2 μM were slowly achieved, then maintained throughout the course of the experiment. Predosing a rat with 200 mg/kg ornithine 30 and 45 minutes prior to perfusion also inhibited the primary rise in uridine concentration in a manner similar to that achieved when ornithine is added directly to the perfusate.

Effect of an Inhibitor of Uridine Phosphorylase on the Metabolism and Output of Circulating Uridine by the Isolated Rat Liver

In addition to studying endogenous modifiers of hepatic uracil nucleotide anabolism, we have investigated the effect of an inhibitor of uridine catabolism on circulating levels of uridine in the perfusate. We previously demonstrated that the isolated liver efficiently removes uridine from the perfusate and metabolizes the majority of it to products beyond uracil in the catabolic pathway of uridine, while maintaining circulatory levels by constant excretion of the nucleoside. Inhibition of the breakdown of uridine in the isolated liver with a new inhibitor of uridine phosphorylase [(5-benzyl-1,2'-hydroxyethylmethyl)uracil, BZHU] changed the pattern and kinetics of circulating uridine in the perfusate. 20 μM BZHU increased the half life of circulating uridine from 8 to 20 minutes, while 100 μM BZHU increased the half life to 60 min. Thus, concentrations as low as 20 μM BZHU inhibit the breakdown of uridine and dramatically slow its removal from the perfusate by the liver which, apparently, cannot efficiently salvage preformed pyrimidines. In terms

of circulating levels of uridine, 20 μM BZHU caused a concentration of 4-5 μM uridine (higher than control levels of 1-2 μM) to be maintained in the perfusate. 100 μM BZHU resulted in a linear increase in the perfusate uridine concentration, such that after 3h it had reached 19 μM uridine which is equivalent to an output rate of 154 nmol/h/g wet weight maintained over 3h. Thus, in the presence of this inhibitor, output of uridine by the liver is apparently unaffected, but the liver's ability to catabolize uridine is inhibited, causing a net increase in the concentration in the perfusate. It is therefore possible to manipulate the role of the isolated liver as a donor organ of circulating uridine. Further investigation is required to extend these results from the isolated organ into the intact animal.

Export of Purines and Regulation of Circulating Purines by the Isolated Rat Liver

The role of the liver as a supplier of purines to other tissues for salvage has long been an accepted premise. The results of Pritchard et al. (Am. J. Physiol. 229: 967-972, 1975) indicated that the liver removes hypoxanthine from the plasma and exports adenosine. With the development of new purine analogues as antitumor agents, we initiated studies in the isolated rat liver to determine the role of this organ as a supplier and regulator of purines that could modify the toxicity of these analogues in purine-requiring organs and tumors. Purine bases and nucleosides were quantitated by HPLC analysis and metabolic interconversions of added purines were determined by radiotracer methodology. Hypoxanthine and inosine were rapidly metabolized to uric acid and approximately 2% of each of these tracers was salvaged by the liver and incorporated into liver purines. Adenosine was rapidly deaminated and the resulting inosine metabolized to uric acid. Adenosine was salvaged to a greater extent by the liver with 20% of the total radioactivity being incorporated into liver purines. Adenine was found to be exported by the liver in increasing concentrations until it reached 1 μM and then plateaued. No adenosine was found in the effluent of the perfused liver, even when the liver was exposed to a 10 μM concentration of hypoxanthine. Thus, it appears that the liver exports adenine and not adenosine as had been previously postulated. Experimentation is underway to explain the discrepancy between our results and those of Pritchard et al. and to determine the importance of adenine export by the liver as a modifier of the action of purine antimetabolites.

Stable Isotope Tracer Methods Development for Measurement of the Flux Through the De Novo Pyrimidine Biosynthetic Pathway In Vivo

A method was developed to measure the flux through the de novo pyrimidine biosynthetic pathway of normal and tumorous tissue in vivo using stable isotope tracers. This technique requires that the isotopic enrichment of atoms contained in the uracil nucleotide pool be quantitated with sufficient accuracy at isotopic enrichments as low as 2%. A gas chromatographic mass spectrometric technique was developed to quantitate the isotopic abundances of atoms in uracil. The sensitivity of this method was approximately 100 times greater than that previously developed for quantitating isotopic contents in the uridine molecule. With this increased sensitivity, isotopic enrichment measurements at the 2% level were demonstrated. Uracil was formed from uridine contained in the dry tissue extracts by adding uridine phosphorylase isolated

from P-388 tumor cells. Before analyzing the uracil for isotopic content a suitable uracil derivative was required to allow gas chromatographic separation of the uracil from other contaminants contained in the tissue extracts. In addition, it was necessary that the derivative produced ions containing the proper molecule fragments under electron impact. Numerous derivatives were investigated and the 1,3-diheptyluracil derivative was selected based on its gas chromatographic and mass spectral characteristics. The electron impact mass spectrum of this molecule (M.W. 308) produced an ion at M/Z 291 suggesting a loss of OH. This ion was confirmed by synthesizing 4-[^{18}O]-diheptyluracil which also produced an ion at M/Z 291 confirming the loss of OH. The technique developed can measure the incorporation of either ^{13}C or ^{15}N into the uracil moiety of the uracil nucleotide pool.

In conjunction with the gas chromatographic mass spectrometric method developed for isotopic measurements of uracil, a method of administering $^{13}CO_2$ to mice with tumors was designed so that the flux through the de novo pyrimidine biosynthetic pathway in mouse tumors could be quantitated in the presence and absence of drugs which inhibit this pathway. Since a single administration of $^{13}CO_2$ to the mouse results in rapid clearance of the tracer from the mouse mainly through the respiratory process, a chamber was designed in which the mice were exposed to a continuous 10% $^{13}CO_2$: 90% air environment for 20 minutes. Isotopic measurements of the $^{13}CO_2/^{12}CO_2$ ratio during the course of the experiment showed rapid equilibration of the expired CO_2 with the $^{13}CO_2$ within the first 5 minutes and a constant ratio of approximately .55 was maintained throughout the remaining 15 minutes.

Studies were also conducted in tumored mice with the labeled precursors $^{15}NH_4Cl$ and ^{15}N-glutamine administered by IP injection and flux through the de novo pyrimidine biosynthetic pathway was quantitated by measuring the ^{15}N incorporation into the uracil nucleotide pool. The results of these studies and others in which rat liver hepatocytes were exposed to $^{15}NH_4Cl$ and ^{15}N-glutamine showed that the ^{15}N from the labeled precursors enters both the N_1 and N_3 positions of the uracil molecule. Since multiple labeling of the uracil molecule by its ^{15}N precursors was observed it was necessary to develop a gas chromatographic mass spectrometric technique that would measure the isotopic enrichment of each nitrogen atom separately. Additional uracil derivatives were synthesized and examined to see if the uracil molecule mass spectral pattern could be altered to produce a suitable fragment ion for quantitating the isotopic enrichment of nitrogen in the 1-position. This investigation led to 1,3-dibenzyluracil which produced a single ion fragment at M/Z 158 containing only the N_1 nitrogen and with no other ions in the M/Z 157-M/Z 161 region. The M/Z 158 and M/Z 159 ions were used to quantitate the incorporation of ^{15}N into the 1-position of uracil in liver and tumor tissue extracts obtained from mice given either $^{15}NH_4Cl$ or ^{15}N-glutamine. Only liver samples obtained from mice treated with $^{15}NH_4Cl$ showed incorporation of ^{15}N in the 1-position. The mole % determined was 8.6 + 1.6% SD (n = 3). The molecular ion of dibenzyluracil was used to measure the mole percent ^{15}N enrichment in uracil as nonlabeled in either the N_1 or N_2 position and that which was doubly labelled in both the N_1 and N_2 positions. The results of this analysis showed that uracil contained in liver and tumor tissue extracts from mice receiving ^{15}N-glutamine and in tumor tissue extracts from mice receiving $^{15}NH_4Cl$ was labeled in only the 3-nitrogen position.

The mole percent ^{15}N enrichments measured in these samples were 2.5 ± .01% SD (n = 3) and 9.6 ± .52% SD (n = 3) for the liver and tumor tissues, respectively, from mice receiving ^{15}N-glutamine and 6.4 ± 1.2% SD (n = 3) for tumor tissues from mice receiving $^{15}NH_4Cl$. Incorporation of ^{15}N into both the 1- and 3-position of uracil was observed in liver tissue extracts obtained from mice receiving $^{15}NH_4Cl$. The mole % uracil which was singly labeled in either the 1- or 3-position and that which was doubly labelled in both the 1- and 3-positions was 6.9 ± 1.6% SD (n = 3) and 7.1 ± 1.35 SD (n = 3), respectively.

In experiments in which di-labelled uracil is formed, the following components of the uracil nucleotide pool can be determined: (a) the amount of labelled product produced by the pathway, (b) the amount of non-labelled product produced by the pathway, and (c) the amount of product not produced by the pathway during the time course of the experiment. These calculations are based on probability theory and the necessary mathematical equations were derived and computer programs written to handle the computations. This is a new approach for determining such values and should prove valuable in determining the contribution of the de novo pathway to uridine pools in normal and tumorous tissues in vivo.

The Role of NH_4Cl and Glutamine as Substrates for De Novo Pyrimidine Biosynthesis In Vivo and In Vitro

The mammalian liver contains two separate carbamyl phosphate synthetase enzymes (CPS-I and CPS-II). CPS-II is a cytoplasmic enzyme which catalyzes the initial rate limiting reaction of de novo pyrimidine biosynthesis. CPS-I, which catalyses the initial step in the urea cycle, is found almost exclusively in the mitochondria of hepatocytes and is in greater abundance than CPS-II in these cells. The nitrogen donating substrates with the strongest affinity for these two enzymes are glutamine (CPS-II) and ammonia (CPS-I). However, ammonia has been reported to stimulate de novo pyrimidine biosynthesis in the liver as measured by increased $H^{14}CO^-_3$ incorporation into uracil nucleotide pools (∑uracil).

In an effort to elucidate the role of ammonia and glutamine as substrates for de novo biosynthesis of pyrimidines in the liver, we used the GC/MS technique described above to measure the incorporation of ^{15}N from these two precursors into ∑uracil in isolated hepatocytes, cultured L1210 cells, and also into L1210 tumors and livers in the intact animal. $^{15}NH_4Cl$ and ^{15}N-glutamine are both substrates for de novo pyrimidine biosynthesis in isolated hepatocytes, cultured L1210 cells, and in mouse livers and s.c. tumors in vivo. ^{15}N-glutamine forms only mono-labelled uracil molecules in vivo and in L1210 cells in vitro, presumably at the N_3 position by acting directly as a substrate for CPS-II. In isolated hepatocytes however, although mono-label predominates, di-labelled uracil is also present. Thus, ^{15}N-glutamine labels aspartate pools of these cells, a process which requires the formation of NH_3 as an intermediate.

Administration of $^{15}NH_4Cl$ results in only mono-labelled uracil molecules in L1210 tumors in vivo, but there is evidence that this label may be in either the N_3 or the N_1 position of the uracil moiety indicating labelling of both aspartate and carbamyl phosphate pools. Although ^{15}N-glutamate is an excellent

substrate for in vitro L1210 pyrimidine biosynthesis, high concentrations of $^{15}NH_4Cl$ in these cells had an inhibitory effect and neither substrate stimulated de novo synthesis as measured by $^{14}HCO^-_3$ incorporation. In hepatocytes increasing $^{15}NH_4Cl$ concentrations increased the incorporation of labelled precursors into ∑uracil. This lack of an inhibitory toxic effect of NH_4Cl in hepatocytes would be due to more efficient conversion of $^{15}NH_4Cl$ to glutamine in the liver. Alternatively, $^{15}NH_4Cl$ is a substrate for CSP-I which could be donated as carbamyl phosphate for utilization by aspartate transcarbamylase and this may not be subject to the feedback control of UTP as is CPS-II. Evidence for the validity of this latter theory is that in the presence of high concentrations of an inhibitor of glutamine synthetase (methionine sulfoximine) the pattern of label incorporation in ∑uracil from $^{15}NH_4Cl$ remains unchanged, indicating no dependence or glutamine formation. $^{15}NH_4Cl$ readily labels aspartate and carbamyl phosphate pools to a high degree in hepatocytes, as is evidenced by the predominance of double labelled uracil molecules.

From our data, there is no evidence that either glutamine or NH_4Cl is a better substrate for pyrimidine biosynthesis in the liver. However, their incorporation into ∑uracil may occur by different mechanisms. At concentrations approximating those found in the liver, glutamine appears to be more readily utilized than NH_4Cl. However at equimolar concentrations, NH_4Cl appears to be more stimulatory and is perhaps able to circumvent feedback control mechanisms of pyrimidine de novo synthesis. An understanding of hepatic control mechanisms for pyrimidine biosynthesis, which may be unique for this organ, could be important for pyrimidine antimetabolite chemotherapy.

Effect of Uridine on the Activity of the De Novo Pyrimidine Pathway in Cultured L1210 Cells

The relative dependence of cultured L1210 cells on de novo synthesis of pyrimidines versus salvage of pyrimidines was examined by measuring the incorporation of carbon-14 labelled bicarbonate into uracil nucleotides at varying media uridine concentrations. Since L1210 cells rapidly deplete uridine from culture media (11-14 nmol/ml/hr/10^6 cells), a method was devised to maintain constant uridine levels by infusing uridine into tubes incubating in a shaking water bath. The de novo synthesis of uracil nucleotides by L1210 cells was inhibited >95% at media uridine concentrations >12 μM. At 3 to 5 μM uridine, de novo synthesis of uracil nucleotides was inhibited approximately 50%, and below 0.5 μM uridine no effect was observed on de novo synthesis. Inhibition of de novo synthesis was related to expansion of the uracil nucleotide pool such that >95% inhibition occurred when the pool size was increased by 100% or greater. As the pool size expanded from 0 to 100%, there was a corresponding increase in the extent of inhibition of de novo synthesis of 0 to >95%. Since circulating uridine concentrations in humans are generally in the range of 2-9 μM, these results suggest that the concentration of circulating uridine may be sufficient to substantially inhibit de novo pyrimidine biosynthesis in cells in vivo. In addition, the lack of therapeutic response by humans towards inhibitors of de novo pyrimidine biosynthesis may be explained by a reduced dependency of tumor cells on the de novo pyrimidine biosynthetic pathway.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06149-12 LCHP

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Pathology of Drug-Induced Alternations in Animals and Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. C. Lowe	Cancer Expert	LCHP	NCI
Other:	C. R. Creveling	Research Pharmacologist	LBC	NIAMDD
	T. E. Gram	Supervisory Pharmacologist	LMCB	NCI
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COOPERATING UNITS (if any)

Laboratory of Bioorganic Chemistry, NIAMDD

LAB/BRANCH
Laboratory of Chemical Pharmacology

SECTION
Pathopharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of the mechanism(s) by which anthracyclines are toxic to isolated heart cells have resulted in the isolation by gel electrophoresis of a protein that may be related to the phenomenon. The protein is present in mitochondrial fractions, has a molecular weight over 120,000, and covalently binds doxorubicin in a time-dependent fashion which correlates with the drug's toxicity to these cells. Efforts are underway to identify the high affinity protein, and to determine if it is involved in the development of the unusual, delayed cardiotoxicity of anthracyclines in vivo.

Project Description

Methods Employed:

Standard techniques of morphological and biochemical analysis have been employed in these studies.

Objectives:

To follow morphological leads and use morphological and biochemical approaches to investigate the mechanisms by which various antitumor agents can induce toxicities. Such information should be of value in 1) designing new, less toxic, analogs of currently useful oncolytic agents, and 2) developing clinical protocols that minimize toxicological manifestations of antitumor agents.

Major Findings:

In Vitro Investigations of Anthracycline Cardiotoxicity.

Using a rat cardiac myocyte system developed in our laboratory, we have isolated a high affinity mitrochondrial protein that binds doxorubicin in a time-dependent manner. The binding is highly specific, as demonstrated by SDS gel electrophoresis, and is associated with what appears to be a single protein (mol.wt. - approximately 120,000). Identification of this protein and more complete characterization of the circumstances under which it binds doxorubicin may provide important clues as to the mechanisms by which anthracyclines are cardiotoxic *in vivo*.

Significance of Biomedical Research and the Program of the Institute:

As stated in the objectives it is our intent to integrate pharmacological and morphological data to develop a better understanding of the mechanisms of toxicity of oncolytic agents. The importance of using morphological approaches to study toxicology would be difficult to overstate. It is often the descriptive data that helps to narrow the range of other types of investigations into certain phenomena. Out of these studies should come new information that will improve our ability to chemotherapeutically treat cancer with minimal toxicity.

The development of a rapid, inexpensive cardiac myocyte model to assess the cardiotoxic potential of new oncolytic agents should make it possible to make programmatic decisions in the drug development area much more expeditiously than in the past.

Proposed Course:

Studies are currently underway to determine the mechanism by which the cardiotoxic anthracyclines adversely affect isolated rat cardiac myocytes.

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ANNUAL REPORT OF THE LABORATORY OF EXPERIMENTAL THERAPEUTICS AND METABOLISM

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 - September 30, 1982

General Background; Research Program Objectives

The Laboratory of Experimental Therapeutics and Metabolism (LETM) is an organizational entity established within the Developmental Therapeutics Program (DTP), Division of Cancer Treatment (DCT), during FY 82. It represents both a continuation and expansion of the research program of the Molecular Toxicology Section (MTS) Clinical Pharmacology Branch, Clinical Oncology Program, DCT. With the establishment of the LETM, all personnel and resources of the MTS were incorporated into the new Laboratory and the MTS was abolished.

The original MTS research program was initiated on February 1, 1977. The main research goals of this section were to develop an analytical pharmacology/chemistry unit to interface with clinical pharmacology studies within the DCT, and to develop a research program in biochemical toxicology relevant to cancer etiology and treatment. As the result of these initiatives, new strategies for predicting adverse drug reactions were derived and new approaches for the development of drugs with greater tumor selectivity and less toxicity were undertaken. To further support these research avenues, and also to provide an additional focus for integration of DCT preclinical and clinical programs, the LETM was established.

The major areas of emphasis in the LETM research program are fourfold: a) to investigate basic and applied aspects of metabolism and disposition of anticancer drugs or candidate drugs, particularly as it relates to modulation of antitumor activity and/or toxicity of drugs for cancer therapy, b) to investigate biochemical mechanisms of adverse reactions to drugs or drug combinations, or drugs combined with other forms of cancer therapy; these studies emphasize development of: (1) better strategies for predicting, preventing, or treating adverse reactions in patients and (2) new strategies and/or new drugs with greater tumor-directed selectivity, c) to develop a strong program of interactions and collaborations with DCT clinical and preclinical research laboratories, and d) to aid the preclinical toxicology program of the DTP through consultation, when appropriate, on toxicological problems or data of relevance to ongoing drug development activities.

General Methodology and Techniques Employed

Research problems are approached in an interdisciplinary manner, and a wide variety of techniques are employed. Analytical facilities within the Laboratory include a Varian MAT 44 quadrupole mass spectrometer with multiple inlet systems. A Varian System 200 computer is interfaced with the mass spectrometer system to facilitate the analysis of both qualitative and quantitative analytical data. Other analytical techniques in use include conventional gas chromatography,

pyrolysis gas chromatography, high-pressure liquid chromatography, and radiochromatography. In biological studies, modern analytical techniques and approaches are used in the study of drug metabolism and drug kinetics both in vitro and in vivo. Toxicologic investigations employ chemical and biochemical evaluations of drug induced abnormalities as well as light- and electron-microscopic evaluations of structural alterations. Autoradiographic techniques frequently are employed in studies of the cellular and intracellular sites of toxin interactions. The Laboratory contains a complete electron microscopy facility, currently including a JEOL 100B transmission electron microscope and all necessary support equipment. Additionally, a new JEOL 100CX scanning/transmission analytical electron microscope is presently being installed and is expected to be fully operational within the present year. An X-ray microanalytical system will be interfaced with the latter instrument. Complete facilities for organic synthesis also are available in the Laboratory and are employed for the preparation of isotopically-labeled standards for analytical procedures and drug metabolism investigations, as well as in drug development studies.

Research Program of the LETM; Summary of Projects and Accomplishments, FY 1982

This year's Annual Reports are grouped into three major project categories: A. Biochemical Toxicology (Z01 CM 07125-01 LETM), B. Pathobiology/Experimental Chemotherapy (Z01 CM 07126-01 LETM), and C. Chemistry/Drug Metabolism (Z01 CM 07127-01 LETM). The subprojects described in detail in each of the above were grouped under the respective categories to emphasize the major program directions presently under way in the Laboratory. Presently these categories facilitate the organization and description of ongoing activities but their longer-term purpose is to provide a structure for organizing the Laboratory into specific work groups, and, in the future, into formal Sections, with well-defined areas of responsibility represented by each of the above project areas.

The project categories A. and C. substantially represent the continuation and expansion of the two major facets of the research program of the Molecular Toxicology Section. Category B., however, is a new facet that will allow more effective integration of basic chemical/biochemical studies with relevant biological systems. For more detailed summaries of individual subprojects, reference should be made to the individual reports attached. The following is a brief outline of progress described therein.

A major focus of effort continues to be the biochemical toxicology of the lung. A compound of central interest in our laboratory has been the furan derivative, 4-ipomeanol, a highly selective pulmonary alkylating agent and cytotoxin. This compound is preferentially activated in most animal species by metabolism in a specific family of bronchiolar cells, the Clara cells. Because the cytochrome P-450 monooxygenase system involved in activating 4-ipomeanol is also responsible for activating many pulmonary carcinogens, we have proposed the Clara cell as a potential site of origin for bronchogenic lung cancers and suggested such cancers might be susceptible to cytotoxic agents such as 4-ipomeanol. Much of our current work with 4-ipomeanol is being directed toward this question. Indeed, preliminary studies suggest 4-ipomeanol is cytotoxic against some human lung cancers in vitro, a finding which, if confirmed, may open a new avenue for development of new types of drugs effective against lung cancer.

Aside from its promise for development as a new class of cytotoxic anticancer drug, 4-ipomeanol has provided an extremely useful tool for investigations of basic pulmonary pathobiology. The discovery that pulmonary Clara cells are a major site of cytochrome P-450 enzymes in lung, a conclusion reached initially through our studies with 4-ipomeanol, has been confirmed subsequently by related investigations in several other laboratories. Particularly important were results from investigators at the National Institute for Environmental Health Sciences (NIEHS). These scientists, using immunohistochemical techniques with antibodies against purified lung cytochromes P-450 showed direct localization of these hemoproteins in Clara cells. In the present reports we describe results of new studies, done in collaboration with NIEHS investigators, of the metabolism of 4-ipomeanol by isolated lung cytochromes P-450 and in isolated pulmonary cell populations. 4-Ipomeanol was metabolized much more efficiently by either of the two major pulmonary cytochromes than by the major hepatic cytochromes P-450. Isolated Clara cells were far more active toward 4-ipomeanol than any other cell types examined (e.g., type II pneumocytes, pulmonary macrophages). These studies provided key information to fully integrate prior chemical and biological studies of 4-ipomeanol, and further strengthened the conceptual basis for a mechanism of pulmonary toxicity involving direct *in situ* metabolic activation of the protoxin in target lung cells.

The cellular specificity of the metabolic activation of 4-ipomeanol also has been studied *in vitro* in intact lung tissue. Isolated whole lungs or lung slices rapidly metabolized the compound to an alkylating agent, and high-resolution autoradiography showed that, as *in vivo*, most of the covalently bound 4-ipomeanol metabolite was found in the bronchiolar Clara cells. Further *in vitro* studies with lung tissues from animals pretreated *in vivo* with metabolic inhibitors, or a GSH depletor (diethylmaleate) showed decreases or increases, respectively, in the intensity of Clara cell alkylation but no change in the Clara cell specificity. This further supported the view that the alkylating metabolite of 4-ipomeanol was actually formed *in situ* in those target cells.

Isolated lungs and lung slices, in conjunction with high-resolution autoradiography, proved to be a useful way to investigate the cell-specific activation of 4-ipomeanol, and this technique will be tested with other pulmonary cytotoxins. However, certain studies planned for the future will require highly purified preparations of viable lung cell types. The development of isolation techniques for lung cells has proved to be exceedingly difficult, but one in which we shall invest considerable effort in the coming year. Clara cells are of obvious interest and we have considerably improved and refined previously published methods for isolation of this population. These higher purity preparations will be essential for planned studies of APUD properties of Clara cells. Further attention also is being focused on improving isolation methods for type II pneumocytes, and on developing isolation procedures for pulmonary endothelial cells, type I pneumocytes, and other major lung cell populations.

A recently completed set of adjunct studies in avian species showed that bird lungs were practically devoid of the cytochrome P-450 system, and that 4-ipomeanol did not cause lung toxicity in these species. Instead 4-ipomeanol caused severe damage to the liver of avians, a site where the agent is rapidly activated by metabolism. Interestingly, avian lungs do not have Clara cells nor ciliated bronchiolar cells, and lung tumors are exceedingly rare in this species.

Studies with 4-ipomeanol also have indicated the potential importance of pulmonary glutathione (GSH) as a protective factor against lung damage by highly reactive alkylating agents or metabolites. GSH presumably protects by combining with the reactive species to form less reactive, less toxic conjugates that can be eventually excreted.

Because the cytochrome P-450 mediated metabolism of 4-ipomeanol leads to an alkylating species that binds covalently at or near its site of formation, the compound may help elucidate cellular sites of cytochrome P-450 activity in tissues other than the lung. In this regard, preliminary studies have been performed with skin, a target site for many carcinogens that require activation by metabolism. Autoradiography of mouse skin incubated with radiolabeled 4-ipomeanol suggested the presence of bound metabolites over the differentiated epidermis, the hair follicles and the sebaceous glands.

Ipomeanol-4-glucuronide was shown to be the major urinary metabolite of 4-ipomeanol in the rat. Production of this metabolite was markedly enhanced by phenobarbital (PB) treatment, but not by methylcholanthrene (MC). The latter result explained the contrasting effects of these two inducers on the in vivo target tissue specificity of covalent binding and toxicity of 4-ipomeanol. PB treatment decreases both the hepatic and the pulmonary covalent binding and toxicity of 4-ipomeanol, whereas MC decreases pulmonary binding but markedly enhances the hepatic binding and toxicity. It is apparent that both MC and PB increase the systemic clearance of 4-ipomeanol through an enhancement of hepatic metabolism. However, the enhancement by PB is predominantly via a "nontoxic" pathway (e.g., glucuronidation) but with MC predominantly via a "toxic" pathway (e.g., formation of alkylating metabolite).

The effect of diethylmaleate (DEM) on the in vivo metabolism and tissue pharmacokinetics of 4-ipomeanol was also investigated. DEM did not alter the amounts of ipomeanol-4-glucuronide excreted, nor did it significantly change the tissue distribution of the unmetabolized 4-ipomeanol. These findings were consistent with the view that the marked enhancement of tissue covalent binding and toxicity of 4-ipomeanol caused by DEM in vivo is due to the depletion of tissue GSH by DEM, and not due to a major change in the exposure of the tissues to parent 4-ipomeanol.

Our studies have been extended to other potentially cytotoxic and/or carcinogenic furan compounds. 3-Methylfuran reportedly is a major component present in certain urban smogs. We have found that this compound, like 4-ipomeanol, is metabolized in situ in the lung to a highly reactive product that destroys the bronchiolar epithelium. The metabolism of 2-methylfuran, which has similar toxicity to 3-methylfuran, also is being investigated since potential metabolite standards for this isomer are more readily accessible to organic synthesis. Detailed histopathological studies recently completed for 3-methylfuran show that this compound not only causes profound disturbances of lung architecture and cellular turnover, but also can cause multiorgan damage involving especially the liver, the kidneys and the lymphoid system.

With both 3-methylfuran and 4-ipomeanol we are exploring methods of noninvasive evaluation of pulmonary function in small laboratory animals. We have found that whole-body plethysmography, a technique previously developed for evaluation

of irradiation-induced lung damage in rodents, is useful for monitoring certain types of chemically-induced lung damage, such as that caused by the above furan compounds.

We also are investigating perilla ketone, another naturally occurring pulmonary cytotoxin that is structurally similar to 4-ipomeanol. Initial investigations indicate that, in certain species, this agent may have even more potent and selective pulmonary alkylating activity than 4-ipomeanol. Therefore, this compound will also receive careful attention in studies of lung tumor-directed cytotoxicity that are currently under way.

We have had a continuing interest in the drug nitrofurantoin. This is a commonly used urinary tract antibacterial agent. It has caused more reported instances of serious lung disease than any other therapeutic agent used in humans. We have investigated the influence of inspiratory O_2, oral antibacterial treatments and dietary vitamin E deficiency on the metabolism and distribution of the compound in rats. The results showed essentially no effect of any of these treatments, and suggested that the markedly potentiated pulmonary toxicity of nitrofurantoin seen in vitamin E deficient rats and in rats exposed to hyperbaric oxygen is not due to alterations in the tissue distribution or covalent binding of the drug. The results are also consistent with the view that nitrofurantoin lung injury in this model involves an "oxygen activation" mechanism resulting from cyclic reduction/oxidation of the drug in lung tissue in the presence of O_2.

Studies of mechanisms of lung injury by known anticancer drugs also are currently receiving emphasis in our laboratory. Investigations are centered on BCNU and bleomycin, the two antineoplastic agents responsible for the majority of drug-induced lung disease in cancer patients. A reliable animal model for BCNU-induced lung fibrosis has been developed and biochemical/morphological correlates are being defined. Similarly, an improved model for bleomycin-induced lung fibrosis is being developed using continuous drug infusion with indwelling minipumps. Ongoing studies with each of these models seek to define the critical chemico-biological interactions (e.g., macromolecular alkylation; lipid peroxidation; depletion of enzyme cofactors) which may initiate fibrogenesis and to develop means for pharmacologic intervention. BCNU causes a marked depletion of pulmonary glutathione, suggesting this substance may have a protective role against lung damage by BCNU. The latter finding is the basis for current studies of the effects of sulfhydryl depleting agents (e.g., diethylmaleate) and sulfhydryl enhancing agents (e.g., cysteine, WR-2721) on BCNU lung injury.

Concerning tissue free-SH enhancing drugs, WR-2721 is one of current interest in our laboratory. Originally developed as a radioprotective agent, this compound also has been shown to protect certain normal tissues, to a greater extent than tumor tissues, against drug-induced cytotoxicity. To explore the basis for this apparent differential protection, the pharmacokinetics and enzymology of tissue sulfhydryl enhancement by WR-2721 are under study. Preliminary results show that free-SH containing metabolites of WR-2721 appear rapidly and in relatively high concentrations in tissues protected from irradiation injury. Protective effects correlate with presence of these metabolites, not necessarily with concentrations of the parent drug. The relative sparing of tumor sensitivity may possibly result from deficient dephosphorylation capacity in tumor tissue.

The role of GSH in other tissues also has been of interest. In earlier studies we found that the glandular mucosa of the rodent stomach contains exceedingly high concentrations of GSH, sometimes exceeding levels found in any other tissues of the body, including the liver. The physiological role of gastric GSH is unknown. In a final set of studies completed and reported this year, we showed that a behavioral stressor (cold/restraint), which causes severe gastric ulcers, caused a marked depletion of gastric GSH when measured during certain times of the day. Similarly, drug treatment (s.c. administration of diethylmaleate) which was shown to markedly deplete gastric GSH caused severe gastric ulceration. These results suggested a possible role for endogenous GSH in maintaining the integrity of the gastric mucosa. Although we have terminated this project in our own laboratory, several laboratories elsewhere are pursuing similar lines of investigation and the above hypothesis continues to appear promising.

A major new initiative undertaken this year in collaboration with the Surgery Branch, NCI, is the development of an *in situ* lung perfusion protocol for treatment of metastatic soft-tissue sarcomas and osteogenic sarcomas in lung. The set of subprojects comprising this endeavor include: a) *in vitro* biochemical studies of the pulmonary metabolism of candidate drugs, b) preclinical studies of drug perfusion pharmacokinetics, metabolism, and toxicology in isolated rodent lungs and in living dogs, and c) Phase I clinical trials of the procedure in patients. The initial drug under study is adriamycin. The pulmonary pharmacokinetics have been defined in the preclinical experiments, and an initial starting dose for patient studies determined. Clinical trials have begun and three patients have thus far been perfused using a starting concentration of 1 μg/ml. Although no tumor regressions have yet been observed, the procedure seems to hold great promise since surgical and pharmacologic/toxicologic problems appear to be manageable. Dose escalations are continuing with adriamycin, and future investigations will also explore the possible value of other types of drugs used in this setting.

In support of these lung perfusion protocol development studies, are ongoing investigations of suitable laboratory models for chemosensitivity testing in a short term pulmonary perfusion setting. A human lung melanoma metastasis model in athymic mice is being adapted for these studies, which will include *in vivo* and *in vitro* (e.g., soft agar) chemosensitivity assays as well as normal/tumor tissue pharmacokinetic studies of candidate drugs. Identification of optimal candidate drugs hopefully will be aided through the development of a panel of soft-tissue and osteogenic sarcomas *in vitro* and in nude mice, a project that is currently under way and showing considerable promise. Sarcomas from the three patients in the above clinical trial are being successfully grown and propagated at the present time. We also plan to begin development of a representative panel of primary human lung cancer lines for use in these and other chemosensitivity studies.

With regard to target tissue toxicity by anticancer drugs, another organ of major interest is the kidney. Similar to our experimental approaches to biochemical toxicology of lung we are developing relevant animal models of drug-induced renal injury and are attempting to elucidate the chemico-biologic events underlying their pathogenesis.

Methyl-CCNU is a nephrotoxic drug of current interest. We have developed a reliable model of Me-CCNU renal damage in mice and rats and have shown that histopathological changes produced by the drug are closely paralleled by marked changes in biochemical parameters (e.g., PAH uptake and TEA transport) measurable in vitro in biopsy specimens, as well as by certain in vivo functional tests (e.g., urinary osmolality, kidney-derived urinary enzymes). Further refinement and study of these models may lead to improved approaches for predicting, monitoring, or treating such reactions in patients.

In another study the nephrotoxicity of 4-ipomeanol which occurs (along with pulmonary toxicity) exclusively in the adult male mouse is of interest. In previous reports we showed the renal cortical necrosis occurring in this species was caused by an alkylating metabolite formed in situ in the kidneys. Recently completed investigations of the in vivo metabolism and distribution of 4-ipomeanol in immature and adult mice of both sexes add further confirmation to this mechanism of toxicity and emphasize the potential importance of species, age and sex differences as determinants of susceptibility to drug-induced renal injury.

Basic tumor biology is an area of emphasis in several new project areas we have undertaken. Three new strains of athymic mice having double or triple immune defects are being carefully defined immunologically and the growth of human tumors in such animals is being investigated. Interestingly, some of these mice appear highly prone to develop lymphomas and the basis for this susceptibility is being probed. The LOX human melanoma metastasis model developed initially by investigators in Norway is being further examined here with particular regard to host factors and tumor cell characteristics for lung colony formation.

The further development and refinement of these and other relevant testing models provide an essential backbone for chemosensitivity evaluations of new classes of drugs of high priority interest in our laboratory. Such drugs include abrin, ricin, pulmonary cytotoxic organometallics (e.g., methylmanganese tricarbonyl), and furan derivatives (e.g., 4-ipomeanol, perilla ketone).

With the addition of an electron microscopy facility to the Laboratory, several other new project areas are under way or soon to be initiated. These include: 1) ultrastructural studies of malignant human cancer cell lines maintained in vitro or in vivo in athymic mice, 2) physiology, pharmacology and toxicology of the pulmonary Clara cell, 3) cellular/molecular factors in cell death by chemically reactive drug metabolites, and 4) cellular dynamics and structural/physiological consequences of drug-induced damage to discrete cell populations in extrahepatic tissues. These new project areas are described in detail in the attached FY 82 Annual Report ZO1 CM 07126-01 LETM. Collaborative studies involving electron microscopy also have been ongoing between LETM staff and other DTP investigators. These projects have included the structural characterization of tubulin and the investigation of liposome structure.

Other major areas of continuing activity in our laboratory include a) the synthesis of new candidate drugs and/or potential metabolites for laboratory and preclinical investigations, and b) the development and/or application of microanalytical methodologies to basic and/or clinical studies of selected anticancer drugs. Exemplifying a) we have reported this year new procedures

for synthesis of high specific activity ^{14}C-labeled and ^{3}H-labeled 4-ipomeanol necessary for studies requiring EM autoradiography. Similarly, with regard to b), a new HPLC assay, developed in our laboratory for the assay of tissue phospholipids, was applied to a collaborative study of the role of phospholipid methyltransferases in control of membrane microviscosity, as described in detail in the attached report Z01 CM 07127-01 LETM.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07125-01 LETM

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biochemical Toxicology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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7.5	5.5	2.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies completed, under way or planned include: Biochemical toxicology of A. lung, and B. kidney, with particular emphasis on in vivo model development for anticancer drugs, and elucidation of importance of metabolic activation and detoxification mechanisms; current studies are focused on nitrosoureas (e.g., BCNU, Me-CCNU), furan derivatives, and bleomycin, C. Xenobiotic metabolism and toxicity in avian species, D. Preclinical and clinical studies related to development of in situ chemotherapy perfusion protocols for treatment of pulmonary metastatic cancers, E. Investigations of acute and chronic toxicology of lung-damaging environmental chemicals, F. Drug/irridation interactions; implications for normal tissue damage, G. New drug candidate development; lung cell selective cytotoxins and lung cancer, and H. Glutathione depletion in glandular gastric mucosa; possible relationship to ulcerogenic effects of chemical (diethylmaleate) and physical stressors.

A. Biochemical toxicology of lung

1. Mechanisms of chemical-induced lung injury; role of metabolic activation (Boyd and staff)

A major aim of our research program over the past several years has been the elucidation of the importance of metabolic activation in the pathogenesis of chemically-induced pulmonary toxicity. Three different mechanistic models of lung injury involving reactive metabolites can be differentiated on the basis of the major site(s) of formation of active metabolites and/or the nature of the ultimate toxic products. In Mechanism I, the parent compound is metabolized to a highly reactive, ultimate toxin in situ in the lung. In contrast, with Mechanism II the ultimate toxic metabolite is produced by the bioactivation of the parent compound primarily in the liver, followed by transport of the active material to the lung by the circulation. In Mechanism III, the cyclic reduction/oxidation of the parent compound through a free-radical product leads to the depletion of cellular reducing equivalents, and the generation of potentially toxic "activated" oxygen species such as superoxide and/or derivatives thereof. The pulmonary bronchiolar Clara cell has been shown to be a major site of cytochrome P-450 monooxygenase activity in lung, making this cell population a prime target for the metabolic activation of potentially cytotoxic and/or carcinogenic chemicals that act via Mechanism I. Clara-cell damage by 4-ipomeanol, 3-methylfuran, and carbon tetrachloride exemplifies Mechanism I, whereas the pulmonary vascular endothelial cell toxicity by certain pyrrolizidine alkaloids probably exemplifies Mechanism II. Lung toxicities by paraquat and by nitrofurantoin are possible examples of Mechanism III. These mechanistic models have important potential implications and applications in pulmonary toxicology and in lung cancer pathogenesis as well as in the development of new or improved cancer chemotherapy protocols. Our continuing efforts at further defining these potential applications of such knowledge are described in several subsequent sections of this report as well as in parts of our FY 82 Annual Reports (Z01 CM 07126-01 LETM and Z01 CM 07127-01 LETM).

Previous studies from our laboratory have contributed substantially to the knowledge base from which the above conceptual mechanisms of pulmonary toxicity have evolved. During the past year considerable effort has been invested in the preparation of several invited chapters and reviews of our work (see Publications 1 - 10). Members of the group also have given invited presentations of this work at various workshops, seminars and symposia.

Past investigations leading to the conceptualization of the various roles of metabolism in leading to lung injury have emphasized "model" compounds which generally are chemically homogeneous, stable, simple in structure and which cause lung injury with a high degree of reproducibility in laboratory animals. Such studies have been valuable both for more clearly defining the mechanistic schemes described above as well as for facilitating the development of potentially useful and efficient experimental approaches to the elucidation of such mechanisms with other lung-toxic compounds.

In our future studies, increased emphasis will be placed on the investigation of anticancer drugs which are known to cause or would be predicted to cause pulmonary injury. These investigations will require an integrated approach involving the development and characterization of appropriate toxicity models in laboratory animals, and the systematic investigation of the distribution and target organ metabolism of the test agents in these in vivo models and in the appropriate in vitro study systems. These studies may prove to be more difficult and less readily interpretable than the past studies with simple model compounds, largely because of the relative structural complexity of the drugs, and/or their relatively complex metabolic profiles, and/or the difficulty in reproducibly eliciting pulmonary toxicities in experimental animals. Nevertheless, the mechanistic models which have been described should provide useful initial working hypotheses for these studies. Compounds of particular interest in our continuing or future investigations include BCNU (see parts 2 and 3, below), bleomycin (see part 4, below), cyclophosphamide, mitomycin C, and procarbazine.

2. Studies of acute lung injury by BCNU; ACE as a potential monitor of injury (Smith and Aiken)

The antitumor activity of bis-chloroethylnitrosourea (BCNU) was initially described in the early 1960's. Since then, BCNU has been found useful in combination with other chemotherapeutic agents in the treatment of various malignancies and as the sole therapeutic agent in the treatment of brain tumors. Recently, there have been a number of reports linking BCNU administration to the production of progressive pulmonary fibrosis. Larger clinical studies indicate that a serious life-threatening pulmonary fibrosis develops in 10-30% of the patients undergoing prolonged BCNU therapy.

We are attempting to define the pulmonary toxicity of BCNU in an appropriate animal model. In order to study acute pulmonary injury by BCNU, a number of different biochemical parameters will be measured. Preliminary experiments are being conducted to assess the effect of BCNU on angiotensin converting enzyme (ACE) levels. ACE is a membrane bound enzyme located primarily on pulmonary endothelial cells. Other investigators have shown that the administration of potent pulmonary toxins, such as paraquat and thiourea, cause a dose- and time-dependent elevation of serum and lavage ACE levels and concomitant decrease in lung tissue ACE levels. These changes in ACE levels occur before there is any histological evidence of cell damage, suggesting that changes in ACE levels may be an early biochemical indication of pulmonary cell damage. The effect of a single dose of BCNU on ACE levels in serum, lung lavage fluid and lung tissue is being determined.

Additional studies will explore the effect of BCNU on collagen synthesis. The production of collagen, the major component of connective tissue, has been shown by others to be markedly stimulated by a variety of agents (such as paraquat, bleomycin and butylated hydroxytoluene) which produce pulmonary fibrosis. The amount of collagen synthesis will be estimated by quantifying the formation of radiolabeled hydroxyproline after the in vivo administration of ^{3}H-proline to BCNU-treated and control rats. Lung explants will be used to determine the effect of BCNU treatment in vivo on the collagen synthetic

enzymes measured in vitro. Also to be investigated will be the effects of BCNU on lung cell turnover. This will be evaluated through the study of ^{3}H-thymidine incorporation into specific cell types following BCNU administration in vivo.

3. Development of a chronic pulmonary disease model with BCNU (Aiken, Smith and staff)

The purpose of this project is to develop a reliable in vivo animal model of chronic pulmonary fibrosis by BCNU. The model must be reproducible, and the degree of pulmonary damage quantifiable. This is necessary to define an end-point in studies designed to elucidate preventative measures or treatment strategies for safer use of the drug in patients.

A wide variety of doses, dosing regimens (single-dose; multi-dose; various dosing intervals), dosing vehicles (EtOH/H_2O; sesame oil; various surfactants), test animal species (rats, mice, hamsters), and several routes of administration (i.v., i.p., s.c.), were systematically tested for the ability to produce lung fibrosis. Lungs were examined by light microscopy of fixed sections stained with H & E, reticulin stain, and collagen stains and were assayed biochemically for total lung hydroxyproline content.

Of all the various treatment regimens tested, the most reliable one producing the desired effects consisted of weekly, s.c. injections of 5 mg/kg BCNU (in 10% EtOH/H_2O vehicle) in male, F-344 rats for 6 weeks, for a total accumulated dose of 30 mg/kg. Animals were sacrificed 11 weeks following the last injection. Lungs showed striking changes indicative of fibrosis and cellular hyperplasia. Pulmonary hydroxyproline levels were elevated to 170% of control values, providing further evidence of fibrosis and suggesting hydroxyproline as a potentially useful quantifier for the lesion.

Further studies are aimed at better defining this very promising model and to begin using it to explore methods for preventing BCNU lung fibrosis. Further evaluation of both the dose- and time-dependencies of pulmonary fibrogenesis will be performed in an attempt to obtain maximal lung effects with but minimizing lethal toxicity to the animals. Treatments subsequently to be evaluated as potential sensitizers or as potential protectors against BCNU-induced chronic lung injury include: combinations of BCNU with other anticancer drugs; $\pm O_2$; WR-2721 and other thiol compounds, proline analogs, anti-inflammatory steroids.

4. Lipid peroxidation and bleomycin-induced lung injury (Smith)

Bleomycin, a complex group of water soluble glycopeptides, is an important drug for treatment of squamous cell neoplasms of the neck, head and cervix, and for testicular tumors and lymphoproliferative disorders. However, the clinical usefulness of bleomycin has been severely limited by dose-related, life threatening diffuse pulmonary fibrosis which develops in a large percentage of patients.

The mechanism by which bleomycin produces pulmonary fibrosis is unknown. However, there is increasing evidence that bleomycin exerts its cytotoxic effects by the generation of activated oxygen species which cause DNA degradation.

These same oxygen species stimulate lipid peroxidation, and therefore, bleomycin, might initiate peroxidative changes in lung lipids. The generation of lipid free radicals may lead to structural damage of both lipids and proteins. This structural damage might ultimately cause cell death and/or stimulate the deposition of fibrous tissue.

The possibility that bleomycin stimulates lipid peroxidation will be explored using several experimental approaches. First, an improved animal model for bleomycin pulmonary toxicity will be sought. Previous methods of bleomycin administration (intratracheal and subcutaneous) will be compared to administration using the Alzet® osmotic minipump system. Pulmonary toxicity will be assessed by measuring lung hydroxyproline content, serum and lung levels of angiotensin converting enzyme and by histopathology. The pulmonary toxicity of bleomycin will be evaluated both in control animals and in animals receiving antioxidant (vitamin E and/or selenium) deficient diets.

After establishing an appropriate animal model, the effect of bleomycin on in vitro and in vivo lipid peroxidation will be determined. Other investigators have demonstrated that lipid peroxidation promotes the formation of conjugated diene moieties in cellular lipids. The conjugated diene content of pulmonary lipids will be measured in vivo and in lung slices exposed to various concentrations of bleomycin.

Another possible measure of lipid peroxidation is the production of ethane and pentane. For example, carbon tetrachloride (CCl_4), a potent hepatotoxin, may cause its toxic effect on the liver by increasing lipid peroxidation. Other laboratories have shown that animals administered CCl_4 expire high levels of ethane and pentane presumably due to increased levels of hepatic lipid peroxidation. In the present study ethane and pentane production by bleomycin-treated and control animals will be determined in vivo, and also in vitro in isolated, perfused lung preparations to assess the direct effect of bleomycin on lung lipid peroxidation.

Bleomycin might also promote lipid peroxidation in pulmonary tissue by altering cellular defense mechanisms. Compromise of these mechanisms could leave the cell unprotected against the deleterious effects of lipid peroxidation. Vitamin E and other endogenously occurring antioxidants (e.g. ascorbic acid and glutathione) prevent the propagation of lipid proxidation reactions and, therefore, protect the cell from uncontrolled lipid peroxidation. These antioxidants will be quantified in bleomycin-treated and control animals in addition to the determination of the activity of the enzyme systems involved in detoxication of free radicals (e.g., superoxide dismutase and glutathione peroxidase). These studies will assess the effect of bleomycin on the cellular defense mechanisms which ameliorate the deleterious effects of lipid peroxidation.

Information concerning the role of lipid peroxidation in bleomycin-induced pulmonary toxicity will help elucidate the biochemical mechanism of this toxicity and its resultant pulmonary fibrosis. Ultimately, an understanding of the mechanism of bleomycin-induced toxicity could establish preventative and therapeutic measures to increase the clinical usefulness of this drug.

5. Pulmonary GSH as a protective factor in drug-induced lung injury (Boyd and Statham)

a. Studies with 4-ipomeanol. Chemically reactive, electrophilic chemicals or metabolites may be involved in drug-induced pulmonary injury as they are in hepatotoxicity. Therefore, an important question is whether glutathione (reduced form; GSH) may be involved in the in vivo detoxification of such substances in lung and thereby may play a protective role against pulmonary damage, analogous to the protective role it plays in the liver. However, there are several difficulties inherent in the design of in vivo studies to explore this hypothesis. Important among these are the problems of determining site(s) (e.g., liver vs. lung) of formation and/or detoxification of the ultimate toxic products, as well as the possible occurrence of treatment-related translocations of GSH between the various tissues. However, it can be argued that many of these experimental problems can be avoided through the study of appropriate model compounds. Accordingly, we have studied the role of GSH as a modulator of pulmonary alkylation and toxicity in rats by the furan derivative, 4-ipomeanol.

4-Ipomeanol was an especially attractive model agent for these experiments. The compound is a highly selective pulmonary alkylating agent and cytotoxin in several species, including rats. This extraordinary pulmonary specificity results from the metabolic activation of 4-ipomeanol in situ in the lung. The highly reactive, toxic product binds immediately to pulmonary macromolecules, or alternatively, as suggested by the present studies, it may be detoxified by binding covalently with low molecular weight substances (e.g., primarily GSH) present in the cytosol to form less reactive, excretable conjugates.

The possibility that GSH might modulate the pulmonary covalent binding and damage by 4-ipomeanol was suggested by previous in vitro and in vivo studies in our laboratory. The addition of GSH to incubation mixtures containing radiolabeled 4-ipomeanol, NADPH, and rat pulmonary microsomes prevented the covalent binding of 4-ipomeanol metabolites, and resulted in the formation of GSH/4-ipomeanol conjugates. The rates of conjugate formation were not enhanced by pulmonary or hepatic cytosol preparations, indicating that the conjugation reactions did not require catalysis by GSH-transferase enzymes present in the soluble fractions. Preliminary in vivo experiments indicated that pretreatment with diethylmaleate, an agent known to deplete tissue GSH, caused a marked enhancement of pulmonary alkylation and toxicity by 4-ipomeanol in rats.

Thus, the specific goals of the present in vivo studies were: 1) to explore further the possible protective role of GSH against alkylation and damage of the lungs of rats by reactive 4-ipomeanol metabolites, and 2) to test whether exogenously administered sulfhydryl reagents could alter the pulmonary covalent binding and toxicity of 4-ipomeanol in vivo.

Toxic doses of 4-ipomeanol preferentially depleted rat lung glutathione. Pretreatment of animals with piperonyl butoxide, an inhibitor of the metabolic activation of 4-ipomeanol, prevented both the depletion of lung glutathione and the pulmonary toxicity of 4-ipomeanol. Prior depletion of lung glutathione by diethylmaleate increased both the pulmonary covalent binding and the toxicity

of 4-ipomeanol, whereas administration of cysteine and cysteamine decreased both the covalent binding and the toxicity. These in vivo studies, in conjunction with previous in vitro studies which showed inhibitory effects of sulfhydryl compounds on the covalent binding of 4-ipomeanol, are consistent with the view that pulmonary glutathione plays a protective role against pulmonary alkylation and lung toxicity by 4-ipomeanol, probably by reacting with the toxic metabolite(s) to form nontoxic conjugate(s).

Because there is evidence that many instances of chemical-induced lung injury may be due to the highly reactive, electrophilic nature of the agent involved or the ultimate toxic products derived therefrom, it appears likely that GSH could have an important general protective role against pulmonary injury by such materials. Therefore, any factors (e.g., other drugs or chemicals, nutritional status, circadian rhythms, irradiation, etc.) which conceivably could alter the availability of pulmonary GSH may likewise be important modulators or determinants of susceptibility to drug-induced lung injury.

A manuscript describing these studies has been published this year (see Publication 11)

b. Studies with BCNU. Studies have been undertaken with BCNU in accord with the above hypothesis that highly reactive lung-damaging drugs may be detoxified by GSH present in the lung. Any factors that might alter GSH, therefore, might be important in determining pulmonary sensitivity to such drugs.

BCNU is a potent alkylating/carbamoylating agent in vivo. It is known to cause marked depletion of hepatic GSH. We recently found that the drug similarly depletes pulmonary GSH in a dose-related, time dependent fashion. Further studies will be undertaken to determine if prior GSH depletion (e.g., by diethylmaleate treatment) enhances BCNU lung injury in our new chronic injury model (see part 2, above). We shall also explore whether enhancement of lung GSH levels (e.g., by administration of GSH precursors), or administration of other exogenous sulfhydryl compounds, will protect against BCNU lung damage.

6. Metabolism and covalent binding of 4-ipomeanol in lung slices and isolated whole lungs (Boyd and staff)

We are exploring the potential value of isolated lungs and lung slices in studies of the metabolic activation of pulmonary cytotoxins in intact lung cell populations. Our initial investigations utilized the model pulmonary cytotoxin, 4-ipomeanol, a compound which selectively attacks pulmonary bronchiolar Clara cells in vivo.

4-Ipomeanol was actively metabolized and covalently bound in incubation mixtures containing mouse lung slices and in isolated whole mouse lungs suspended in, and perfused intratracheally with, oxygenated Kreb's solution. Covalent binding was time-dependent and was concentration-dependent up to 0.25 - 0.50 mM 4-ipomeanol, at which concentrations the maximal rates of binding were achieved. Heat-denatured tissues did not mediate the covalent binding of 4-ipomeanol. In vitro covalent binding was markedly decreased in lung preparations from animals

pretreated in vivo with the inhibitor of 4-ipomeanol metabolism, piperonyl butoxide. In lung tissue preparations from animals pretreated in vivo with diethylmaleate (an agent which depletes lung glutathione), the in vitro covalent binding of 4-ipomeanol was markedly enhanced. Preliminary autoradiographic studies indicated that the 4-ipomeanol covalently bound in these in vitro systems was located primarily in the nonciliated bronchiolar (Clara) cells. Thus, all of these in vitro results are fully consistent with previous in vivo studies which indicated the involvement of in situ metabolic activation in the preferential damage to pulmonary cells by 4-ipomeanol.

High-resolution autoradiographic studies are under way to evaluate in these systems the possible effects of several factors on the lung cell specificity for metabolism of 4-ipomeanol: a) variations in 4-ipomeanol concentrations and times of exposure, b) treatments with metabolic inducers and inhibitors and c) prior exposure to other lung-toxic chemicals.

A manuscript describing these initial investigations is in preparation.

7. Development of new and/or improved methodologies for the isolation of viable, defined lung cell populations (Minchin)

Pulmonary metabolic systems are capable of activating and detoxifying a range of potentially toxic compounds as well as modifying the kinetics of many circulating endogenous and exogenous substrates. These non-respiratory functions of lung appear to be localized within specific cell-types although studies in this area have been limited by the heterogeneous nature of lung tissue. A number of recent studies have been aimed at isolating and identifying various cell-types, in particular the alveolar type II and nonciliated bronchiolar epithelial (Clara) cells. However, most established procedures are limited by the low yield of cells, a complex and time-consuming isolation procedure, or a poor final purity of the cell population. The objectives of this study are to develop improved methods for the isolation of defined lung cell populations which will allow the investigation of the biochemical mechanisms of pulmonary toxins at a cellular level.

At present, we have only investigated rabbit pulmonary Clara cells. A cell population of 7-10 x 10^6 cell/lung of 85% purity (range 73-91%) can be obtained within 4-5 hours. Viability is greater than 95%. The method employs a 0.1% collagenase solution containing 5.4 mM Ca^{+2} to produce a single-cell suspension of 4-5 x 10^8 cells/lung. These figures are generally much higher than those reported in the literature.

Attempts to isolate and identify other lung cell-types are under way. Capillary endothelial cells, interstitial fibroblasts and alveolar type I cells are of particular interest as all have been shown to undergo histological changes in the presence of a variety of lung toxins. At present, methods for identifying these cell-types at the light and electron microscope level are being investigated.

B. Biochemical toxicology of kidney

1. Studies with Methyl-CCNU and other nitrosoureas (Kramer)

The chloroethyl nitrosoureas (BCNU; CCNU; methyl-CCNU) are an important class of alkylating agents with demonstrated activity against malignant brain tumors. A problem resulting from the longer-term survival of patients receiving such therapy has been the development of previously unseen, cumulative dose-limiting toxic effects. Whereas, lung is the target organ for the cumulative dose-limiting toxicity of BCNU therapy (Weiss, R.B. and Poster, D.S., Cancer Treat. Rep. 8:111, 1981), nephrotoxicity limits the therapeutic potential of Me-CCNU (Harmon, W.E. et al., New Engl. J. Med. 300:1200, 1979; Schacht, R.G., et al. Kidney Int. 14:661, 1978; Micetich, K.C. et al., Am. J. Med. 71:967, 1981). From these studies it has been concluded that renal damage is dependent upon a cumulative dose of around 1,400 mg/M^2, and that children are much more susceptible to renal injury than adults. A noteworthy feature of Me-CCNU nephrotoxicity is an apparent lack of a clearly defined acute phase. Clinical evidence of renal dysfunction (i.e., elevated BUN or serum creatinine) occurred only after prolonged therapy, and frequently was not evident until long after the drug had been discontinued. The mechanism of the chloroethyl nitrosourea-induced nephrotoxicity remains unknown, although one or more metabolites might mediate the renal effect (Weiss, R.B. and Poster, D.S., Cancer Treat. Rep. 9:37, 1982). There is, as yet, no explanation for the delayed onset of nephrotoxicity.

Although acute renal toxicity was noted in preclinical toxicologic studies of the chloroethyl nitrosoureas (Oliverio, V.T., Cancer Chemoth. Rep. Part 3, 4:13, 1973; Harrison, S.D. et al., Pharmacologist 18:172, 1976; Schaeppi, U. et al., Cancer Chemoth. Rep. Part 3, 5:53, 1974), the absence of any significant renal damage in early clinical trials precluded the necessity for further studies specifically addressing this problem. Therefore, a primary goal of the LETM research program is the elucidation of the chemicobiologic interactions which underly the nephropathy produced by long-term nitrosourea chemotherapy. To achieve this, we are developing a comprehensive program integrating histopathologic, in vitro and in vivo methodologies for the assessment of renal function. Briefly, histopathology is used in conjunction with accumulation of organic ions (PAH and TEA) in kidney slices to determine both functional and morphologic changes in Me-CCNU treated animals. Non-invasive tests of renal function include the measurement of kidney derived urinary enzymes (e.g., LDH, γ-glutamyltranspeptidase and N-acetylglucosaminadase); renal concentrating capacity; PAH and creatinine clearance; and the measurement of serum BUN and creatinine levels. A more detailed explanation of renal function tests employed by our laboratory has been recently reviewed by Kluwe, W.M. (Tox. App. Pharmacol. 57, 414, 1981).

We are able to produce acute renal toxicity by Me-CCNU in mice and are currently developing a rat model. Test animals, dose, route and vehicle of drug administration, as well as time after drug administration are all important factors in establishing this laboratory model. In mice, S.C.-administered Me-CCNU produced a sharp lethality curve between 150-180 mg/M^2, with no deaths occurring before day 6. Doses below 120 mg/M^2 produced a small but transient decrease,

whereas, doses > 120 mg/M^2 resulted in a chronic decrease in renal function (at least 2 weeks). Doses in excess of 150 mg/M^2 did not result in a significantly greater decrease in renal function (~60% inhibition of PAH accumulation), only greater mortality. Furthermore, the decrease in PAH transport preceded that of TEA transport. This observation correlates roughly with preliminary histopathologic data suggesting that with time, renal damage progressed from the inner medulla to the cortex.

Future experiments are designed to explore stategies for minimizing or preventing Me-CCNU-induced nephropathy by the following manipulations: hydration-diuresis; thiol protective agents; radioprotectors; and inhibition of renal organic ion transport. In addition, we plan to investigate the possible additive and/or synergistic nephropathic effects produced by multiple dosing of Me-CCNU, and of other anticancer drugs (e.g., adriamycin) used in conjunction with Me-CCNU or other presumably less nephrotoxic nitrosoureas. We shall also attempt to develop a chronic model of Me-CCNU nephropathy using multiple-dose regimens and/or combinations with other drugs or conditions that may predispose to development of chronic nephropathy with histologic characteristics (e.g., sclerosis/fibrosis) similar to those produced typically in patients with chronic Me-CCNU therapy. Biochemical correlates and mechanisms of renal fibrogenesis will be investigated. Finally, studies addressing other chemico-biologic conditions necessary for the susceptibility of kidney towards nitrosourea-induced nephropathy (i.e., renal metabolism, covalent binding, GSH conjugation, age and sex differences) will be initiated.

2. Role of drug metabolism in renal injury (Boyd and staff)

The kidney, like the lung, is situated anatomically and functionally in a site of high exposure to xenobiotics and/or their metabolites. Also, like the lung, the kidney contains drug-metabolizing enzyme activity, albeit at levels generally lower than the liver (but in some cases equal or exceeding those in the lung). Because of the clinical importance of drug-induced renal injury, particularly with certain cancer chemotherapeutic agents, we are exploring the possible role of metabolic activation in the pathogenesis of chemical-induced renal toxicity.

Because of the many experimental advantages provided by use of 4-ipomeanol in previous studies of extrahepatic (pulmonary) metabolic activation and toxicity, and because 4-ipomeanol was found to cause acute proximal renal tubular necrosis highly reproducibly in mice, we have investigated the mechanism of this toxicity as a possible model for kidney damage due to *in situ* metabolic formation of an alkylating agent.

In previously reported studies we demonstrated the role of the renal cytochrome P-450 monooxygenase system in the metabolic activation and toxicity of 4-ipomeanol in the mouse. Administration of toxic doses of radiolabeled 4-ipomeanol to mice resulted in the preferential alkylation of the kidneys by a 4-ipomeanol metabolite. Correspondingly, unlike other species we had studied, the mouse responded to 4-ipomeanol with a striking renal cortical necrosis, in addition to pulmonary bronchiolar necrosis. Autoradiographic studies demonstrted that the covalently bound 4-ipomeanol metabolite was highly localized in those

renal cortical tubules which became necrotic. In vitro studies demonstrated that 4-ipomeanol was converted to an alkylating metabolite via cytochrome P-450-dependent mixed-function oxidase activity present in mouse liver, kidney, and lung microsomes. Renal slices also catalyzed the in vitro metabolic activation of 4-ipomeanol. Formation of an alkylating metabolite of 4-ipomeanol was greatly increased in liver microsomes, but not renal microsomes, from C57Bl/6J mice pretreated with 3-methylcholanthrene (MC). In vivo hepatic alkylation by a 4-ipomeanol metabolite was markedly elevated in the induced mice, while renal alkylation was markedly decreased. 4-Ipomeanol produced striking centrilobular hepatic necrosis in the induced mice, but caused much less renal necrosis, as compared to noninduced controls. In contrast, MC pretreatment had no significant effect on target organ alkylation and toxicity by 4-ipomeanol in "noninducible" DBA/2J mice. These results support the view that the highly reactive metabolite of 4-ipomeanol that causes renal necrosis in mice is actually formed within the target tissue, and not in the liver.

In present extensions of these studies we are utilizing high-resolution autoradiography and electron microscopy, to elucidate the specific renal cortical cell type(s) which are capable of metabolically activating 4-ipomeanol and which cells are damaged by the compound. These studies may permit conclusions regarding cellular loci for cytochrome P-450 enzymes in the kidney, and they may therefore have important general implications for renal toxicology. We also intend to explore the possible utility of 4-ipomeanol as a tool for the investigation of renal cellular biochemistry, physiology and pathophysiology.

3. Factors modifying susceptibility to drug-induced renal injury; species, age and sex differences (Boyd and staff)

Species, age and sex differences may be prominent determinants of susceptibility to nephrotoxic chemicals and drugs. In this project we are interested in identifying and characterizing such differences in susceptibility with certain model nephrotoxins and with anticancer drugs that appear to be potentially nephrotoxic. Additionally, we are investigating the extent to which metabolic and dispositional factors may underlie the above differences in susceptibility.

4-Ipomeanol (see part 2, above) shows striking species, age and sex dependency. The adult, male mouse is the only species whose kidneys are capable of rapidly metabolizing 4-ipomeanol to an alkylating agent in situ. Kidneys of nonsusceptible species (e.g., rats, guinea pigs, hamsters, rabbits) as well as female mice or immature male mice do not show the capacity to metabolically activate 4-ipomeanol. Moreover, in vivo tissue distribution studies of 4-ipomeanol in mice show that comparable doses of the compound actually yield much higher renal concentrations of the parent compound in the young, nonsusceptible male mice compared to the susceptible older mice. These and related studies have supported the view that, as demonstrated with 4-ipomeanol, renal toxicity can occur as the result of in situ activation of certain compounds to yield highly reactive, nephrotoxic metabolites. It also is apparent that species and/or age and/or sex-related differences in renal metabolism and/or renal disposition of potentially nephrotoxic chemicals can determine susceptibility to the toxic effects.

Manuscripts describing the above studies with 4-ipomeanol are in preparation. Similar investigations are being extended to other appropriate model agents and to anticancer drugs (e.g., methyl-CCNU; see Section 1) or new drug candidates.

C. Studies of xenobiotic metabolism and toxicity in avian species

1. In vivo studies on the target tissue metabolism, covalent binding, glutathione depletion and toxicity of 4-ipomeanol in birds (Boyd, Statham and staff)

For several reasons, studies of the target organ specificity for covalent binding, glutathione depletion and toxicity by 4-ipomeanol in the bird offered a unique and potentially valuable approach to extend and support previous investigations in the rodent. We recently found that the cytochrome P-450-dependent enzyme activity necessary for metabolically activating 4-ipomeanol was very low or absent in bird lung microsome preparations in vitro (see part 2, below). This activity is very high in pulmonary microsomes from other species, where 4-ipomeanol ia a potent lung toxin that selectively attacks pulmonary Clara cells. Bird lungs anatomically are also considerably different from mammalian lungs. Lungs in the bird do not have terminal airways as such but rather have a continuous set of conducting airways. Cells analogous to the ciliated and nonciliated bronchiolar (Clara) cells of the mammal are not present in bird lung. Moreover, the occurrence of primary tumors in the lung of birds is exceedingly rare. It seems that a lack of ability by bird lung to metabolically activate potential carcinogens might at least partially explain the rare incidence of primary pulmonary tumors in this species, but this possibility has never heretofore been explored.

In birds, the target organ for toxicity and covalent binding by 4-ipomeanol was the liver. Tissue damage was not observed in lungs or kidneys of either Japanese quail or roosters at any dose of 4-ipomeanol tested. Likewise, the levels of irreversibly bound 4-ipomeanol metabolites were highest in liver, with much lower levels in lungs, kidneys and all other organs studied. In addition, toxic doses of 4-ipomeanol markedly depleted hepatic, but not pulmonary or renal glutathione in Japanese quail. The covalent binding of 4-ipomeanol metabolites and concomitant depletion of tissue glutathione in the liver were both time- and dose-dependent. In quail, over a dose range of 5 to 75 mg/kg, no dose threshold for covalent binding or hepatic damage which depended upon substantial glutathione depletion was observed. Pretreatment of quail with phenobarbital had no effect on liver cytochrome P-450 levels, nor did it have any effect on tissue covalent binding or toxicity by 4-ipomeanol. Although 3-methylcholanthrene pretreatment nearly tripled the microsomal cytochrome P-450 levels in quail liver, it had relatively little or no effect on the hepatic covalent binding or toxicity by 4-ipomeanol. Prior treatment with the drug metabolism inhibitor, piperonyl butoxide, markedly decreased the covalent binding, glutathione depletion and the toxicity of 4-ipomeanol. Treatment of quail with diethylmaleate produced a dose-dependent depletion of hepatic, renal and pulmonary glutathione, and it markedly increased hepatic covalent binding by 4-ipomeanol, but had no effect on renal or pulmonary covalent binding. Diethylmaleate markedly enhanced the hepatic necrosis by 4-ipomeanol. The results are consistent with the view that 4-ipomeanol is metabolized preferentially in quail liver to highly reactive metabolites which can be detoxified by

glutathione. It appears unlikely, however, that differenes in detoxification via glutathione are the primary determinants of the remarkable tissue selectivity for covalent binding and toxicity of 4-ipomeanol in different animal species in vivo. The present studies support the view that the tissue selectivity of 4-ipomeanol resides primarily in the ability of the target tissue to rapidly metabolize 4-ipomeanol to its ultimate toxic metabolite(s). These investigations also suggest that the bird may be a potentially useful animal model in studies on the relationship of target organ toxicity to the formation of reactive metabolites by other chemicals requiring metabolic activation.

2. Metabolic activation of 4-ipomeanol by avian tissue microsomes (Boyd and staff)

The formation of highly reactive metabolites from 4-ipomeanol was studied in tissues from roosters and Japanese quail in vitro. Consistent with previous in vivo studies on the target organ selectivity for covalent binding and toxicity of 4-ipomeanol metabolites in birds (see part 1, above), the rates of reactive metabolite formation were very high in bird liver microsomes compared to avian pulmonary or renal microsomes where this activity was relatively very low or absent. The formation of reactive 4-ipomeanol metabolites in quail lung and liver microsomes was enzyme-mediated and dependent upon cytochrome P-450 monooxygenases. The covalent binding of 4-ipomeanol metabolites to microsomal protein was strongly inhibited by boiling the microsomes, by deleting the NADPH generating system or by incubation in a $CO:O_2$ atmosphere. Incubation of quail liver or lung microsomes, NADPH and reduced glutathione resulted in low levels of covalent binding and in two water-soluble glutathione conjugates which had identical high-pressure liquid chromatographic characteristics to those produced in similar incubations with rat lung or liver microsomes. The K_m for 4-ipomeanol covalent binding was nearly 4-fold lower in quail liver as compared with quail lung microsomes while the V_{max} was 8 times higher in liver than lung; these kinetic results were in sharp contrast to those obtained previously in simlar studies with rat microsome preparations, where the pulmonary system was much more active. These results indicate the potential value of in vitro investigations in evaluating biochemical mechanisms underlying target tissue selectivity of toxic damage by xenobiotics acting via a highly reactive metabolite(s) produced in situ.

Several manuscripts describing the above in vitro as well as the preceding in vivo studies in avians are in press (see Publications 12, 13, 14 and 15).

3. NADPH- versus arachidonic acid-dependent metabolism of benzpyrene in avian lung and liver (Boyd and staff)

Microsomes prepared from tissues with high prostaglandin synthetase (PS) activity are capable of cooxidizing substrates such as benzo(a)pyrene (BP) (Canc. Res. 41:1834, 1981) and 3-hydroxymethyl-1-((3-(5-nitro-2-furyl)-allylididene)amino)-hydantoin (HMN) (Canc. Res. 41:2032, 1981) to potentially carcinogenic metabolites. Indeed, studies with human lung microsomes have demonstrated that arachadonic (ARA) dependent metabolism of BP occurs at 45% of the NADPH dependent rate. Moreover, rat kidney (a target for the carcinogenic effects of HMN) metabolizes HMN by ARA but not NADPH dependent routes. Thus, cooxidative

metabolism of certain substrates may play an important part in chemically-induced cytotoxicity/carcinogenicity in tissues with high PS activity. During the course of studies on the in vitro metabolism of 4-ipomeanol in rooster tissues, we noted that rooster lung microsomal preparations lacked detectable cytochrome P-450 and the ability to metabolize 4-ipomeanol to covalently bound metabolites. Since primary lung tumors are exceedingly rare in birds, it was important to determine whether rooster lung microsome preparations were capable of catalyzing the metabolic activation of substrates like BP by routes other than NADPH.

In two separate experiments with rooster lung microsomes, ARA but not NADPH dependent metabolism of BP to covalently bound metabolites was observed. Binding was inhibited by Vit E (70% of control), and BHT (35% of control) but not by indomethacin. Both ARA and NADPH dependent AHH activity was observed in rooster lung microsomal preparations. In contrast, in rooster liver microsomes, ARA dependent covalent binding of BP metabolites occurred at only 10% of the NADPH dependent rate. Likewise, ARA dependent hydoxylation of BP (AHH) was undetectable but NADPH dependent metabolism occurred at an easily measurable rate.

This project is essentially completed and no further experiments are planned. Descriptions of the results of these studies are included in LETM publications 12-15.

D. Development of in situ chemotherapy perfusion protocols for treatment of pulmonary metastatic cancers (Boyd and staff)

1. Metabolism of candidate drugs in vitro in pulmonary subcellular fractions; toxicological implications (Tsang)

Accumulating evidence has demonstrated the lung's capability to metabolize drugs. In instances where drug is converted enzymatically to active toxic metabolites in situ, toxicity may be manifested specifically on certain cells or nonspecifically depending on the site of metabolism and action. A better understanding of the metabolic pathways may shed light on the drug action and/or potential toxicity.

The antibiotic adriamycin is one of the most clinically active drugs for the treatment of a variety of neoplasms. Its mechanism of action is still unclear. The antineoplastic action correlates with strong intercalative binding to duplex nucleic acids, and inhibition of DNA replication and synthesis. Adriamycin's clinical application is limited by cumulative cardiotoxity. It has been postulated that an active intermediate, a semiquinone, is produced in the presence of NADPH cytochrome P-450 reductase. The semiquinone under anaerobic conditions would be converted to an inactive aglycone. Alternately, in the presence of O_2 the semiquinone may be reoxidized, leading to the production of reactive oxygen species that may play a role in cardiotoxicity or possibly the therapeutic action.

The lung is a tissue with high oxygen tension as well as substantial microsomal NADPH cytochrome P-450 reductase activity. It therefore would be a likely

target of redox recycling of adriamycin and possibly lipid peroxidation resulting therefrom. Alternately, the generation of the semiquinone may lead to covalent binding of adriamycin to macromolecules. Preliminary findings in other laboratories have demonstrated the lung's capability to metabolize adraimycin in both the cytosol and microsomal fractions. A thorough in vitro study on the relative importance of the various pathways under different conditions such as different oxygen tension has now been undertaken in our laboratory. The investigation of the biochemical pharmacology of adriamycin in the lung is of value for the understanding of potential lung toxicity, and its possible amelioration, especially in preparations where the isolated perfusion of the organ is used in the treatment of lung metastases.

The past year has been concentrated on establishing reliable methodology to analyze adriamycin and its potential metabolites by HPLC and measuring lipid peroxidation by malonaldehyde or pentane formation. Baseline comparative data on the time courses, protein linearity and kinetics of adriamycin metabolism in lung and liver subcellular fractions have been obtained.

Following the studies with adriamycin, similar in vitro investigations will be undertaken with other drugs under consideration for use with in situ lung perfusion protocols.

2. Investigation of non-invasive measures for evaluation of peroxidative injury in drug-perfused lungs (Grygiel)

Considerable evidence has accumulated which strongly associates pentane production by mammalian tissue as a measure of cytotoxicity via the mechanism of lipid peroxidation. Correlations have been established between in vitro measures of lipid peroxidation and in vivo pentane content of exhaled breath collected from animals subjected to peroxidative stress.

The use of anthracycline antibiotics, a group of agents with a broad spectrum of antitumor activity, is restricted by dose-limiting cardiotoxicity thought to occur via the mechanism of lipid peroxidation. It is of considerable interest to establish whether pentane production is altered following anthracycline administration. The aim of this study is to explore whether in vivo production of pentane and anthracycline-induced lipid peroxidation are related. The nature of such a relationship could then be further explored.

In vivo production of pentane can be measured by a gas chromatographic analysis technique described by Dillard et al. [Lipids, 12(1): 109-114]. In brief, this technique involves trapping of pentane from exhaled breath under cold conditions then diversion of the gas onto the GC column following high temperature volatilization. At the present time, this assay has just been established with a standard curve and animal experimentation is about to begin.

It is proposed that a normal range of pentane production from unstressed rat be first established which will provide a baseline to gauge the effects of anthracycline and other stressful factors on pentane production in vivo. Lipid peroxidation is known to be accelerated by selenium and vitamin E deficiencies so that animals with dietary induced deficiences of these factors will also be tested.

Synergism of cytotoxicity in multi-agent chemotherapy may also be explored using this noninvasive technique with immediate application in rational designing of new multi-agent protocols by avoiding agents with common modes of toxicity.

Finally, this technique may be useful in assessing lipid peroxidative damage to lungs using the isolated perfused organ preparation. The practical details of this line of investigation remain to be demonstrated in the face of difficulties in maintaining viable preparations of isolated lung for a sufficient period of time. The purpose of this methodology, however, lies in its potential applicability as a "clinical" monitor for the evaluation of peroxidative lung injury by adriamycin within the context of the ongoing in situ lung perfusion studies in dogs (see part 5, below) and humans (see parts 5 and 6, below).

3. Preclinical studies of anticancer drug pharmacokinetics (uptake, accumulation, efflux) and metabolism in isolated, perfused rodent lung preparations (Minchin)

The pharmacokinetics of adriamycin in isolated perfused rat lungs have been studied as part of the development of an in situ lung perfusion protocol which may be useful for the treatment of pulmonary metastatic soft-tissue sarcoma patients who are unresponsive to both surgery and chemotherapy. Since soft-tissue sarcomas almost exclusively metastasize to the lungs, an isolated perfused lung technique may offer a means to deliver tumoricidal drug concentrations while avoiding systemic toxicity. Adriamycin has been shown to be a potent anticancer agent with activity against most sarcoma cell-types. The objectives of the present study were to investigate the uptake, metabolism and efflux of adriamycin in perfused rat lung.

Results indicated that lung accumulated adriamycin very slowly and that the rate of accumulation could be altered by manipulating perfusion parameters such as flow rates and perfusate pH. Significant metabolism occurs within 10 min. of perfusion although the metabolites could not be identified as either doxorubicinol or adriamycin aglycone. Covalent binding to trichloroacetic acid - precipitated proteins was also observed and efflux rates of the drug were considerably less than net uptake rates. The linearity of uptake with both time and concentration allows for accurate prediction of final lung concentrations. Extraction of adriamycin by rat lung was similar to that seen both in dogs and in man, suggesting that this model is appropriate for preclinical development studies.

At present, the effect of oxygen tension on both the kinetics and toxicology of adriamycin in lung is under investigation. Since adriamycin undergoes redox cycling in the presence of appropriate reductases and cofactors, the presence of oxygen may be a vital factor in determining antitumor or cytotoxic activity of the drug. Further, should adriamycin's anticancer activity be related to its ability to covalently bind to macromolecules while its cytotoxic effects result from the activation of molecular oxygen and/or lipid peroxidation, manipulation of oxygen tensions in the perfused lung setting may represent a procedure for augmenting the tumoricidal activity of the drug while minimizing or possibly eliminating tissue cytotoxicity.

Following the completion of studies with adriamycin, we shall similarly investigate other anticancer drugs, in particular 5-fluorouracil, abrin and ricin, and other potential candidates for the in situ perfusion treatment of pulmonary soft-tissue sarcomas.

4. Development of non-invasive techniques for in vivo measurement of pulmonary clearance and pharmacokinetics of anticancer drugs (Tsang)

Ideally, the choice of an anticancer agent and route of administration should be one that would achieve a therapeutic level of the agent at the target organ yet minimize the residence time of the drug in the other tissues. For tumors in the lung, a possible way to accomplish this is by pulmonary arterial infusion of an agent(s) that would be cleared rapidly (e.g. by in situ metabolism to less toxic metabolites) so as to minimize systemic toxic effects.

Accumulating evidence demonstrates the lung's capability of metabolizing endogeneous and exogeneous substrates. In spite of its smaller mass and generally much lower concentration of drug metabolizing enzymes, the lung's strategic position as the recipient of the total cardiac output on one side and accessibility to environmental influence (e.g., the atmosphere) on the other, makes it a potentially important organ of metabolism. Identification of highly extracted antineoplastic agents in terms of pulmonary clearance would be of value to identify candidate drugs for direct intraarterial infusion.

Intraarterial infusion of 5-flurouracil (FU) has been used for treatment of metastatic tumors in the liver. FU has very interesting pharmacokinetics with a total body clearance greater than cardiac output. After constant i.v. infusion hepatic clearance only accounts for 50% of the total body clearance, suggesting the importance of extrahepatic clearance. FU is metabolized to essentially inactive metabolites (e.g., CO_2, urea, α-fluoro-β-alanine). A high rate of pulmonary clearance of 5-fluorouracil might, therefore, be of importance for the treatment of primary or metastatic tumors in lungs by intraarterial infusion.

In vivo pulmonary extraction for FU and other candidate drugs will be studied by comparing the area under the plasma concentration-time curves following intravenous and intra-aortic administration. Serial plasma samples will be analyzed for percent drug and metabolites. Analysis of the biological fluids will be accomplished by established analytical techniques.

Effort has been put into perfecting the necessary cannulation techniques in small animals, and in analytical and computational methods for the study of pulmonary clearance of 5-fluorouracil.

The study of pulmonary clearance of 5-fluorouracil should be achieved in the coming year. Continued effort will be made to identify agents with high rates of pulmonary extraction. Effect of various factors that may alter cardiac output, distribution of pulmonary flow and rate of metabolism should also be identified for better control and predictability of pulmonary arterial infusion of highly extracted antineoplastic agents.

5. Preclinical studies of anticancer drug pharmacokinetics, metabolism and toxicity in in situ perfused lungs in living dogs (Minchin, Aiken, McMenamin and Hall)

In order to investigate the long-term effect of in situ isolated lung perfusion, it was necessary to develop an appropriate model representative of the proposed human procedure. A canine left lung hemiperfusion was found to be most appropriate. The model involved isolated perfusion of the left lungs of dogs for periods up to 50 min. During the developmental stage, problems were encountered in standardizing several aspects of the model including dosage regime, surgical procedures and reproducible perfusion conditions. A high incidence of post-operative deaths occurred due to both surgical and drug-related causes. More recent studies have produced a very reliable procedure which has been used to investigate the kinetics and toxicity of adriamycin in perfused lungs. These studies are being pursued in collaboration with the Surgery Branch, NCI (Dr. M. Johnston and colleagues).

Briefly, the left pulmonary artery and left venous return are isolated and the left lungs perfused with donor blood and varying concentrations of adriamycin for 50 min. Lung and blood samples are collected for drug analysis. At the end of each experiment, the chest of the dog is closed and the dog allowed to recover. Two weeks post-perfusion, a right-lung pneumonectomy is performed in order to assess the functional capacity of the perfused lungs. Plasma angiotensin converting enzyme, a parameter previously shown to change during lung damage, is also measured daily.

Uptake of adriamycin in dog lung was found to closely resemble that seen in rat lung. General uptake was slow and linear with time at lower blood concentrations. The study has shown that the functional integrity of dog lung is maintained after perfusion with adriamycin concentrations in excess of 25 µg/ml. No consistent drug-related pathological changes in the lung tissue were observed. Initial studies have suggested that the time course of plasma angiotensin converting enzyme post-perfusion may be dose related and possibly a useful measure of lung degradation in this setting.

Following completion of the study with adriamycin in dog lung, it is proposed to investigate 5-fluorouracil and other candidate drugs in a similar manner.

A manuscript describing the initial dog studies is in press (see Publication 16).

6. Phase I clinical trial of in situ isolated pulmonary perfusion protocol using adriamycin in patients with metastatic soft-tissue sarcomas in lungs (Minchin and Aiken)

Soft-tissue sarcomas frequently metastasize to the lungs and multiple resection of these nodules has yielded a 25-30% five year survival rate. However, a considerable proportion of sarcoma patients are unresectable and chemotherapy remains the only alternative. Adriamycin is considered a potent antitumor agent against sarcoma and remission has been seen in 33-40% of these patients.

However, cardiotoxicity often limits adriamycin dosage with the result that a significant number of patients are neither resectable nor responsive to chemotherapy.

As a possible treatment protocol for this group of patients, an isolated lung hemiperfusion technique was developed in dogs. The method proposed administering high concentrations of adriamycin to only the lungs over a short treatment period. Successful preclinical experiments in the dog model have led to a Phase I clinical trial being established and which is now under way. The present study is being pursued in collaboration with Dr. M. Johnston and colleagues (Surgery Branch, NCI).

The technique involves perfusing the left lungs of unresectable sarcoma patients with increasing adriamycin concentrations commencing at 1 μg/ml. Pulmonary function tests and chest x-rays are used to determine possible pulmonary toxicity.

At present, 3 patients have undergone treatment with no surgical complications. Adriamycin kinetics in the lungs resembled that seen in the dog and rat models. Generally, tumor drug levels were significantly lower than normal lung tissue. Ventilation-perfusion scan ratios are unchanged after 3 weeks post-perfusion in the first 2 patients. Chest x-rays indicated a progression of tumor growth in both left and right lungs although tumors biopsied at the end of perfusion seemed to show a lower soft agar growing efficiency compared to nonperfused tumors.

Several problems inherent to the procedure are currently under investigation. Firstly, determination of post-perfusion lung toxicity is dependent on several criteria with unknown sensitivity in the present setting. While these tests may prove useful in deciding on the extent of lung damage, other biochemical measurements are being studied. Secondly, inconsistent interpatient bronchial blood flows have resulted in some difficulty in maintaining appropriate perfusion drug levels. This may well prove an important parameter if the therapeutic index of adriamycin is small.

E. Pulmonary Toxicology

1. Acute toxicity and chronic toxicity/carcinogenesis, and effects on lung cell proliferative kinetics, of 3-methylfuran administered by inhalation (Boyd)

3-Methylfuran (3-MF) has been reported as a major atmospheric contaminant during a smog alert in Washington, D.C. (Saunders et al., Biomed. Mass. Spec. 1, 192, 1974). It was suggested that atmospheric photooxidation and degradation of naturally occurring volatile hydrocarbon precursors such as isoprene and terpenes could be the source of the 3-MF. These precursors are released in large amounts from deciduous forests such as those covering the Appalachian Mountains and may be concentrated during certain weather conditions.

3-MF is similar structurally to 4-ipomeanol [1-(3-furyl)-4-hydroxypentanone], a highly lung toxic furanoterpenoid. In earlier studies we have shown that, like 4-ipomeanol, 3-methylfuran is activated *in vivo* to a potent pulmonary toxin

when administered to mice by either i.p. injection or by inhalation. It is converted by a cytochrome P-450-dependent pathway in the mouse lung to a highly reactive metabolite which becomes covalently bound to the epithelium of terminal bronchioles and is followed by striking bronchiolar necrosis within 24 hours of administration. At very high concentrations 3-MF is covalently bound and toxic to both nonciliated (Clara) and ciliated bronchiolar cells. At lower concentrations 3-MF is preferentially toxic to the Clara cell, an important lucus of mixed function oxidase enzyme activity in the lung.

Thus, 3-MF and structurally similar compounds are toxic environmental agents which following inhalation or ingestion could play a significant role in the pathogenesis of human lung disease either by initiating primary disease, by exacerbating acute or chronic bronchiolar disease, or by interacting with other pulmonary toxicants.

We have undertaken a series of morphologic studies to examine in greater detail the pulmonary and extrapulmonary toxicity of 3-MF following inhalation exposure. These studies are being pursued in collaboration with Drs. W. Haschek and H. Witschi of the Oak Ridge National Laboratory (Oak Ridge, TN).

We initially investigated the acute inhalation toxicity of 3-MF in mice, rats and hamsters by determination of the LC50 values and by histologic examination of animals killed at varying times following a 1 hr exposure. In addition, in mice respiratory rate measurements and cell kinetics were used to quantitatively assess pulmonary damage and repair.

In mice, there was relatively selective necrosis of nonciliated bronchiolar epithelial (Clara) cells after 1 day, with virtually complete regeneration by 14 to 21 days. The LC50 in the mouse was 37 µmol/l. ^{3}H-Thymidine incorporation studies showed that peak bronchiolar cell proliferation occurred at 3 days with a labeling index (LI) of 5.0% compared to 0.4% in controls. An increase in parenchymal cell proliferation was also noted correlating with a mild interstitial pneumonitis. This parenchymal proliferation, peaking at 10 days with an LI of 1.4% compared to 0.2% in controls, consisted primarily of type II epithelial and endothelial cell proliferation indicating possible delayed damage and repair of type I epithelial and endothelial cells. The respiratory rate showed an initial transient increase followed by a more prolonged decrease with eventual return to control levels, correlating with observed clinical signs. 3-MF toxicity was also evidenced by a necrotizing suppurative rhinitis, centrilobular hepatic necrosis, lymphocyte necrosis in the thymus and spleen, sialoadenitis and otitis media.

The Fischer rat was more sensitive to 3-MF-induced lethality than the CD/CR rat, with an LC50 of 81 µmol/l compared to 222 µmol/l. No sex difference was found. The hamster was relatively resistant with no lethality at 326 µmol 3-MF/l. Pulmonary damage was present in both species. In the hamster, selective necrosis of nonciliated bronchiolar epithelial (Clara) cells was seen at 1 day with virtually complete regeneration by 14 days. In the rat, the bronchiolar epithelial damage was more extensive and was followed by scattered peribronchiolar fibrosis and epithelial mucous metaplasia suggestive of "small airway disease." Relatively selective 3-MF-induced necrosis of

olfactory epithelium occurred in the nasal mucosa of both species with resolution of this lesion by 14 days in the hamster. In the rat the necrosis was much more extensive, resulting in partially occlusive fibrosis of the nasal cavity at 14 days. 3-MF toxicity also was evidenced in both species by centrilobular hepatic necrosis and by lymphocyte necrosis in the thymus and spleen. Two manuscripts describing these acute 3-MF toxicity studies are in press (see Publications 17 and 18).

Further studies are underway to assess the long-term toxicity/carcinogenicity of 3-MF administered to animals by repeated inhalation exposures. The total duration of these studies is 6 mos - 2 yrs, and the majority of animals in these experiments will be sacrificed and evaluated in detail during the coming year.

2. Evaluation of usefulness of whole-body plethysmography and breathing rate measurement as a noninvasive means of assessing drug-induced pulmonary injury in rodents (Boyd and staff)

Methods for the detection and/or quantitation of drug-induced pulmonary toxicity in small laboratory animals generally are invasive or involve sacrifice of the animals. This presents serious limitations on studies requiring sequential assessment of lung injury in the same groups of animals over a period of time. Dr. Elizabeth Travis and colleagues (Radiation Oncology Branch) have developed a new, non-invasive, functional assay for radiation-induced lung damage in mice. A whole-body plethysmograph is used to measure breathing frequency and amplitude. Excellent correlations have been shown between increased breathing frequency, histologically observable pulmonary damage and lethality in mice subjected to a wide range of doses of thoracic irradiation. Not only was the method sensitive to the relatively early-occurring pneumonitis following irradiation, but also reliably detected the later phase of pulmonary fibrosis. The breathing rate assay is relatively simple and rapid and, therefore, has practical applicability to long-term studies and/or studies which may involve large numbers of animals.

Because of the demonstrated value of whole-body plethysmography in studies of lung injury by irradiation, we were interested in the possible applicability of this approach to the investigation of lung injury by drugs or other chemicals. Therefore, we have undertaken collaborative investigations with Dr. Travis to evaluate this potential application. We have investigated initially the effects of the pulmonary alkylating agent and cytotoxin, 4-ipomeanol (IPO) on breathing rate in mice.

The effects of graded single doses of 4-ipomeanol on the breathing rate were observed up to 4 days after treatment with non-toxic doses of 4-ipomeanol (0.6 of the maximum tolerated dose), giving a clear dose-dependent increase in breathing rate at 8 hours and 4 days after treatment. Time-course and dose-response studies showed good agreement between changes in breathing rate and pulmonary alkylation after 4-ipomeanol treatment. Pretreatment of the mice with diethylmaleate (DEM) increased pulmonary alkylation and toxicity of 4-ipomeanol. However, breathing rate was severely depressed by all doses of DEM that were examined. This decrease was not dose dependent within the range of

doses studied. These studies suggest that breathing rate is useful for monitoring the effects of non-lethal doses of 4-ipomeanol on the lungs of mice and does not require sacrifice of the animal. The technique may be similarly applicable to studies of other pulmonary toxins, but careful attention must be given to time-and-dose-relationships and the possible effects of other drugs or pretreatments.

In further collaborative studies with Dr. Travis (who has recently joined the staff of M.D. Anderson Hospital, Houston, TX) we shall continue to explore the utility of the breathing rate measurement technique for assessment of lung injury by drugs and by drugs in combination with irradiation.

A manuscript describing the initial phase of these studies with 4-ipomeanol is in press (see Publication 19).

F. Drug/irradiation interactions; implications for normal tissue damage

1. Role of tissue sulfhydryl concentrations as a determinant of susceptibility to radiation injury of skin, gut and lung; effects of diethylmaleate (Boyd and staff)

It has been generally viewed that the presence of endogenous free sulfhydryl compounds (primarily glutathione [GSH]) serves as an important protective factor against radiation injury of tissues. Presumably, this nucleophilic sulfhydryl compound could interrupt tissue destruction by chemically trapping and/or reducing highly reactive free-radical products formed through the effects of irradiation on tissue constituents. This concept has provided the basis for development of free-SH containing drugs (e.g., cysteamine, N-acetylcysteine) or drugs that are converted in vivo to free-SH derivatives (e.g., WR-2721) for use as radioprotective agents. However, although such agents do provide some protection of varying degrees to different tissues, the molecular basis of such protective effects is not entirely clear. We have undertaken the present project to attempt to clarify the role of GSH as a possible protective factor against tissue injury by irradiation. These studies are being pursued in collaboration with Dr. Elizabeth Travis (formerly with the ROB, NCI; now staff member of M.D. Anderson, Houston, TX).

Our initial experiments have been designed to explore the effects on irradiation injury of lowering the endogenous GSH levels. Diethylmaleate (DEM) was used to deplete tissues of GSH. The dose- and time-dependencies of GSH decreases by DEM first were determined in lung, ileum, and skin. An optimal DEM dose of 500 mg/kg, s.c., was determined; doses greater than this did not produce further depletion of GSH, only greater toxicity (lethality). Maximal GSH depletions were obtained 1-2 hr after DEM administration. Typical GSH values measured 2 hr following s.c. 500 mg/kg doses of DEM were 40, 40, and 60 percent of control GSH values in lung, ileum, and skin, respectively.

Preliminary studies have indicated that irradiation-induced injury of the lungs and gut was not enhanced by DEM treatment. This finding was somewhat unexpected, and further experiments should confirm or disprove this initial observation. In contrast to the negative effect of DEM on lung and gut, the treatment did enhance

irradiation-induced injury of the skin. The possible role that the relative tissue oxygenations may play in these apparently contrasting effects of DEM on radiation injury in different tissues will be considered in future experiments.

2. Pharmacokinetics of free-SH metabolites of WR-2721 in gut and lung; correlations with radioprotective effects (Boyd and staff)

WR-2721 (S-2-[3-aminopropylamino]-ethylphosphorothioic acid) protects certain tissues, including the lungs and the gut, against radiation-induced injury. The drug is dephosphorylated in vivo to form a free-SH containing metabolite that presumably is the active radioprotective moiety. However, the key temporal relationships between production of the active WR-2721 metabolite and the radioprotective effect have not been clearly elucidated. Therefore in this project we sought a means to measure the enhancement of tissue-SH content after WR-2721 and to see if the tissue pharmacokinetics of this active moiety correlates with its tissue protective effects.

An adaptation of Ellman's procedure (Arch. Biochem. Biophys. 82, 70, 1959) for measurement of tissue nonprotein sulfhydryl NPSH levels proved useful for measuring increased NPSH following WR-2721 treatment in mice. Following a range of i.p. doses of WR-2721, NPSH levels were significantly elevated to peak values within 30-60 min and remained somewhat elevated for up to several hours. A WR-2721 dose of 200 mg/kg produced an elevation of 50-60% of control NPSH values in the lung and the ileum. In continuing studies we are examining in detail the time-dependency of the relative degree of radioprotection in lungs and gut of mice receiving WR-2721. These studies should help clarify the mechanism of radioprotection by WR-2721 and provide a basis for development of more rational dosing regimens to produce maximal radioprotection and minimal toxicity to normal tissues.

G. New drug candidate development and/or exploration; lung-cell selective cytotoxins and lung cancer

1. Studies with chemically-induced lung tumors in rodents (Boyd and staff)

The pulmonary Clara cell may have an important role as a possible site of origin of bronchogenic lung cancers (see Annual Report FY 82, Project No. Z01 CM 07126-01 LETM). In this project, experiments will be undertaken with the following specific aims:

a. Do pulmonary "Clara cell" tumors induced in hamsters by nitrosoheptamethyleneimine or by transplacental induction in mice with urethane or ethylnitrosourea contain cytochrome P-450 enzymes necessary for metabolically activating 4-ipomeanol?

Tumors will be induced as already described in detail in the literature; the metabolic activation of 4-ipomeanol in vivo in tumor-bearing animals, and in vitro in slices of tumor-containing lungs, will be assessed by high-resolution autoradiographic techniques. One preliminary set of experiments along these lines currently is in progress in collaboration with Dr. Shirley Kauffman (Downstate Medical Center, Brooklyn, NY).

b. Can such tumors be isolated and propagated in vivo in immune-deficient mice and/or in vitro in culture?

Standard in vitro cell culture techniques will be explored initially. In in vivo studies attempts will be made initially to develop tumor xenograft systems using athymic (nude) mice.

c. Do the above tumor systems retain characteristics of normal Clara cells?

The in vitro and in vivo Clara cell tumor preparations will be assessed regularly for their monooxygenase activity, specifically as to their capacity to metabolize and covalently bind 4-ipomeanol which will serve as the primary Clara cell "marker" activity. Maintenance and stability of this measure then will justify more detailed investigation of substrate specificity characteristics for the monooxygenase system, and relative activities of the individual components of the monooxygenase system (e.g., cyt. P-450, NADPH cyt. P-450 reductase; cyt. b_5 and NADPH cyt. b_5 reductase). Activities of other important drug detoxification enzymes, including expoxide hydrase and GSH-transferase, also will be assessed. Careful morphologic comparisons also will be made of the tumor-derived Clara cells and normal Clara cells; these studies will employ both light- and electron-microscopy. Results of these investigations should permit an assessment of the comparability of the tumor cells and the normal Clara cells, and an estimate of the potential utility of chemically induced Clara cell tumors as a bulk source of cells required for further investigations of metabolic functions of the Clara cell. Such an approach has proved very useful for another pulmonary cell type; pulmonary type II-cell tumors have been investigated extensively to gain insight into the metabolic functions of the type II aveolar pneumocyte.

d. Is 4-ipomeanol cytotoxic to Clara cell-derived tumors?

This question will be examined in vitro, as well as in vivo by evaluating animal survival and/or tumor implant size and/or viability in athymic mice bearing Clara cell tumors. Tests will be performed using a wide range of dosages and treatment schedules with 4-ipomeanol. For comparisons, the relative effectiveness of other cytotoxic agents, such as bleomycin, adriamycin, cytoxan, bischloronitrosourea, pentamethylmelamine, and hexamethylmelamine, also will be tested in these systems. These studies may give valuable information as to the potential utility of 4-ipomeanol as a new type of chemotherapeutic agent for bronchogenic lung cancers.

2. Studies with human lung tumors (Boyd)

This project, scheduled to begin in FY 82, will be pursued by Dr. Boyd in collaboration with Drs. T. Connors (MRC Toxicology Unit) and K. Harrap (Royal Marsden Cancer Hospital) in Surrey, England. It is the result of approval of Dr. Boyd by the Officer of International Health (Department of Health and Human Services) for a 6 mos. - 1 yr. Foreign Work/Study assignment with the above laboratories.

The Surrey investigators have developed a panel of human lung tumors, maintained in vitro in culture and in vivo in immune-deficient mice. These systems appear to be ideal for studies of metabolism and evaluation of antitumor activity of a series of cytotoxic furan derivatives which have been studied extensively in our laboratory. These compounds produce highly selective alkylation of pulmonary bronchiolar epithelium in normal animals, due to their activation by metabolism in situ.

In vivo testing of 4-ipomeanol, perilla ketone and 3-methylfuran will be carried out in 7 human lung tumor xenograft lines currently available in the Surrey labs. Also, in vitro and in vivo metabolic studies will be performed with 4-ipomeanol to explore the extent to which the compound may show tumor-directed metabolic activation and alkylation in the human lung tumor preparations.

H. Glutathione content of glandular gastric mucosa - influence of diethylmaleate and stressors; possible implications for acute ulcerogenesis and/or response to chemical carcinogens

1. Studies with diethylmaleate (Boyd and staff)

In a recent communication (Science 205, 1010, 1979), we reported that the glandular gastric mucosa of laboratory rodents contains very high concentrations of glutathione (reduced form; GSH). In rats, for example, levels as high as 7-8 mM frequently were observed. The gastric GSH levels sometimes even exceeded those in the liver, depending upon the time of day the tissues were sampled. The gastric and hepatic glutathione concentrations varied diurnally, but these variations did not occur in parallel; the highest gastric tissue concentrations generally occurred in the late afternoon and the lowest at night, while hepatic GSH usually was highest at night and early morning and lowest in the late afternoon. Animals fasted for 24-48 hrs had significantly lower gastric and hepatic GSH concentrations. The physiological significance of the high GSH content in gastric mucosa is unknown.

In the present study with rats we found that the subcutaneous administration of diethylmaleate (DEM), a drug known previously to deplete liver glutathione (GSH) caused a severe dose-dependent, rapid and persistent decrease in the glutathione content of the glandular gastric mucosa. This effect of DEM was accompanied by the occurrence of severe ulcerative lesions of the gastric lining and sometimes also a marked gaseous inflation of the stomach. The acute ulcerative lesions appeared identical to those previously shown to be induced by a variety of physical and/or behavioral stressors in rodents. Also, a pretreatment (i.e., starvation) that decreased gastric GSH enhanced the DEM-induced ulcerogenesis. These studies suggest that a possible role for GSH in maintaining the normal homeostasis and integrity of the gastric mucosa should be considered.

A manuscript has been published this year which describes the above studies in detail (see Publication 20).

Because of other priorities we do not presently plan to further pursue this project.

2. Studies with an experimental stressor (Boyd and staff)

In this study we examined the effects of an experimental stressor (cold-restraint) on gastic GSH. This stressor rapidly produces acute gastric ulcerative lesions apparently identical to those produced by diethylmaleate (see part 1, above).

Before attempting to determine the effects of the stressor on GSH and the possible influence of time of day and/or food deprivation on any stressor-induced effects, we were intersted in the effects of time of day and/or food deprivation alone on the normal diurnal cycles of gastric and hepatic GSH. Starvation consistently caused decreases in hepatic GSH at all of the times of day studied but it caused less consistent alterations in glandular gastric GSH concentrations. Significant decreases in gastric GSH were seen only when the measurements were taken early in the day.

The concentrations of GSH in the livers and stomachs of either fed or starved animals subjected to the stressor were significantly decreased, compared to nonstressed controls, at all of the various times of day for stressor exposure that were tested. The degree of depletion in the liver was significantly greater in the starved animals only in the group stressed at 2:00 p.m.-6:00 p.m. The depletion in the stomach was significantly greater in the starved animals stressed either at 9:00 a.m.-1:00 p.m. or 2:00 p.m.-6:00 p.m., whereas the degree of depletion of gastric GSH was significantly less in the starved animals stressed at 8:00 p.m.-12:00 a.m., when these groups were compared to their respective fed controls.

Thus, the present study clearly showed that the cold-restraint stressor caused marked decreases in both hepatic and gastric GSH concentrations. It therefore appears that a stressor can alter a crucial cellular substance involved in modulating susceptibility to cellular toxins and carcinogens. The system we have studied here seems to offer an ideal approach to further explore the hypothesis that stressors may indeed alter the responses of animals to chemical toxins and carcinogens whose activation or detoxification requires GSH. Moreover, the present studies have demonstrated that future work should also take into account the possible additional modifying influences of nutitional status and time of day as potential determinants of the magnitude of stressor-induced alterations in GSH as well as the adverse biological effects of the chemicals under study.

A manuscript has been published this year which describes the above studies in detail (see Publication 21).

Because of other priorities we do not presently intend to further pursue this project.

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Pathobiology/Experimental Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ø. Fodstad, M.D., Ph.D.	Visiting Scientist	LETM	NCI
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COOPERATING UNITS (if any)

Norsk Hydro's Institute for Cancer Research, Oslo, Norway; Litton Bionetics; BRMP, FCRC; NCI-Navy; Surgery Branch, DCT, NCI; VRB, NIH

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Projects currently under way or soon to be initiated include the following: A. Establishment, characterization and studies of a human melanoma lung metastasis model in athymic mice, B. Development and pharmacologic studies of a short-term lung perfusion model for chemosensitivity studies using human melanoma lung metastases, C. Immunological and tumor biology studies in new strains of mice with multiple immune defects, D. Studies of lymphoma development in beige-nude mice, E. Chemosensitivity studies of new drug candidates in human lung cancer systems, F. Establishment of a panel of human soft-tissue sarcomas and osteogenic sarcomas in nude mice and in culture, G. Ultrastructural studies of human cancer cell lines, H. Physiology, pharmacology and toxicology of the pulmonary Clara cell, I. Cellular/molecular factors in cell death by chemically reactive metabolites, J. Cellular dynamics and structural/physiological consequences of drug-induced damage of discrete extrahepatic cell populations, and K. Collaborative ultra-structural studies with other DTP laboratories. More detailed descriptions of each of these projects are presented herein.

A. Establishment and characterization of a human melanoma lung metastasis model in athymic, nude mice; host factors and tumor cell characteristics of importance for lung colony formation (Fodstad, McMenamin and Sanders)

Human tumors growing as xenografts in athymic nude mice are very useful in the study of various aspects of tumor biology and tumor therapy. However, since the tumors metastasize rarely and only as a very late event, such mice have so far not provided a good model for investigating mechanisms involved in metastasis formation.

Recently, cells from one of our melanoma xenografts (LOX) have been shown to grow progressively in the lungs of adult nude mice upon intravenous injection, killing the mice in 30-35 days when 1 x 10^6 cells are injected. Hence, this human melanoma provides a unique opportunity to study factors of importance for lung colony formation, factors such as: a) tumor cell characteristics and b) host immunology.

a) The following tumor characteristics will be examined and compared with corresponding data obtained on non-colony forming melanomas.

   Growth in vivo (S.C.) and in vitro (monolayer, soft agar)
   Morphology
   Cytogenetics (Litton Bionetics)
   Cell membrane/tumor antigens (in collaboration with Dr. C. Morgan, FCRC)
   Cytofluorometry (in collaboration with Dr. Paul Bunn, NCI-Navy)

b) The growth of the LOX melanoma in strains of mice with different immune defects will be compared (see: C.) in order to study the importance of different cell types expected to play a role in the host defense against tumors. This can be further elucidated by the use of agents that selectively stimulate or suppress the activity of specific cell types such as macrophages and NK-cells.

These studies are in progress and the results obtained have revealed important differences in tumor antigen expression between LOX and the other melanomas. A chromosome banding has shown characteristic markers, making it easy to identify the LOX tumor. Tumor growth data in mice with different immune defects indicate that some of the current views on the importance of NK-cells in tumor metastasis prevention may need reevaluation.

This project will be continued along the lines indicated above. In addition, sublines of the LOX tumor will be established in order to further investigate characteristics influencing the ability of the tumor cells to form lung colonies. Tumor cells will be labeled with ^{125}IUdR and the tissue distribution of such cells studied.

B. Development of a short-term lung perfusion model for chemosensitivity studies using the human melanoma lung metastasis model; comparisons with conventional chemotherapy; drug pharmacokinetics studies (Fodstad, Minchin, McMenamin, Sanders and Boyd)

Several human tumors do primarily metastasize to the lungs of the patients. This emphasizes the need for a model in which a human tumor actually grows in the lung of the host animals. The LOX melanoma provides such an opportunity to assess the effect of therapy on the tumor colonies actually residing in the lungs of nude mice.

In coordination with the projects described in a separate report (see Section D., Annual Report FY 82, Project No. Z01 CM 07125-01 LETM), a short-term lung perfusion model will be developed in nude mice. The objectives will be to compare the effects of adriamycin and other selected drugs administered conventionally (i.v.) with the effects of lung perfusion. Concurrently, drug distribution to normal and tumor tissue as well as drug pharmacokinetics will be studied.

The lung perfusion will be performed on isolated mouse lungs with/without LOX lung colonies. Drug concentrations will be measured by HPLC and the therapeutic effect assessed by the use of a soft agar clonogenic assay.

Initial experiments on s.c. growing tumors have shown that LOX is moderately sensitive to adriamycin given intravenously. The drug also prolonged the life span of mice with LOX lung colonies. The soft agar method to be used is now being tested on LOX tumor cells.

Some of the major problems involved in the project have been overcome and the experiments continue. In addition to adriamycin, it will be of interest to study similarly the effects of other new drugs (e.g., abrin or ricin [See: F.], as well as other established drugs (e.g., 5-FU, hexamethylmelamine) which may become candidates for clinical trial in patients in protocols utilizing isolated in situ pulmonary perfusion.

C. Immunological characterization of three new strains of athymic, nude mice with double or triple immune defects (Fodstad, McMenamin and Sanders)

The athymic, T-cell defective, nude mouse has in recent years proved its importance for significant advances in the fields of tumor biology and therapy, as well as in immunology. Dr. Carl Hansen, VRB, NIH has recently introduced the Beige and XID genes into nude mice. The Beige mouse is known to exhibit a very low NK activity whereas XID mice are reported to have a poor B-cell response. By backcrossing, Dr. Hansen has also managed to breed some nude mice with both the Beige and the XID genes. To our knowledge, the Beige-nude and the Beige-XID-nude mice are so far the only ones of their kind being bred, giving us a unique opportunity to obtain valuable information on the importance of the different cell types thought to be important in the immunological defense against tumors.

The objectives of this project fall into two parts:

a) Immunological characterization of the new mouse strains

b) Determination of tumor growth characteristics in such mice

a) These studies are mainly performed in collaboration with Dr. Grace Cannon (Litton Bionetics) and will include both homozygote and heterozygote mice of the different strains. NK-tests, proliferation and plaque assays and macrophage tests are employed.

b) The growth of several mouse and human tumors is investigated, looking at tumor take and growth rate of subcutaneous tumors as well as the metastasizing abilities of some of the tumors.

The immunological mapping has shown:

1. Beige-nude mice have a low NK-activity and somewhat lowered B-cell reactivity.

2. XID-nude mice have a low B-cell response.

3. Beige-XID-nude mice have NK- and B-cell reactivities between 1 and 2 but significantly lower than conventional nude mice.

The tumor studies indicate that NK-cells are much less important in control than previous reports have indicated. The studies will continue in order to obtain further data on various aspects of the immunological tumor defense mechanisms.

D. Investigation of factors involved in lymphoma development in beige-nude mice (Fodstad, McMenamin and Sanders)

The Beige-nude mice (see: C.) are defective in T-lymphocytes, NK-activity and, to some degree, in their response to B-cell mitogens. Such defects could theoretically render the mice particularly prone to developing spontaneous tumors. Lymph node enlargements (tumors) have been observed in about 25% of the Beige-nude mice exposed to LOX tumor cells injected intravenously. In some instances these tumors have been growing progressively. These tumors may represent lymphoproliferative disease or lymphomas, possibly induced by the antigenic stimulation caused by the tumor.

Studies have been initiated to identify these tumors and attempts made to detect factors possibly involved in tumor induction.

The tumor morphology will be examined, the tumor cells tested for response to T- and B-cell mitogens, and attempts made to grow them in culture.

One non-tumor bearing mouse has developed a similar tumor. This particular mouse was not healthy, possibly because of infection, indicating that antigenic stimulation other than transplanted tumors can be effective. The role of different immune defects will be investigated and the possibility that the lymphoproliferation may be virus-related should be examined.

E. Evaluation of the in vitro and in vivo chemosensitivity of human lung cancers to the new drug candidates abrin, ricin and 4-ipomeanol, perilla ketone, and methylmanganesetricarbonyl (MMT) (Fodstad, McMenamin, Sanders and Boyd)

Abrin and ricin are plant toxins inhibiting protein synthesis in eukaryotic cells. They have been shown to possess selective antitumor effects in various tumor systems. 4-Ipomeanol and perilla ketone produce pulmonary cytotoxicity due to a selective metabolic activation by the bronchiolar Clara cells. MMT is selectively cytotoxic to Clara cells, but apparently does not require metabolic activation in these target cells.

To screen for possible antitumor effects of these agents on lung cancers, two systems will be used:

a) In vitro clonogenic assay

b) Tumors growing in nude mice

These studies will be performed in collaboration with Dr. Des Carney, NCI-Navy, who will provide the tumor lines and do most of the in vitro work.

Preliminary in vitro results indicate an extremely high degree of responsiveness to abrin and ricin of lung adenocarcinomas and small cell lung cancers (SCLC), whereas, large cell tumors were less sensitive. The clonogenic cells of the two sensitive tumor types were killed by drug exposure for 1 hr at doses as low as 10 pg/ml. Interestingly, SCLC-tumor lines were also somewhat sensitive to 4-ipomeanol.

The results obtained are very promising and of interest from a biological point of view, as well as from a therapeutic one. The in vitro studies will be extended and the in vivo experiments are under way. If in fact 4-ipomeanol's apparent cytotoxicity in human lung cancer cells is confirmed, this implies that such tumor cells have the metabolic capacity to activate 4-ipomeanol. The presence of such activity would be highly significant, since it would indicate a parent cell of origin having similar metabolic activity (e.g., Clara cells). This in turn could have important chemotherapeutic implications. Following the conclusion of the present in vitro and in vivo tumor cytotoxicity studies, we shall explore the metabolism and activation of 4-ipomeanol and/or perilla ketone in human lung cancer lines if susceptible cell types are identified.

F. Establishment of a panel of human soft tissue and osteogenic sarcomas as xenografts in nude mice. Characterization and chemosensitivity testing (Fodstad, McMenamin and Sanders)

Human soft-tissue sarcomas and osteosarcomas are currently attracting special interest because of improved therapeutic results in the clinic, including a possibility to cure some patients with metastatic disease by using aggressive multimodality treatment. However, further progress may depend on exploration of the biology and chemosensitivity of such tumors in model systems, and so far this has been done only to a very limited extent.

The objectives of this project are:

Establish 5-7 sarcomas as xenografts in nude mice and in tissue culture, if possible.

Characterize the tumors in a similar way as described for melanomas (A).

Test the tumor chemosensitivity to established drugs and to abrin and ricin or other new agents.

Four sarcomas are growing as tumor lines in nude mice and their growth characteristics are determined. Cells from the two tumors tested show a high cloning efficiency in soft agar and can be grown in monolayers for some weeks. Chemosensitivity studies are initiated.

The sarcomas seem to be well suited for these studies. Attempts will be made to obtain a sarcoma line that could form tumor colonies in the lungs of nude mice, making an even more relevant model for the lung perfusion studies (see: B.). If abrin and ricin show good activity on the sarcomas, these agents may be explored as potential candidates for lung perfusion studies as described in detail in a separate report (see Section D., Annual Report FY 82, Project No. Z01 CM 07125-01 LETM).

G. Ultrastructural studies of malignant human cancer cell lines maintained in vitro or in vivo in athymic host xenograft systems (Fodstad and del Campo)

Having established several human melanomas and sarcomas as xenografts in nude mice and as monolayer and soft agar cultures in vitro, we have the opportunity to compare ultrastructural characteristics and heterogeneity between:

a) Different tumors of the same type

b) The same tumor growing in different environments or as sublines (clones) in vivo and in vitro

The heterogeneity found can then be correlated with differences in biological behavior as determined by the methods described in A.

Three variants of the LOX melanoma are established, all growing both in vivo and in vitro. These sublines have different chromosome numbers and histology, and one of them grows more slowly in the mice. These LOX lines will be compared with four other melanomas.

Similar studies will be applied to the sarcoma lines described in F.

H. Physiology, pharmacology and toxicology of the pulmonary Clara cell (Boyd)

1. Possible use of 4-ipomeanol as a tool for study of Clara cell functions

The nonciliated bronchiolar cell (Clara cell) is a major pulmonary cell type present in the terminal bronchioles of mammalian species. However, the

physiologic function(s) of the Clara cell remains unknown, and its potential role in the pathogenesis of pulmonary diseases largely remains to be elucidated.

We found that 4-Ipomeanol selectively damaged or destroyed the pulmonary Clara cells of several laboratory animal species (Nature 269, 713, 1977). This observation suggested that the toxin might provide a tool to study pathophysiological events related to Clara cell damage as well as the normal physiological role of the Clara cell. To further establish the possible validity, criteria and limitations on this use of 4-ipomeanol in experimental studies, we are performing detailed ultrastructural investigations of the time-courses and dose-dependencies of the cellular pathology in lungs of rats, mice, guinea pigs, rabbits and hamsters receiving 4-ipomeanol. Both the acute, destructive phase of toxicity and the resolution or reparative phases are being examined. Along with the morphologic investigations, autoradiographic studies of the incorporation of [^{3}H]-thymidine (DNA precursor) and [^{3}H]-δ-aminolevulinic acid (hemoprotein precursor) into specific lung cell types during both the destructive and repair phases also will be investigated.

Our previous studies have indicated that an initial alkylation and destruction of Clara cells by lethal doses of 4-ipomeanol frequently is followed by extensive pulmonary edema (and/or pleural effusions in some species), vascular congestion, hemorrhage and eventual deaths of the animals. In the terminal stages of the acute toxicity, necrosis of other major cell types (including capillary endothelium, and types I and II pneumocytes) sometimes occurs, although there is little or no alkylation of these cell types by a 4-ipomeanol metabolite. It is possible that these changes (e.g., edema, hemorrhage) occurring after the initial Clara cell damage are not primary events, but instead represent secondary or tertiary pathological changes. However, the ongoing studies will be essential to establish and further characterize the relationships between all of these pathological responses to 4-ipomeanol, and to define the framework within which 4-ipomeanol may provide a useful experimental tool in further studies of pulmonary cell functions and pathophysiology.

We shall also investigate the possible interactions of 4-ipomeanol and other pulmonary toxicants that cause damage to different pulmonary cell types. The latter investigations are of particular interest because of the apparent role the Clara cell plays in repairing damage to pulmonary bronchiolar epithelium. For example, in response to noxious inhalants such as NO_2 or ozone, the ciliated airway cells become necrotic. The ciliated cells are highly susceptible to these kinds of toxicants, but they are not capable of proliferation and repair of the airways after such injuries. Instead, the damage to ciliated cells stimulates a proliferative response to the Clara cells. Many of the proliferated Clara cells subsequently transform into normal ciliated cells, and the normal bronchiolar morphology eventually is recovered. Thus, the Clara cells appear to have a major role in pulmonary airway repair and defense mechanisms which is analogous to that performed within the alveoli by the type II epithelial cells in response to injury to the highly susceptible (and nondividing) type I pneumocytes. An important question is the possible effect of perturbations of these crucial defense mechanisms of the lung. The use of 4-ipomeanol may provide an approach to such studies. Of interest initially will be the investigation of the pathophysiologic sequelae of simultaneous damage to highly susceptible,

nondividing cell populations (e.g., ciliated bronchiolar cells) and the corresponding reparative cell populations (e.g., Clara cells). Indeed, it is conceivable that combinations of pulmonary toxins causing damage to these kinds of cells could be encountered in atmospheric exposures. For example, NO_2 and O_3 (toxic to ciliated cells) are prevalent atmospheric contaminants, while 3-methylfuran (a Clara cell poison) also has been suggested to be a major constituent of certain urban smogs. It is possible that combined lung cell insults of this type could lead to fibroblastic proliferation and overgrowth. Thus, we plan to explore the possible role of combined bronchiolar epithelial cell injuries in the pathogenesis of bronchiolar fibrosis, and to elevate the possible implications such findings may have for the pathogenesis of environmentally-associated and/or iatrogenic chronic obstructive pulmonary disease (COPD) in man.

2. The Clara cell as a target for pulmonary cytotoxins requiring activation by mixed-function oxidase enzymes

Our studies with 4-ipomeanol provided the first definitive evidence of cytochrome P-450 monooxygenase activity in a specific lung cell type (Nature 269, 713, 1977). These results led to the view that Clara cell would be an especially susceptible target cell for other toxic chemicals requiring activation by similar monooxygenase activities. Subsequent investigations with 3-methylfuran and carbon tetrachloride added additional support for this view. In continuing studies, we are examining the pulmonary metabolism and cellular toxicity of other chemicals that are known, or are likely, to undergo metabolic activation by monooxygenase enzymes. Agents under study (or to be studied in the future) include naphthalene, urethane, variously substituted furans, thiophenes and pyrroles, and cyclophosphamide.

3. The Clara cell as a site of activation and/or neoplastic transformation by chemical carcinogens

It is now generally believed that many, if not most, pulmonary cancers are caused by exposures to cancer-inducing chemicals present in the environment. The additional realization that many carcinogens require metabolic activation has stimulated much interest in the cellular localization of xenobiotic-metabolizing enzymes in the lung. Based on results of our previously discussed studies with 4-ipomeanol, we proposed that the Clara cell is an important potential site of activation of carcinogens by MFO enzymes.

Certain nitrosamine derivatives are known to induce tumors of apparent Clara cell origin. But, unfortunately, definitive metabolic evidence is not available to indicate that the cellular binding or the pulmonary tumorigenicity of nitrosamines such as nitrosoheptamethyleneimine is due to a metabolite of these compounds formed in situ in Clara cells. Moreover, there presently is no direct evidence that the lung-toxic furans, such as 4-ipomeanol and 3-methylfuran, are pulmonary carcinogens. Taken together, however, the research on these two classes of compounds provides strong evidence that the pulmonary Clara cell indeed is a potential site of oxidative metabolic activation of carcinogens. Therefore, not only is it likely to be a potential cell of origin for tumors

induced by certain classes of chemicals, it also is possible that other bronchiolar or bronchial cell types could be targets for carcinogenic metabolites having sufficient stability to pass from one cell to another following formation in Clara cells.

To pursue these concepts further, we plan to examine the possible carcinogenicity of 4-ipomeanol and related compounds. Likewise, we plan to initiate detailed studies of the in vitro and in vivo metabolic activation of nitrosoheptamethyleneimine and related nitrosamines to determine if these agents are activated in situ in the lung by enzyme systems which are the same as or similar to those involved in the activation of 4-ipomeanol.

In other ongoing studies, described in greater detail elsewhere, we are attempting to define further the biochemical characteristics of normal Clara cells, and to compare these properties with those of pulmonary tumors (see Annual Report FY 82, Project No. Z01 CM 07125-01 LETM, part G.1.). The identification of biochemical similarities within specific histological types of human bronchogenic carcinomas may implicate specific cell(s) of origin. We are particularly interested in screening a wide variety of human and animal lung tumor specimens for the capacity to metabolize 4-ipomeanol, since this metabolic activity appears to be uniquely associated with Clara cells in normal lungs.

In this regard, we presently are investigating, or plan to study, the metabolism of 4-ipomeanol: (a) in urethane-induced mouse pulmonary tumors and nitrosamine-induced hamster pulmonary tumors in vitro and in vivo, (b) in vitro in slices and/or microsome preparations of human bronchogenic lung tumors obtained at surgery and (c) in vitro and in vivo in human and animal pulmonary tumors propagated and maintained in vivo in athymic (nude) mice.

I. Cellular/molecular factors in cell death by chemically reactive drug metabolites (Boyd)

Although it is now apparent that chemically reactive drug metabolites may cause necrosis of target tissues such as the liver, the lungs an the kidneys, the precise mechanisms of chemical/cellular interactions in the pathogenesis of cell death is unknown. In this project we are approaching these questions using a combination of biochemical measures coupled with morphologic techniques, including high-resolution light microscopy, scanning and transmission electron microscopy, x-ray microanalysis, and light- and EM-autoradiography. Through correlations of the time courses and dose dependencies of the metabolism of protoxins, the subcellular site(s) of interactions of reactive metabolites, changes in selected biochemical parameters and morphologic alterations, we hope to elucidate critical chemicobiological events leading to cell death.

Studies will be performed on tissues from animals dosed in vivo with compounds of interest, as well as with isolated, defined cell populations (from lungs, livers and kidneys) exposed in vitro. Compounds of current interest include acetaminophen (liver) 4-ipomeanol, α-naphthylthiourea and cyclophosphamide (lung and kidney).

J. Cellular dynamics and structural/physiological consequences of drug-induced damage to discrete cell populations within extrahepatic tissues (Boyd)

The purpose of this project is to define the ultrastructural/cellular pathology in extrahepatic target tissues (primarily lung and kidney) exposed to antitumor drugs producing toxic lesions in such tissues. The temporal progression of such lesions will be followed carefully from the acute phase through the resolution phase(s) with a view toward defining changes in cell dynamics (turnover/repair) and the possible consequences this may have for altered physiological function(s) of the tissues. The possibility of altered susceptibility to further injury of such tissues by simultaneous and/or subsequent exposure to additional doses of the same drug or to different drugs which may affect other cell populations will also be explored. These investigations hopefully will provide some insight into the pathogenesis of adverse extrahepatic tissue lesions that result, or which might be predicted to result, from different dosing regimens and/or combinations of drugs used in cancer chemotherapy. Compounds of particular interest in these studies include BCNU, methyl CCNU and other nitrosoureas, cyclophosphamide, bleomycin and adriamycin.

K. Ultrastructural investigations in collaboration with other DTP Laboratories (del Campo)

1. Microtubule - associated protein (MAP)-dependent polymerization of purified tubulin with a GDP analog and ribose analogs of GTP

Guanosine nucleotides are intimately involved in the structure and function of tubulin, the major component of the mitotic spindle. Since understanding the role of nucleotides in tubulin polymerization may aid in the rational design of effective antineoplastic agents, Dr. E. Hamel and coworkers in the LMCB have been studying the effects of GDP and GTP analogs. The LETM (Mr. del Campo) has collaborated in these studies using electron microscopy to evaluate the nature of the polymer formed after polymerization induced by nucleotide analogs of GDP and GTP.

Both negative stain and sectional material were examined in the electron microscope. In some cases, specimens were prepared utilizing a special EM technique involving fixation in tannic acid - glutaraldehyde and embedded in a high resolution epoxy resin. This permitted the subunit structure of the microtubules to be readily observed.

Deoxy GTP (dGTP) analogs and arabinosyl GTP all supported the polymerization reaction in which microtubules were formed in the presence of MAP's. However, the reaction began earlier and required a lower nucleotide concentration when compared to GTP-supported reaction. 2',3'-Dideoxyguanosine-5'-triphosphate (ddGTP) was unique among the analogs tested in that it supported polymerization without MAP's, although a higher nucleotide concentration was required. The polymer was a mixture of microtubules and open sheets. In addition, microtubules formed with ddGTP plus MAP's were significantly more cold stable than microtubules formed with GTP plus MAP's.

Analogs with an open ribose ring bearing a methyl or phosphate group at the 2' or 3' hydroxyl were also examined. 2',O-Methylguanosine-5-triphosphate (2',O-MeGTP) was comparable to GTP in its ability to support MAP dependent polymerization as a function of nucleotide concentration. Progresively decreasing activity was observed with acycloguanosine-5'-triphosphate (3',O-MeGTP) and the periodate-oxidized, borahydride-reduced derivative of GTP (ox-redGTP). Microtubules were formed with acyclo GTP and 3',O-MeGTP, but thick-wall enlarged tubules about 80-82 nm in diameter were formed with 2',O-MeGTP and Ox-redGTP. No definite protofilaments could be distinguished in these bizarre structures. If nucleodiphosphate kinase was included in the reaction mixture, microtubules became the predominate product with ox-redGTP.

This project is essentially complete and two manuscripts are in preparation.

2. Development of methods to produce consistent preparations of liposome entrapped Melphalan

Melphalan (MPL) is a commonly utilized cytotoxic drug active against a variety of cancers including mammary cancer in which the regional lymph nodes are the common site of metastasis. However, it is difficult to remove all of the involved lymph nodes, especially those containing micrometastases, at the time the primary tumor is resected. Liposomes containing MPL may be useful in the therapy of lymph node metastasis because it is known that lymphatic capillaries absorb fatty substances.

Previous studies conducted by Dr. Juneji Khato of the Laboratory of Chemical Pharmacology on lymph node uptake of liposomes containing MPL revealed the importance of producing preparations of liposomes reproducibly with respect to size and drug entrapment rate. Small liposomes are usually prepared by sonication until the sonicate becomes "clear." However, the time required for clarification of a sonicate is not consistent because of several variables involving the sonicator. Therefore, it has been difficult to determine a suitable endpoint for sonication. Obviously, a more quantitative way to assess the duration of sonication is desirable. The present work evaluated optical density as an index to monitor sonication time so that more reproducible liposome preparations can be obtained.

Liposome suspensions were observed in the electron microscope (Mr. del Campo) to assess the morphology of the vesicles and make size measurements. A negative stain technique incorporating ammonium molybdate as the contrasting stain was utilized.

Electron microscopic examination of suspensions produced by prolonged sonication revealed vesicles that were small and round and relatively uniform in size and shape. The mean diameter of these vesicles was 36.9 ± 7.7 nm. Short sonication times (2-4 min) OD = 1.8-2.0 produced cloudy suspensions with higher entrapment rates for both MPL and the aqueous phase. These suspensions contained mostly large vesicles but were notably variable in size and shape.

This project is essentially completed and two manuscripts are submitted for publication.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07127-01 LETM

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemistry/Drug Metabolism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies completed, under way or planned include: A. In vitro metabolic activation of cytotoxic agents by microsomal enzymes and purified cytochromes P-450 from liver and lung, B. In vitro metabolic activation of cytotoxic chemicals in isolated lung cells, C. APUD-cell-associated properties in pulmonary Clara cells, D. Effects of vitamin E deficiency, O_2, and antibiotics on nitrofurantoin metabolism and distribution, E. Effects of metabolic inducers on extrahepatic activation and toxicity of xenobiotics, F. Metabolism of 3-methylfuran, G. Activated metabolites of 2-methylfuran, H. In vivo glucuronidation of 4-ipomeanol, I. Effect of diethylmaleate on metabolism and disposition of 4-ipomeanol, J. Cytochrome P-450 cellular localization in skin, K. Phospholipid methyltransferases and control of membrane microviscosity, L. Development of microanalytical methodologies, M. Synthesis of new drug candidates or metabolites, N. Metabolic activation and cytotoxicity of perilla ketone, O. Metabolic activation (dephosphorylation) of the radioprotector WR-2721 and related drugs.

A. In vitro metabolic activation of cytotoxic compounds by lung and liver microsomal enzymes and by reconstituted systems containing purified pulmonary and hepatic cytochromes P-450; implications for target tissue/cell specificity; applicability to in vitro antitumor test systems with drugs requiring activation by metabolism (Statham, Boyd and McMenamin)

The formation of toxic metabolites from many environmental chemicals is catalyzed by the cytochrome P-450 dependent monooxygenase system (P-450 system). Toxic effects are often confined to one or a limited number of the mammalian tissues that contain the P-450 system. Although the factors that control the tissue specificity of toxicants are not yet fully understood, it is clear that they are both numerous and complex. One of these factors may be differences in the properties of the P-450 systems in susceptible as compared to resistant tissues or cell types. We are studying the P-450 system of rabbit lung and liver to determine if differences in their characteristics represent controlling factors in specific toxic reactions that involve metabolic activation.

The pulmonary-specific toxicity of 4-ipomeanol has been studied previously in considerable detail. This compound is metabolized by the P-450 system to a product(s) that binds covalently to protein, particularly in the pulmonary Clara cell, of a number of mammalian species. We have now investigated further the in vitro metabolic activation of this model pulmonary aklylating agent/cytotoxin. Part of the studies were performed in collaboration with Drs. R. Philpot and J. Bend, NIEHS, NIH, Research Triangle Park, NC.

The rate of covalent binding of 4-ipomeanol in microsomal preparations from rabbit lung is about 12 times faster than in preparations from rabbit liver. The pulmonary reaction requires NADPH, is blocked by GSH, and is inhibited by antibodies to purified rabbit lung cytochromes P-450I or P-450II.

Autoradiograms of pulmonary microsomes incubated with ^{3}H-4-ipomeanol and subjected to polyacrylamide gel electrophoresis with SDS, show covalent binding to virtually all of the observed proteins. Decreases in binding caused by GSH or antibodies do not appear to be selective with respect to individual proteins.

The purified pulmonary cytochrome P-450 isozymes mediate the covalent binding of 4-ipomeanol at rates of 3.6 (P-450I) and 2.7 (P-450II) nmol product bound/nmol P-450/min. The addition of phospholipid to the incubations increases these rates by about 50%. The metabolism of 4-ipomeanol in reconstituted monooxygenase systems results in apparent cross-linking as well as fragmentation of the protein components.

It is clear that rabbit pulmonary microsomal preparations metabolize 4-ipomeanol more efficiently than do hepatic preparations, and that both P-450I and P-450II catalyze this reaction. However, these findings must be related to the Clara cell before any conclusions can be reached about the role of the P-450 system as a determinant in the cell-specific effect of this toxin. To some extent, the finding in Dr. Philpot's laboratory that P-450I and P-450II, as well as NADPH cytochrome P-450 reductase, appear to be highly concentrated in the rabbit Clara cell (as determined in tissue sections by immunohistochemical techniques) further defines a relationship between the specific toxicity of 4-ipomeanol

and the P-450 system. Additional evidence for this relationship has been obtained by results of experiments carried out with cells isolated from rabbit lung. In Dr. Bend's laboratory, cytochromes P-450I and P-450II have been identified in isolated Clara cells by immunochemical methods and proteins with the same monomeric molecular weights as P-450I and P-450II are present in the microsomal fraction from these cells. The pulmonary forms of P-450 are also present in isolated alveolar Type II cells, but they appear to be at lower concentrations than in the Clara cells. No P-450-related components were detected in isolated rabbit pulmonary macrophages.

We believe that the difference in substrate specificity between the P-450 systems of lung and liver and the highly localized nature of the pulmonary system are important factors in the tissue and cell-specific toxicity of 4-ipomeanol. Studies are continuing to ascertain the importance of relationships of target tissue/P-450 systems with respect to tissue specific toxicities.

Several manuscripts describing various phases of this work are in press or have been published this year (see Publications 1 - 3).

A new direction for this project being initiated for the development in vitro of antitumor test systems involving P388 and soft agar methods incorporating these reconstituted or microsomal P-450 preparations. This hopefully may allow for for the improved testing of antineoplastic activity of chemotherapeutic agents which require metabolic activation.

B. In vitro metabolic activation of cytotoxic chemicals in isolated, defined lung cell preparations (Statham and Boyd)

The initial goal of these studies was to determine the abilities of isolated, defined cell populations to activate 4-ipomeanol to its cytotoxic alkylating intermediate in vitro. The metabolism and covalent binding of 4-ipomeanol (IPO) were investigated initially in pulmonary lung cells from rabbits. ^{3}H-Labeled IPO was incubated with alveolar type II cells (83% purity), non-ciliated bronchiolar epithelial (Clara) cells (77% purity) and alveolar macrophages (>90% purity).

Covalent binding of radioactive material to type II and Clara cells was observed by autoradiography and by a biochemical method. IPO binding to cells was almost totally prevented by 1 mM piperonyl butoxide, an inhibitor of the cytochrome P-450-dependent metabolism of 4-ipomeanol. No covalent binding was observed with alveolar macrophages in the presence or absence of piperonyl butoxide. The maximal rates of enzyme-mediated covalent binding of IPO to protein were greater in the Clara cells (135 pmoles/10^{6} cells/min) than in the type II cells (13 pmoles/10^{6} cells/min). Incubation of either sonicated Clara or type II cell fractions with ^{3}H-IPO, glutathione (GSH) and NADPH (20 min, 37°C) resulted in the formation of two distinct radiolabeled GSH conjugates.

The studies showed that 4-ipomeanol is metabolized to some extent in vitro by alveolar II type cells, although to a much lesser extent than by Clara cells. But, in vivo, the alveolar type II cells are not damaged by 4-ipomeanol. The in vitro findings may correlate with the lower levels of GSH in isolated cells

than might be found in vivo. Studies will proceed to evaluate the importance of GSH concentrations in isolated cells and its role in detoxification of reactive metabolites. Also, studies will be extended to include other compounds such as paraquat and carbon tetrachloride which produce pulmonary damage to other cell populations in the lung, and also to certain anticancer drugs (e.g., cyclophosphamide) which are known to be activated by metabolism and which therefore might be toxic to metabolically active cells in the normal lung.

The studies with 4-ipomeanol in isolated cells are essentially complete and a manuscript has been published (see Publication 4).

C. Investigations, in isolated pulmonary Clara cells, of endocrine properties (e.g. dopa decarboxylase, histaminase, calcitonin, β-endorphin) associated with APUD-type cells, small cell lung tumors and certain non-small cell tumors (Statham and Boyd)

Small-cell lung cancer (SCC) has in the past been related to an origin from cells of the APUD (amine precursor uptake and decarboxylation) type because of their frequent association with endocrine-related properties. But this pathogenesis has not been clearly established. In fact, a recent report by Berger et al. (J. Clin. Endocrinol. Metab. 53: 422-429, 1981) indicates that many endocrine-related properties occur throughout the spectrum of human lung cancer, and biochemical differences between the major histopathological types are quantitative rather than qualitative; this may reflect the fact that the major forms of lung cancer represent a continuum of differentiation within a common cell lineage which includes both SCC and non-SCC lung tumors.

Previous studies from our laboratory have implicated the pulmonary bronchiolar Clara cell as a likely cell of certain origin for carcinogen-induced lung tumors (see Section H., Annual Report FY 82, Project No. Z01 CM 07126-01 LETM). It is not known if the Clara cell has any APUD-cell characteristics. Therefore in the present study we shall investigate in isolated pulmonary Clara cells some particular endocrine properties that are typically associated with APUD-type cells as well as with both SCC tumors and with non-SCC tumors. This may provide additional key information relevant to elucidation of the cell(s) of origin of lung tumors. Biochemical parameters to be measured will include dopa decarboxylase, histaminase, calcitonin, and β-endorphin.

D. Effects of vitamin E deficiency, inspiratory O_2 and antibiotic pretreatments on nitroreductive metabolism; implications for nitrofuran cytotoxicity (Statham and Boyd)

Nitrofurantoin (N-[5-nitro-2-furfurylidine]-1-aminohydantoin) (NF) is an antibacterial drug used widely to treat acute urinary tract infections as well as for long-term prophylaxis against certain urinary-tract pathogens. Both acute and chronic pulmonary reactions have been described after standard therapeutic regimens of the drug. Nitrofurantoin is capable of producing a direct, dose-related pulmonary toxicity in rats. The acute lethality of nitrofurantoin in rats has been shown to be greatly enhanced in animals maintained in a vitamin E-free diet (LD50 - 35 mg/kg compared to 400 mg/kg in the control rats). Also the lethality and degree of pulmonary damage produced by nitrofurantoin were

greatly enhanced in rats kept in oxygen-enriched atmospheres. In vitro studies with rat lung and liver microsomal preparations have shown that nitrofurantoin is enzymatically converted, to a greater extent under anaerobic conditions, to reactive metabolites which are capable of alkylation of biological macromolecules.

The present investigations were undertaken to determine whether the increased toxicity of nitrofurantoin seen in vitamin E deficient and 100% O_2 treated rats is due to alterations in the tissue distribution or covalent binding of nitrofurantoin. Dr. H. A. Sasame (LCP, NHLBI) was a collaborator on this project.

The distribution and metabolism of NF and its metabolites were studied in normal and vitamin E deficient (VED) rats. VED had no effect on the levels (pmoles/mg) of covalently bound NF metabolites in tissues and the order of binding was kidney > liver > lung > blood. The levels of covalently bound NF metabolites increased with an increase in the dose of NF administered. Analysis of tissue extracts by HPLC indicated that the tissue distribution of unmetabolized NF was not affected by VED but that normal rats had lower tissue concentrations of NF metabolites after 16 hr in all tissues except the lung. Urinary excretion of both NF and NF metabolites in VED rats was significantly decreased at all time periods examined. Pretreatment of VED rats with antibiotics or 100% oxygen did not alter the 1 hr tissue distribution of NF, NF metabolites, or levels of covalently bound NF metabolites as seen in normal and unpretreated VED rats. These data suggest that the potentiated toxicity of NF seen in vitamin E deficient and 100% oxygen treated rats is not due to alterations in the tissue distribution or covalent binding of NF.

These studies are essentially completed and a final manuscript is in preparation.

E. Effects of drug metabolism inducers on the extrahepatic disposition and toxicity of drugs activated by metabolism in situ in extrahepatic target tissues (Statham and Boyd)

Chemicals which exert their toxic effects through metabolism in situ to an active species respond in a complex fashion to treatment with inducers of drug metabolizing enzymes. In general, it may be difficult if not impossible to predict the effects of inducers on the metabolism and toxicity of compounds which are metabolically activated both in hepatic and extrahepatic tissues.

Major factors contributing to this complexity include: 1) the multiple effects of inducers on drug toxification and detoxification pathways, 2) the stability of the reactive intermediates formed within the hepatic and extrahepatic tissues with respect to their ability to be transported via the circulatory system and 3) the overriding effects of hepatic metabolism, due to the liver's large mass and high content of drug metabolizing enzymes; this can be the major influence on the tissue distribution of the parent compound to potential extrahepatic sites of metabolism in vivo. Studies to explore these concepts were designed using 4-ipomeanol (IPO) as a model compound whose toxicity is expressed through metabolism to a reactive intermediate in situ in the target tissues.

Previous in vivo studies in the rat had shown that treatment of rats with mixed-function oxidase inducers phenobarbital (PB) and 3-methylcholanthrene (MC) decreased both the acute toxicity of IPO and the concentrations of covalently bound IPO equivalents in the lung. MC treatment, however, markedly increased the amount of IPO equivalents bound covalently in the liver and produced a striking shift in the target tissue necrosis. Centrilobular hepatic necrosis, accompanied by a marked diminution in the severity of pulmonary lesions, were the major result of IPO administration to MC-treated rats. No such alteration in the target organ specificity of IPO was observed with PB treatment. In vitro studies had shown that both the PB treatment and the MC treatment increased the amount of covalently bound IPO equivalents with hepatic microsomes but produced little or no effect with the lung microsomal subcellular fractions. Investigations, therefore, were undertaken to elucidate the basis for the contrasting in vitro and in vivo effects of PB on hepatic binding, and to explore the effects of PB and MC treatments on the in vivo distribution and metabolism of IPO in the rat.

Analysis of tissue extracts by high-pressure liquid chromatography (HPLC) showed that both PB and 3-MC treatments markedly decreased the concentrations of unmetabolized IPO at all times examined. PB treatment increased the urinary excretion of nonbound IPO metabolites while MC treatment did not alter their excretion. Analysis of urine by HPLC indicated that the increased concentration of urinary metabolites found in the phenobarbital-treated rats was attributable primarily to an increased excretion of IPO-4-glucuronide. These data indicate that the decreased pulmonary covalent binding and lethality of IPO in the rat after MC and PB are caused by alterations in the tissue distribution of the parent compound. Pulmonary concentrations of unmetabolized IPO are decreased by MC through an increased metabolism of IPO in the liver, primarily to toxic products that bind covalently in that tissue and lead to hepatotoxicity. PB produced a similar decrease in unmetabolized IPO concentrations in lung but by an enhanced in vivo metabolism and clearance of IPO, primarily through a "nontoxic" pathway, glucuronidation, and did not lead to hepatotoxicity.

In another study the effects of MC on the covalent binding and toxicity of IPO were examined studied in (B6D2)D2 mice. The mice were segregated into relatively "inducible" or "noninducible" groups based on zoxazolamine (ZOX) sleeping times following MC-treatment. MC treatment decreased the covalently bound IPO metabolite(s) both in the lungs and in the kidneys of "inducible" and "noninducible" mice when compared to nonpretreated controls. Concentrations of covalently bound IPO metabolite(s) in liver were increased in "inducible" mice and decreased in "noninducible" mice by MC pretreatment when compared to nonpretreated mice. Associated with MC pretreatment was a significant decrease in the acute lethality of IPO both in the "inducible" and in the "noninducible" mice when compared to nonpretreated animals (LD50: 213 ± 2, 140 ± 14, and 14 ± 4 mg/kg, respectively). Hepatic necrosis occurred frequently in the "inducible" mice and occasionally in the "noninducible" mice given large IPO doses near the respective LD_{50} values. Hepatic necrosis was never observed in nonpretreated mice receiving near lethal doses of IPO. These results supported previous studies indicating that reactive IPO metabolites binding to extrahepatic tissues are formed in situ and do not reflect binding of blood-borne metabolites formed in the liver.

These data collectively illustrate that when dealing with inducing agents and compounds which are metabolized to highly toxic reactive intermediates in both hepatic and extrahepatic target tissues, it is the balance of the toxification and detoxification reactions or the overall net effect of the inducing agents on these pathways in each of the various tissues that may dictate the overall toxicity of a given compound. Moreover, inducer effects in one tissue clearly can influence those in another. As particularly illustrated by this study, the in vivo effects of inducers on the metabolism and toxicity of compounds activated in situ in extrahepatic tissues may be largely modulated by the effects of the inducers on the liver. Because of its large mass and high drug-metabolizing activity, the liver may predominantly regulate the amounts of unmetabolized compound reaching extrahepatic tissues where they could subsequently be metabolized to toxic products.

These studies are essentially completed, and the resulting manuscripts are in press (see Publications 5 - 7).

F. Metabolism of 3-methylfuran; implications for in vivo toxicity (Statham, Boyd and staff)

Saunders and co-workers (Biomed. Mass Spectrom. 1, 192, 1974) reported that 3-methylfuran (3-MF) was a major atmospheric contaminant during a smog alert in Washington, D.C. Although the source of the 3-methylfuran was unknown, the authors speculated that it could be formed from atmospheric photooxidation and degradation of volatile hydrocarbons such as isoprene and other terpenes. These naturally-occurring precursors are released in large amounts into the atmosphere by certain plants, and may be concentrated during certain conditions. Deciduous forests, such as those covering the Appalachian mountains, are thought to be a major source of these hydrocarbon precursors. The suggestion that 3-methylfuran, which apparently is not present in automobile exhaust emissions, could be a major contributor to certain urban smogs aroused considerable controversy, since automobiles have been widely considered to be the major source of atmospheric pollutants, especially in nonindustrial areas such as Washington, D.C. Previous studies from our laboratory have shown that the furan derivative, 4-ipomeanol is highly toxic to pulmonary bronchioles due to its metabolism in situ to a toxic alkylating intermediate. Our preliminary studies (Nature 272, 270, 1978) indicated 3-MF was activated similarly in situ to a highly toxic metabolite in lung. Further Studies were therefore designed to: 1) examine the metabolism and toxicity of 3-MF in mice in vivo, 2) investigate the in vitro requirements for the metabolic activation of 3-MF to its highly reactive intermediate, 3) obtain dose response relationships between 3-MF administered intrapertoneally, orally and by inhalation with the extent of bronchiolar necrosis, 4) examine by an autoradiographic technique the localization of the covalently bound material within specific lung cell populations and 5) investigate the importance of the furan ring on 3-MF toxicity.

In vitro studies demonstrated that 3-MF is converted to a highly reactive electrophilic metabolite via cytochrome P-450 dependent mixed-function oxidase activity present in mouse lung microsomes. Autoradiographic studies of lungs of mice given radiolabeled 3-MF either i.p. or by inhalation showed highly preferential accumulation of a covalently bound 3-MF metabolite in the pulmonary

bronchioles. Striking bronchiolar necrosis was present in lungs of animals 24 hr after exposure to 3-MF. Administration (i.p.) of piperonyl butoxide, an inhibitor of the metabolic activation of 3-MF, prevented both the bronchiolar alkylation and necrosis by inhaled 3-MF. Prior depletion of tissues of reduced glutathione by diethylmaleate increased both the bronchiolar alkylation and toxicity of 3-MF.

Studies have indicated that at very high concentrations, 3-methylfuran was toxic to both nonciliated and ciliated bronchiolar cells, and it was covalently bound in both of these cell types. At lower concentrations though, 3-methylfuran was selectively toxic to Clara cells.

These results are consistent with the view that a metabolite of 3-MF is formed in situ which alkylates lung bronchioles, leading to their necrosis.

Detailed autoradiographic and electron microscopic studies of the acute and chronic pulmonary responses to 3-methylfuran also are planned for the future. In these studies we hope to determine if covalent binding of 3-methylfuran to ciliated bronchiolar cells after administration of high doses of the compound is due to metabolism of 3-methylfuran in the ciliated cells or, alternatively, whether the alkylating metabolite has sufficient stability so that a portion of it may reach the ciliated cells after formation in nonciliated cells.

The major objectives of these metabolism studies on 3-MF have been completed and the final manuscript is in preparation. Also, a review of these and other studies from our laboratory on biochemical toxicology of furans is in press (see Publication 8).

G. Studies of reactive metabolites of 2-methylfuran (Statham, Boyd and staff)

We have undertaken studies on the chemical nature of toxic, alkylating metabolite(s) produced by cytochrome P-450-mediated metabolism of 2-methylfuran (2-MF). 2-MF causes pulmonary bronchiolar lesions in mice like those produced by other lung-toxic furans such as 3-methylfuran and 4-ipomeanol. We chose to study the activation of 2-MF in greater detail because appropriate reference compounds for the identification reactive intermediate(s), and/or secondary metabolite(s) resulting therefrom, appeared more readily accessible to synthesis.

NADPH-dependent microsomal metabolism of 2-MF results in the formation of a highly electrophilic metabolite which alkylates the microsomal protein; addition of cysteine to the incubations results in the formation of a water-soluble cysteine conjugate and a decrease in the microsomal alkylation. Since an initially formed furan-epoxide of 2-MF could possibly rearrange to α- or β-angelicalactones, we have synthesized both the lactones, as well as their cysteine conjugates. Using a combination of techniques including high-pressure liquid chromatography, gas chromatography and mass spectrometry, we have developed separation and analytical techniques for these standards, as well as for 2-MF itself, and the conjugate formed in microsomal incubations with 2-MF and cysteine. Comparison of the properties of the potential metabolites we have synthesized with those produced from 2-MF in vitro and in vivo should

facilitate the further characterization of the toxic metabolite of 2-MF. High-resolution NMR investigations of the 2-MF/cysteine conjugate(s) will also be undertaken if necessary.

H. In vivo metabolism of the pulmonary cytotoxin 4-ipomeanol; significance of glucuronidation (Statham, Boyd and staff)

Although quantitatively the in situ metabolism of 4-ipomeanol in the target tissue is of prime importance with respect to the development of tissue alkylation and necrosis, it represents only a minor metabolic pathway for 4-ipomeanol. Initial studies indicated that less than 0.1% of the total dose of 4-ipomeanol administered is excreted unchanged in the urine. Urinary metabolites of 4-ipomeanol had not been characterized and our objective was to identify and quantitate the major urinary metabolites of 4-ipomeanol in the rat and elucidate the importance of these metabolic pathways with respect to the overall toxicity of the compound.

Hydrolysis by β-glucuronidase and inhibition of hydrolysis by the specific β-glucuronidase inhibitor, saccharo-1,4-lactone, together with the molecular ion and the fragment ions observed during CI mass spectrometry confirmed the identity of the major urinary 4-ipomeanol metabolite as a glucuronide conjugate. Identical EI mass spectra and identical HPLC retention times of standard 4-ipomeanol and that of the β-glucuronidase-released radioactive compound indicated that the β-glucuronidase hydrolysis product was unchanged 4-ipomeanol. This further confirmed the major metabolite of 4-ipomeanol in the rat as ipomeanol-4-glucuronide.

The glucuronide conjugate of 4-ipomeanol accounted for 74 ± 7% of the total metabolites excreted in urine and for 14 ± 1% of the total dose administered, while total urinary metabolites accounted for only 19 ± 1% of the total administered dose at the 1/2 hr time period. By 2 hours, excretion of metabolites peaked and the urinary radioactivity accounted for 47 ± 1% of the total dose administered, while 55 ± 1% of the total urinary radioactivity was accountable as ipomeanol-4-glucuronide. The glucuronide therefore is quantitatively the predominant in vivo metabolite of 4-ipomeanol in the rat and accounts for as much as 26% of the total administered dose by 2 hrs. An increase or decrease in glucuronide formation could conceivably alter the acute toxicity of 4-ipomeanol in vivo.

The importance of 4-ipomeanol glucuronidation as a detoxication pathway in the rat shall be investigated. Preliminary observations are that pretreatment of rats with phenobarbital significantly increased the urinary excretion of ipomeanol-4-glucuronide. Associated with the phenobarbital-enhanced urinary excretion of the glucuronide were decreased tissue levels of covalently bound 4-ipomeanol and a decreased acute lethality of the compound (LD50 24 mg/kg control and 50 mg/kg phenobarbital-treated).

A manuscript describing part of this work currently is in press (see Publication 9).

I. Influence of the GSH depletor, diethylmaleate (DEM), on the disposition and metabolism of 4-ipomeanol, a cytotoxic furan detoxified via pulmonary GSH (Statham and Boyd)

Because diethylmaleate (DEM) depletes glutathione (GSH) in tissues, there has been considerable use of this agent as an experimental modifier of GSH-dependent drug metabolism and drug toxicity, both in vivo and in vitro. However, there is little information concerning other effects of DEM beyond its action on GSH. In vitro studies in fact have indicated that DEM might produce either inhibition or stimulation of certain microsomal drug metabolism pathways. Therefore, the likelihood that, depending upon the particular chemical substrate involved, DEM might substantially alter GSH-dependent and/or GSH-independent drug metabolism in vivo indicates that a detailed analysis of the influence of DEM on the in vivo distribution and metabolism of each specific parent compound and its metabolite(s) should be made. Such information will facilitate the interpretation of the mechanism of DEM's modifying effect(s) on toxicities by such chemicals, particularly those for which GSH serves as a protective factor.

Our previous studies have indicated that GSH modulates the pulmonary cytotoxicity of 4-ipomeanol. Further, treatment of rats in vivo with DEM, markedly depletes reduced GSH concentrations in lung, liver and other tissues of the rat, and enhances the pulmonary toxicity and covalent binding of 4-ipomeanol (see Section A. 4., Annual Report FY 82, Project No. Z01 CM 07125-01 LETM). To clarify the mechanism of this effect, a detailed analysis of the influence of DEM on the in vivo distribution and metabolism of 4-ipomeanol was carried out.

The distribution of unmetabolized 4-ipomeanol-[5-^{14}C] and its metabolites were studied in tissue extracts by high-pressure liquid chromatography (HPLC) in control and DEM-treated rats. At all time periods examined, DEM treatment produced no significant effect on the tissue distribution of unchanged 4-ipomeanol. In both groups, the relative tissue concentrations of unmetabolized 4-ipomeanol were in the order blood > lung > liver. In control rats, the relative tissue concentrations of nonbound, solvent-extractable 4-ipomeanol metabolites, as well as the covalently bound 4-ipomeanol metabolites were in the order lung > liver > blood. The pulmonary levels of both the covalently 4-ipomeanol and solvent-extractable 4-ipomeanol metabolites were increased markedly by DEM treatment at all time periods examined. The total pool of urinary 4-ipomeanol metabolites was significantly decreased by DEM treatment, but the total amounts of excreted ipomeanol-4-glucuronide, the major metabolite of 4-ipomeanol in rats, were not significantly different in the control and DEM-treated rats. These data suggest that the increases in pulmonary covalent binding and toxicity of 4-ipomeanol produced by diethylmaleate treatment in rats are due to the depletion of pulmonary GSH by the DEM and not a major DEM-induced alteration in the distribution of the parent 4-ipomeanol.

These studies have been completed and the final manuscript is in press. (see Publication 10).

J. Studies of cytochrome P-450 monooxygenase activities with 4-ipomeanol in mouse skin (Statham and Boyd)

These studies are designed to identify cells in skin which metabolize chemicals to products which may bind to protein and possibly DNA and, thereby, may be important in the initiation of carcinogenesis process.

Skin slices from hairless mice were incubated with tritiated 4-ipomeanol. Autoradiography was used to identify cell components in skin which metabolize 4-ipomeanol and potentially other xenobiotics. The metabolite of cytochrome P-450-dependent monooxygenation of 4-ipomeanol in liver and lung binds covalently to cellular proteins at the site of its production. Since cytochrome P-450 catalyzes the monooxygenation of a large variety of substrates, including carcinogens, this reaction was used as a marker for cell-specific metabolism of xenobiotics in skin. These studies were carried out in collaboration with Dr. J. Fouts and R. Pohl (NIEHS).

Silver grains were concentrated over the differentiated epidermis, the hair follicles and the sebaceous glands of hairless mouse skin incubated with tritiated 4-ipomeanol. More grains were seen in skin from mice treated topically with B-naphthoflavone than in that from untreated mice. In contrast to the effects of piperonyl butoxide which completey inhibited 4-ipomeanol binding in liver and lung preparations of control liver and lung tissue groups, piperonyl butoxide (an inhibitor of cytochrome P-450-dependent monooxygenation) caused only a 50% reduction of silver grains in skin slices.

Further inhibitor studies will be done to determine if cooxygenation of 4-ipomeanol by prostaglandin synthetase or by some other mechanism is responsible for the high control metabolism of 4-ipomeanol in skin by isozymes of cytochrome P-450 which are not inhibited by piperonyl butoxide.

K. Role of phospholipid methyltransferases in control of membrane microviscosity (Statham)

The phospholipids are the major component of the lipids of biomembranes and are thought to provide the fluid matrix for protein movement and organization. Cytochrome P-450 is an integral protein in the microsomal membranes and is thought to be enclosed in a phospholipid matrix which extends from the cytoplasmic side to the luminal side of the endoplasmic reticulum. In chemical carcinogenesis, metabolic conversions of procarcinogens to their ultimate carcinogenic forms are mediated within the cell by the cytochrome P-450 monooxygenase enzyme systems located in the endoplasmic reticulum. To determine if the membrane methyltransferases influence the cytochrome P-450 MFO system, we have undertaken studies to: 1) develop a high-pressure liquid chromatographic technique to quantitate the individual phospholipid components, 2) characterize the liver membrane phospholipid methyltransferases and 3) investigate the selective changes in the specific phospholipid components after induction of liver cytochromes with 3-methylcholanthrene and phenobarbital. These studies are in collaboration with Dr. R. Sastry (Vanderbilt University) and Dr. J. Axelrod (LCS, NIMH).

The HPLC technique successfully devised was used for separation and characterization of three major phospholipids (phosphatidyl-N-methylethanolamine, phosphatidyl-N,N-dimethylethanolamine and phosphatidylcholine). The HPLC system consisted of a Waters Associates Liquid Chromatography System, a micro Porasil column (30 cm x 9.9 mm), and a mobile phase (1 ml/min) containing ethanolamine (0.2%), water (2.0%), methanol (20%) and chloroform (77.8%). 1-ml samples of the chloroform phase were evaporated to dryness under a stream of nitrogen and were reconstituted in 25 µl of the mobile phase for HPLC analysis. Sixty samples were collected during 12 min to obtain the HPLC radiochromotographic profile of the phospholipids.

The stepwise methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) occurred in the rat liver. Two methyltransferases were involved. The first methyltransferase converted PE to phosphatidyl-N-methylethanolamine (PME) and the second methyltransferase converted PME to PC. After incubation of rat liver microsomes with the methyl donor, three peaks corresponding to PME, dimethyl-PME (PMME), and PC were found and quantified. The first methyltransferase had low K_m (0.83 µM), pH optimum of 8, and was activated by Mg^{2+}. The second methyltransferase had a high K_m (~67 µM) and a pH optimum of 10. The proportion of the first methyltransferase in the microsomal membranes was increased by repeated washings in hypotonic medium containing EDTA. When the microsomal membranes were subjected to repeated mild sonication and centrifugation at 105,000g a fraction of the second methyltransferase was solubilized (i.e., appeared in the nonparticulate fraction). The solubilized enzyme utilized dipalmitoyl-PME and -PMME as substrates. Both enzymes were also present in mitochondrial and nuclear membranes with highest specific activities occurring in the microsomal membranes.

Phenobarbital and 3-methylcholanthrene increased cytochrome P-450 levels 2- to 3-fold. AT low SAM concentration, the proportion of PME among the total phospholipids formed increased significantly, and at a high SAM concentration, the proportion of PC among the total phospholipids formed decreased significantly in microsomes of treated rats. Treatment of rats with phenobarbital and 3-methylcholanthrene also decreased microviscosities of the microsomal membranes and liposomes which were prepared from phospholipids extracted from the microsomes. In synthetic liposomes containing PE, PME and PC, microviscosity decreased when the proportion of PME was increased or the proportion of PC was decreased. These results suggest that the membrane fluidity increases with phenobarbital and 3-methylcholanthrene treatment, and changes in phospholipid methyltransferases may contribute to the process of enzyme induction. Alteration in phospholipid methylation is possibly the primary cause of the decrease in membrane microviscosity after induction with 3-methylcholanthrene.

The inter-relationship between methylation of microsomal membrane phospholipids and membrane microviscosity changes after 3-methylcholanthrene and phenobarbital induction is under further investigation. The comparison of temporal sequences of these events will be of particular interest. It will be determined if the drug-induced changes on the methyltransferases precede the changes in P-450 content and associated drug metabolizing activities and are causally related or whether they simply accompany the changes in P-450 content.

Two manuscripts on this work have been published this year and another is in preparation (see Publications 12 - 13).

L. Development and/or application of microanalytical methodologies to basic and/or clinical studies of selected anticancer drugs (Boyd, Burka* and Statham)

The purpose of this project is to integrate with and provide analytical chemistry support for a variety of other LETM projects as well as for collaborative projects with other NCI laboratories. New analytical procedures will be developed when required, or existing methodologies will be modified or adapted if appropriate. Procedures may be developed and/or applied for basic laboratory research studies as well as for certain clinical studies in cancer patients. Analytical instrumentation within the LETM that may be employed in such studies include a GC-mass spectrometry facility (including an integral computer system), conventional gas chromatographs, HPLC systems, various spectrophotometric systems, radiochromatography systems, pyrolysis GC, and X-ray microanalysis. Dr. L. T. Burka* is being recruited as a Cancer Expert (to begin in FY 82) to provide essential expertise to support this project.

One paper from this project area was published during the present year (see Publication 14). The latter publication was the final report describing our clinical pharmacologic investigations with pentamethylmelamine, a study described previously in detail in FY 81 Annual Report (Z01 CM 06512-01 CP).

M. Synthesis of new candidate drugs and/or potential metabolites for laboratory and preclinical investigations (Boyd, Burka* and Statham)

This project is intended to support and coordinate with several other LETM projects in drug development and drug metabolism. Synthetic and/or purification procedures will be developed when necessary for compounds of special interest or relevance to LETM studies. Compounds of interest may include model compounds, drug candidates and/or their metabolites.

One area of current interest is the identification, through structure-activity in studies, of compounds which possess maximal potency and selectivity for metabolism and alkylation of pulmonary bronchiolar Clara cells. 4-Ipomeanol serves as a prototype, but it is possible that further candidates may be identified with even greater pulmonary selectivity and less toxicity to nontarget tissues. Such candidates will be evaluated for cytotoxic activity in a human lung tumor test panel (see Section E., Annual Report FY 82, Project No. Z01 CM 07126-01 LETM).

One paper, concerning a recently completed subproject has been published this year (see Publication 15). That report outlined our new procedures for synthesis of high specific activity ^{14}C-labeled and ^{3}H-labeled 4-ipomeanol. The ^{14}C-compound utilized ^{14}C propylene oxide as the source of the radiolabel, whereas, the ^{3}H-derivative was made with an exchange procedure utilizing $^{3}H_2O$. Such material is necessary for high-resolution EM autoradiography studies which we shall be pursuing in the coming year (see Section I., Annual Report FY 82, Project No. Z01 CM 07126-01 LETM).

N. Enzymatic mechanisms of activation (dephosphorylation) and nature of free-SH metabolite(s) of the radioprotective agent WR-2721, in hepatic, extrahepatic and tumor tissues; applications to design of improved congeners (Boyd, Burka* and staff)

WR-2721 (S-2-[3-aminopropylamino]-ethylphosphorothioic acid) originally was developed as a radioprotective agent. More recently it has also been found to protect against normal tissue damage from cytotoxic drugs. Its potential value in cancer chemotherapy lies in possible differential protective effects on normal versus tumor tissues. The compound currently is in clinical trials to evaluate this possibility.

The mechanism(s) whereby WR-2721 could exert such differential protective effects is unclear. It has been suggested, but not clearly established, that the drug penetrates tumors less readily than normal tissue. However, another potentially important factor that has not been considered in any detail is that certain tumors may be relatively deficient in enzymatic activity needed to dephosphorylate WR-2721. Dephosphorylation yields a free-SH metabolite(s) of WR-2721 which likely serves as the nucleophilic trapping reagent necessary for radioprotection or inhibition of tissue alkylation by cytotoxic drugs.

In this project a detailed characterization will be made of the enzymatic dephosphorylation activities for WR-2721 both in normal hepatic and extrahepatic tissues of laboratory animals and in tumor tissues. Tumors selected for study will be those for which in vivo models are available, or can be developed, to show differential tumor/normal tissue protection with WR-2721 against irradiation. Measurements of free-SH metabolite concentrations resulting in vivo in tumors and normal tissues of animals receiving WR-2721 will be performed.

It will also be of interest to examine in greater detail the nature of the free-SH metabolite(s) of WR-2721. It presently is unclear whether one or multiple metabolites are responsible for the total free-SH metabolite pool produced in vivo and what the relative protective effects of such metabolite(s) may be.

Finally, if correlations can be developed that emphasize the importance of enzymatic dephosphorylation in the differential tumor/normal tissue protective effects of WR-2721, consideration will be made for the design, synthesis and evaluation of new congeners or other derivatives that may take advantage of this principle to further enhance normal tissue protection and minimize the loss in tumor sensitivity to irradiation and/or drugs.

O. Investigations of mechanism of activation, and chemical nature of alkylating metabolite(s) of perilla ketone, a new, naturally-occurring lung-specific alkylating agent (Boyd, Burka* and staff)

Perilla ketone (PK) (1-[3-furyl]-4-methylpentanone) is a furan derivative that occurs naturally in the mint plant, Perilla frutescens. The mint plant occurs worldwide, and the presence of PK probably accounts for the severe lung injury in grazing animals that ingest the plant. Moreover, the human use of the mint plant or extracts thereof, particularly in oriental food and medicinal preparations, raises the question of the possible public health significance of PK.

PK is closely related in structure to 4-ipomeanol (1-[3-furyl]-4-hydroxy-pentanone) (IPO). IPO also is a naturally-occurring furan but it is from an entirely different source; it is a product produced in moldy sweet potatoes.

Because IPO is a highly selective pulmonary aklylating agent and cytotoxin, we were interested in the possibility that PK had similar properties. This interest arose both from the potential toxicological significance of PK as well as from our interest in exploring the possible antitumor activity of such compounds. As the initial phase of these studies we have examined the acute toxicity and the tissue covalent binding of PK in several laboratory animal species.

The 36 hr LD50 values for PK (adm. i.p.) in adult male mice (C57BL/6J), rats (SD) and hamsters (Golden Syrian) were (mg/kg) 6 ± 2, 18 ± 9, and 50 ± 15, respectively. Thus, the acute lethality of PK was comparable to IPO in rats, but PK was much more potant than IPO in mice (IPO LD50, 20 mg/kg) and hamsters (IPO LD50, 150 mg/kg). Also of interest was that PK produced only pulmonary necrosis in all three species. In addition to pulmonary necrosis, IPO can produce renal necrosis in male mice and hepatic necrosis in hamsters. However, these nonpulmonary lesions did not occur with PK. Therefore, PK may actually be more selectively lung toxic than IPO.

Radiolabeled PK was synthesized by catalytic reduction of isoegomaketone (EMK) with tritium gas. EMK was prepared from a published method.

In vivo studies of i.p. administered ^{3}H-PK indicated that large amounts of radiolabeled material were covalently bound (1-2 nmol/mg protein), with high selectivity to the lungs of mice, rats and hamsters. The preferential pulmonary alkylation occurred over a wide range of doses studied in each species. Maximal levels of pulmonary alkylation occurred quickly (within 1-2 hr) and declined only very slowly over the following 24 hr.

Pretreatment of rats with piperonyl butoxide (a cytochrome P-450 monooxygenase inhibitor) markedly decreased the hepatic and pulmonary covalent binding of PK, and likewise markedly decreased the lethal toxicity of PK. On the other hand, treatment with diethylmaleate (which depletes tissues of glutathione) sharply increased the tissue covalent binding and toxicity of PK. Thus, the effects of these treatments on PK toxicity supported the view that the lethal toxicity of the compound was mediated by highly reactive, electrophilic metabolite(s) and not the parent PK itself. Moreover, treatments with either phenobarbital or 3-methylcholanthrene significantly enhanced the hepatic covalent binding of PK but at the same time decreased the pulmonary covalent binding and the acute lethality of PK. These results indicated that the reactive metabolite(s) of PK formed in the liver likely did not escape that tissue; therefore the covalent binding of PK to lung was due to metabolic activation of the compound in situ in the lung itself.

Further studies are continuing to characterize the in vitro metabolic activation of PK in microsomes and reconstituted P-450 systems from lung and liver as well as in isolated lung cell populations. Also to be investigated is the nature of the activated metabolite(s) of PK, which will be explored using high-resolution NMR and mass spectrometry of conjugates formed from trapping activated PK in

vitro with electrophilic reagents such as GSH, cysteine and other thiols. In vivo metabolite profiles will also be explored with a view toward elucidating the likely structure(s) of active PK metabolites.

One manuscript from this work is in press (see Publication 11) and another is near completion.

*Dr. L. T. Burka, who is being recruited as a Cancer Expert to begin in FY 82, will serve as co-investigator on the projects indicated.

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ANNUAL REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 - September 30, 1982

The Laboratory of Medicinal Chemistry and Biology is an organizational entity resulting from the consolidation in 1975 of previously established sections from within the Drug Development and Drug Evaluation Branches of the Drug Research and Development Program, DCT; the Laboratory of Chemical Pharmacology, Experimental Therapeutics Program, DCT; and the Laboratory of Biochemistry, DCBD. The aim in establishing the new Laboratory was to bring about a closer relationship between medicinal chemists involved in the design and synthesis of new antitumor agents, and biologists involved in the elucidation of their biochemical pharmacology and in their clinical evaluation. It was felt in proposing this new organizational structure that knowledge of the molecular biology and etiology of cancer is approaching the level at which the biologist can now make useful early contributions to the rational design of new antitumor agents, rather than being restricted almost entirely to biochemical, pharmacological and clinical studies on agents whose antineoplastic activity has already been established on an empirical basis. While it is recognized that it would be premature and over-optimistic to propose that empirically-based screening approaches can in the foreseeable future be abandoned in favor of specific, rational antitumor drug design, the time now appears to have arrived when a mixture of empirical and rational approaches is warranted. Examples of drug classes in which opportunities for specific, target-directed design are now possible are agents affecting structure and function of tumor cell surface glycoproteins; inhibitors of viral oncogenesis; agents with specific or unique DNA-binding properties; transition-state inhibitors for key enzyme sites; and agents with specific tissue-localizing properties. In these and a number of other rapidly expanding research areas, the state of the art appears sufficiently advanced that the biologist with specific expertise can make contributions of value to the medicinal chemist in the design of new antitumor agents and in the modification of existing agents.

Conversely, from the point of view of the medicinal chemist, a closer interrelationship with the molecular biologist and the clinician offers the opportunity of obtaining specific biological assay data with the minimum of delay, in order to guide the course of further synthesis. In addition to providing routine antitumor screening, the biologist can design assay methods tailored to the specific target site under consideration. Assays for parameters such as inhibition of viral replication, immunostimulation, enzyme inhibition or tumor cell membrane binding can be provided where relevant to the activity under consideration. Thus, the juxtaposition within a single laboratory of medicinal chemists and biologists from a variety of different disciplines offers the prospect of accelerating drug development, and of supplementing the traditional empirical approaches which have yielded most of our clinically successful antitumor drugs up to the present time with specific target-site directed drug design programs.

The major organizational entities within the original Laboratory of Medicinal Chemistry and Biology, in addition to the Drug Design and Chemistry Section, were the Molecular Biology and Methods Development Section, with the capacity to assess the effects of new agents on cell growth and cell cycle progression, and including a membrane biology group, with a specific interest in the role of tumor cell membrane alterations in drug resistance, and an Applied Pharmacology Section which is concerned both with the mode of action of new agents at the molecular level and with disposition and metabolic studies of such agents in experimental animals and at the Phase I level. The Office of the Chief, LMCB, in addition to assuming administrative responsibilities for the Laboratory as a whole, also maintains an active research interest in antitumor drug disposition studies, in the mode of action of new agents, and in the identification of potential sites for pharmacological attack. On March 1, 1980, the Laboratory underwent a major expansion, with the addition of two additional organizational components, i.e., the Biochemistry and Drug Interactions Sections. The Biochemistry Section is concerned with the mechanisms by which antitumor agents exert their inhibitory effects on pyrimidine and purine biosynthetic pathways; while the Drug Interactions Section studies the effects on the action of antitumor drugs of factors such as metabolism, absorption, tissue distribution, protein binding and excretion. Throughout the Laboratory, particular stress is given to studies of agents originating from within the LMCB drug synthesis program.

As indicated above, this Laboratory has an interest both in new antitumor agents originating from the scientific community as a whole, and also in agents originating within our own Drug Design and Chemistry Section. Of the latter group, AZQ (NSC-182986) continued Phase II trials at several institutions. This compound appeared to have some effectiveness against human brain tumors. Our research involvement has continued with this drug through collaboration in the clinical trial at NIH and the BCRP. Spirohydantoin mustard (NSC 172112) is scheduled to complete toxicology this year prior to a Phase I clinical study. Toxicology was completed on dihydro-5-azacytidine (NSC 264880) during the current fiscal year and an IND has been opened. A new compound from this Section, ara-AC (NSC 281272), achieved Decision Network 2A status this year. Once formulation research has been completed, this compound, which is active against all three xenograft tumor panel models, will begin toxicological evaluation prior to clinical trial.

A detailed study of the plasma and CSF pharmacokinetics of AZQ in conjunction with BCRP Phase I/II clinical trials was completed using the HPLC assay described in last year's report. Comparison of plasma and CSF areas under the entire concentration-time curves indicated a high degree of drug availability to the CSF. Indeed, when the extensive plasma protein binding (79 ± 1% at 1 μg/ml) was taken into account, free plasma AZQ was seen to be completely available to the CSF. The HPLC assay for AZQ was further refined to increase its efficiency and sensitivity. The improved assay was applied to study both infusion and post-infusion pharmacokinetics in high grade glioma patients in the NCI-MB Phase II trial. No evidence was found for a third elimination phase with an extended half-life after analysis of long post-infusion time samples in this or the BCRP study. Intact AZQ could also be measured in both brain tumor tissue and tumor cyst fluid of patients treated pre-operatively with the drug.

Progress continues to be made in the preparation of cytidine deaminase inhibitors for potential use in combination with antitumor agents such as araC and 5-azacytidine, whose clinical utility is limited by enzyme-catalyzed deamination in vivo. Several compounds of the diazepinone riboside series have been prepared with K_i values in the range 1-5 x 10^{-8}M, i.e., approximately one order of magnitude more active than the reference compound tetrahydrouridine, the most potent cytidine deaminase inhibitor described prior to the present studies. The most active member of this series, NSC-322096, when combined with araC, produced increases in T/C values against murine L1210 leukemia comparable to those obtained with three times the dose of araC alone. The dose of the inhibitor was one-tenth that of tetrahydrouridine required to produce a comparable increase in T/C values. Biological evaluation of these compounds is continuing.

As described elsewhere in this report, recent work in the Biochemistry Section, LMCB, on the mode of action of the antitumor agent 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC-286193) has established that the active form of the latter compound is the fraudulent NAD analogue adenine-D-ribose-phosphate-phosphate-D-ribose-thiazole-4-carboxamide (TAD), a potent inhibitor of the enzyme IMP dehydrogenase, a key step in the purine biosynthetic pathway. The Drug Design and Chemistry Section was instrumental in the determination of the structure of biosynthetic TAD through the use of nuclear magnetic resonance and mass spectrometry. A successful chemical synthesis of TAD has now been developed, and the product shown to be identical to the biosynthetic compound. The compound is an extremely active inhibitor of IMP dehydrogenase (K_i ca. 0.05 μM), and also exhibits significant cytotoxicity vs. P388 cells in vitro. It has not yet been established whether the latter effect is due to the parent compound, or to hydrolysis during assay with release of the active thiazole-4-carboxamide moiety. Future synthetic plans include replacing the labile phosphodiester linkage by hydrolytically stable phosphonate groups.

The synthesis of 2- and 4-pyridones and pyridone nucleosides has continued. The pyridone aglycons possess significant P388 activity, although the mode of action of these compounds is still unknown. In view of the possibility that these agents may be acting at the nucleoside or nucleotide level, the corresponding ribo and arabinofuranosides of the active members of the series were prepared. Several of the ribose analogs were more active than the parent compounds, when tested against P388 in vitro, while the corresponding arabinoside analogs were virtually lacking in activity. These compounds have now been submitted for in vivo testing.

The development of new analytical techniques for agents of interest to LMCB has continued. The objectives of these studies are to establish the structure and purity of new antitumor agents, and also to elucidate reaction mechanisms and to develop assays for the quantitation of these agents in physiological samples. Mass spectrometry, gas-liquid chromatography and HPLC techniques are used. In terms of assay development, compounds of current interest are dihydro-5-azacytidine (NSC-264880), 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC-286193) and 5-azacytosine arabinoside (NSC-281272). The assay for AZQ previously developed has been modified to improve its sensitivity. Extensive studies have been carried out on the identification of the active metabolite of 2-β-D-ribofuranosylthiazole-4-carboxamide.

At the molecular level, studies have continued in the Applied Pharmacology Section on the effects of nucleoside analogues on the synthesis and methylation of RNA and DNA and on the translational activity of mRNA. Compounds studied over the past year include 5-fluorouridine (5-FUR), 5-azacytidine (5-AC), dihydro-5-azacytidine (DHAC) and sangivamycin. 5-AC and DHAC-modified mRNA were isolated from Ehrlich ascites cells and assessed in vitro using a cell-free translation system. The coding capacity of drug-modified mRNA was not affected, but inhibition of rRNA synthesis was found to follow closely the relative antitumor potencies of these two agents. Studies of 5-FU and 5-FUR in a human colon carcinoma (HT-29) cell line refractory to nitrosoureas indicated that the lethal effects of these agents corresponded closely to their levels of incorporation into nuclear RNA. The concentration and time-dependence of these effects have practical relevance in the design of synergistic drug combinations employing 5-FU or 5-FUR. Ongoing studies with sangivamycin, a pyrrolopyrimidine antibiotic, have confirmed its time-dependent lethality in HT-29 cells, in association with its marked incorporation into mRNA. In view of the unusual activity of sangivamycin in the human colon carcinoma line, a collaborative Phase I/II study of this agent in colorectal cancer has been initiated with the Medical College of Wisconsin (Dr. Paul Ritch), with this laboratory providing bioassay support as required.

A new project initiated this year has been a thorough biochemical and cytokinetic study of human fibroblast interferon (IFNβ) in several human tumor cell lines. We have determined whether the IFNβ mediated induction of (2',5') oligo(A) synthetase, an enzyme which is unique to IFN-treated cells and is associated with antiviral activity, is related to the growth inhibitory properties of IFNβ and of double-stranded RNA (poly(I·C); $I_n \cdot C_n$). A thorough study of the time and concentration dependence of (2',5')oligo(A) synthetase induction in association with the lethal and growth inhibitory properties of IFNβ and $I_n \cdot C_n$ used singly and in combination is underway. It is also planned to measure the levels of (2',5')oligo(A) synthetase in patients with CLL being treated with IFNβ, as a measure of their responsiveness to treatment.

Studies have also been initiated on two new anthracyclines recently obtained from the Stanford Research Institute (Dr. E. Acton). These two agents, 5-iminodaunorubicin and 3'-deamino-3'-morpholinyldaunorubicin, both possess a low capacity to spontaneously generate free radical intermediates and show a low level of myelotoxicity and cardiotoxicity. Their lethal effects, pharmacokinetics, inhibitory effects on RNA and DNA, and DNA strand breakage activities have been measured in HT-29 cells. The unusual properties of these two agents make them attractive candidates for further development. Studies have also continued on the lymphocytotoxic properties of the adenosine deaminase inhibitor 2'-deoxycoformycin (2'-dCF), utilizing the mitogen-stimulated mouse splenocyte system. Changes in ribonucleoside mono- and triphosphates and in the synthesis and methylation of nucleic acids consequent on 2'-dCF administration have been characterized. We are presently extending these studies to leukemic lymphocytes obtained from CLL patients receiving the drug on a single-dose weekly schedule in Phase II trials.

In the Biochemistry Section, research emphasis during the past year has been on the mode of action of the C-nucleoside riboxamide or tiazofurin (2 β-D-ribofuranosylthiazole-4-carboxamide; NSC-286193). The latter compound was cytotoxic

toward P388 cells at micromolar levels, but a number of simple substitutions of both the ring and sugar moieties nullified cytotoxicity. Cytofluorimetric analysis revealed that the drug arrests cells in the "S phase" of the cell cycle. At antiproliferative concentrations, the agent inhibited the synthesis both of RNA and DNA. The macromolecular incorporation of preformed pyrimidines, including thymidine, was inhibited by the drug, but among the purines, this effect extended only to members of the adenine family, and in fact, the utilization of guanine and its congeners was reproducibly stimulated.

When an examination was made of the ability of a comprehensive series of preformed purines and pyrimidines to overcome the inhibition of thymidine incorporation provoked by exposure to the thiazole nucleoside, the guanines were notably effective, but xanthosine also was shown to be an active antidote. Confirmation that the drug was producing a state of guanine deprivation was provided by HPLC analysis of acid-soluble extracts: a time-dependent fall in the concentration of GMP and GTP ensued upon exposure to the drug; on the other hand, IMP concentrations increased ~15 fold. Further studies revealed the site of inhibition to be IMP dehydrogenase, rather than GMP synthetase.

In vivo, administration of riboxamide to BDF_1 mice bearing subcutaneous P388 leukemia provokes a sharp depression in the concentration of intratumoral guanine nucleotides and a correspondingly large expansion of the IMP pools. Measurements of IMP dehydrogenase in the tumors of treated mice reveals that this enzyme is inhibited in a dose-responsive way with ~50% inhibition engendered by the administration of the drug at a dose of 25 mg/kg and >90% inhibition by all doses >100 mg/kg; this range of doses (given daily for 5 days) is therapeutically active versus this transplanted leukemia. The inhibition of enzyme activity seen after a dose of 250 mg/kg reaches a maximum 120 min after treatment and has subsided substantially 8 hr after dosing; by 24 hr, enzyme activity is fully restored. This relatively short duration of action when the drug is given on a daily basis would appear to warrant the exploration of more frequent schedules of administration at both the experimental and the clinical level.

Kinetic studies revealed that neither the parent compound nor its 5'-monophosphate was sufficiently active as an IMP-dehydrogenase inhibitor to account for the results observed. The anabolite responsible for the inhibitory effect has now been isolated from homogenates of the tumor by dialysis and ion-exchange HPLC, and its presence monitored by enzyme inhibition assay. Chromatographically, the inhibitory principle exhibits a moderately strong net negative charge at pH 3, and elutes in the general vicinity of the nucleoside-5'-diphosphates; its absorption maximum in aqueous solution (pH 7) lies at 252 nanometers. Exposure of the molecule to snake-venom phosphodiesterase or nucleotide pyrophosphatase destroyed its inhibitory potency, whereas other phosphoesterases were either less effective or inert. Since these results suggested that the anabolite might be a dinucleotide with a phosphodiester linkage of the kind found in NAD, attempts were made to synthesize such an NAD analog from the 5'-monophosphate of thiazole nucleoside and ATP-Mg^{++}, using a purified preparation of NAD pyrophosphorylase; modest yields were obtained of a compound with chromatographic, spectrophotometric and enzyme-inhibitory properties identical to those of the material isolated from P388 tumor nodules. This enzyme-synthesized material was radioactive when [^{3}H]ATP was used as co-substrate, and yielded

both AMP and thiazole nucleoside-5'-monophosphate on treatment with phosphodiesterase. It resisted attack by NAD glycohydrolase. Mass spectral analysis and nuclear magnetic resonance studies were compatible with a dinucleotide structure in which the nicotinamide of NAD has been replaced by thiazole-4-carboxamide, i.e., adenine-D-ribose-phosphate-phosphate-D-ribose-thiazole-4-carboxamide. Versus IMP dehydrogenase, the dinucleotide exhibited a K_i of ca. 0.05 μM and was competitive with NAD as the variable substrate. As described elsewhere in this report, the dinucleotide anabolite (TAD) has now been prepared in this Laboratory by chemical synthesis, and shown to be identical in its properties to the biosynthetic material.

In studies with a P388 line rendered resistant to riboxamide, it was found that the resistant variant forms no TAD *in vitro*, and only one-tenth as much TAD *in vivo*, as its riboxamide-sensitive counterpart. In addition, the specific activity of the enzyme likely to be responsible for the synthesis of TAD, NAD pyrophosphorylase, is 6 to 10-fold lower in the resistant P388 line. Extrapolation of some of these findings, in particular, measurements of TAD formation and of IMP dehydrogenase and NAD pyrophosphorylase, to human clinical samples is planned when Phase I trials with this agent are initiated in COP late in 1982.

Ongoing studies with riboxamide include the evaluation of its pharmacokinetic behavior in rat, mouse and monkey (the latter in collaboration with POB); the exploration of long-term infusion dose schedules; and the further exploration of the pharmacological properties of the riboxamide anabolite TAD.

In the Drug Interactions Section, studies have continued on the molecular toxicology of the clinically important antitumor agents bleomycin and adriamycin. Ultrastructural examination of adriamycin-damaged hearts reveals, among other alterations, intramitochondrial swelling, condensation, and frank degeneration including dissolution of cristae and the formation of myelin figures. Since enzymatic activation of adriamycin to a free radical and subsequent enhanced reactive oxygen generation may play a role in adriamycin cardiomyopathy, we investigated the possibility that the morphologic changes in mitochondria could result from adriamycin-stimulated lipid peroxidation. Using purified rat liver mitochondria as a model biological system, we found that NADPH-dependent lipid peroxidation, measured by the 2-thiobarbituric acid method, was stimulated as much as 4-fold by adriamycin. Marker enzyme analysis indicated that mitochondria were substantially free of contaminating microsomes. NADPH substituted for NADH as electron donor to mitochondrial NADH-dehydrogenase and supported adriamycin-stimulated mitochondrial lipid peroxidation; however, when reducing equivalents were provided by ascorbate, adriamycin potently inhibited peroxidation. Superoxide dismutase, catalase, 1,3-dimethylurea, reduced glutathione, EDTA, and α-tocopherol inhibited adriamycin-augmented mitochondrial lipid peroxidation, indicating that multiple forms of reactive oxygen and possibly trace amounts of endogenous iron participated in the peroxidation reactions. Thus, it is likely that the *in vivo* effects of adriamycin on mitochondrial morphology and function are mediated by adriamycin-enhanced reactive oxygen-mediated lipid peroxidation.

In the case of bleomycin, this agent must undergo activation in order to exert its effects on nucleic acids. Since the mixed-function oxidase system

represents an efficient biological mechanism for the activation of bleomycin, the ability of microsomes isolated from the Lewis lung carcinoma and the B16 melanoma to catalyze bleomycin-mediated DNA deoxyribose cleavage was examined. Microsomes from both tumors were able to catalyze this reaction although those from the B16 melanoma exhibited three times the activity of microsomes isolated from the Lewis lung carcinoma. The addition of ascorbic acid, Fe^{3+} or the redox cycling chemical, paraquat, significantly enhanced this reaction whereas EDTA, superoxide dismutase and glutathione inhibited the cleavage of deoxyribose by bleomycin. In addition to bleomycin A_2, bleomycin PYP (NSC 276381), bleomycin BAPP (NSC 294979), bleomycin PEP (NSC 276382) and tallysomycin A (NSC 279496) were activated to a DNA damaging species by microsomes from the B16 melanoma and the Lewis lung carcinoma.

To ascertain if the inhibition of microsomal lipid peroxidation by bleomycin can be attributed to its sequestering of adventitious iron, the effects of bleomycin A_2 and bleomycin A_2 complexed with copper (1:1) (NSC 260620) were compared. Bleomycin A_2 significantly inhibited rat liver microsomal lipid peroxidation in a concentration-dependent fashion: 5 μM bleomycin inhibited lipid peroxidation by 35%, while 25 μM bleomycin was nearly 100% inhibitory. In contrast, the bleomycin A_2-Cu^{2+} complex had no significant effect on microsomal lipid peroxidation at these low drug concentrations. Thus, the results of these experiments demonstrate that bleomycin inhibits microsomal lipid peroxidation by sequestering the adventitious iron necessary to catalyze the initiation of microsomal lipid peroxidation. However, this inhibition by bleomycin is only transient, since as a result of the channeling of electron flow from the microsomal mixed-function oxidase system to the bleomycin-iron-oxygen complex, bleomycin undergoes an activation-inactivation process resulting in a metabolite which apparently can no longer bind iron. Thus, the iron initially sequestered by bleomycin is available to participate in the generation of reactive oxygen species involved in the initiation of microsomal lipid peroxidation.

Within the Office of the Chief, studies have continued on the interactions of GDP and GTP analogs at the exchangeable nucleotide binding site of tubulin by examining analog effects on tubulin polymerization. Studies with ribose-modified analogs in glutamate-induced polymerization were extended to arabinosylGDP (araGDP) and arabinosylGTP (araGTP), as well as to two sets of open ribose ring analogs, acycloGDP and acycloGTP (phosphorylated derivatives of acyclovir [9-(2-hydroxyethoxymethyl)guanine] synthesized in our laboratory) and periodate-oxidized and borohydride-reduced derivatives of GDP (ox-redGDP) and GTP (ox-redGTP). These compounds extended previous observations: araGTP was more active than GTP in supporting polymerization, acycloGTP was moderately less active and ox-red GTP substantially less active than GTP, and all three GDP analogs were poor inhibitors of polymerization compared to GDP. These observations were consistent with our earlier suggestions that GDP and GTP bind to tubulin by different mechanisms, and that the ribose moiety of GTP is oriented toward the interior of the exchangeable site.

A heterogeneous group of proteins obtained from microtubules, which have been termed microtubule-associated proteins (MAPs), induce tubulin to polymerize into microtubules in 0.1 M 2-(N-morpholino)ethanesulfonate (Mes), a reaction condition in which tubulin will not otherwise polymerize. We have found that

heat-treatment of isolated MAPs, which does not affect their ability to promote polymerization, destroys all contaminating kinase and phosphatase activity. The ribose-modified GDP and GTP analogs were therefore re-examined for their ability to affect microtubule formation with MAPs in 0.1 M Mes.

With one exception, the GTP analogs displayed the same relative ability to support tubulin polymerization with MAPs as with glutamate: deoxyGTP analogs and araGTP were more active than GTP, while ribose-substituted and open ribose ring analogs were less active. The exception was 2'-O-methylguanosine 5'-triphosphate (2'OMeGTP). With glutamate, 2'OMeGTP was significantly less active than both GTP and acycloGTP in supporting polymerization, but with MAPs, it was equivalent to GTP. One analog, 2',3'-dideoxyguanosine 5'-triphosphate (ddGTP) was unique in also supporting polymerization without MAPs in 0.1 M Mes. Microtubules were formed in all cases except three: 1 & 2) with 2'OMeGTP and ox-redGTP bizarre, short tubules three times the diameter of microtubules were formed. These structures even appeared to lack protofilaments, but MAPs were required for their formation; and 3) without MAPs, ddGTP supported formation of a mixture of microtubules and open sheets.

Moreover, one GDP analog not only failed to inhibit polymerization, but was itself able to support polymerization both with MAPs in 0.1 M Mes (microtubules formed) and with glutamate (sheets formed). This analog, 2',3'-dideoxyguanosine 5'-diphosphate (ddGDP) was not hydrolyzed in the course of polymerization but was incorporated into the polymer. This observation is of potential importance since it unambiguously demonstrates that GTP hydrolysis is not essential for polymerization and that a molecule smaller than a nucleoside triphosphate can induce the reaction. The exchangeable site may therefore indeed be subject to pharmacological manipulation by appropriately designed drugs.

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

Because of a report that the methotrexate analog, 5-amino-1,2-dihydro-3-[(N-methylanilino)methyl]pyrido[3,4-b]pyrazin-7-ylcarbamate (NSC-181928) had anti-tumor activity and caused mitotic arrest, we examined the drug for potential interactions with tubulin. We established that NSC-181928 was an antitubulin agent. Structurally, the drug most closely resembles the benzimidazole carbamates, such as nocodazole (an established antitubulin agent), and like them, inhibited colchicine binding to tubulin and tubulin polymerization. Like nocodazole and colchicine, but even more potently, NSC-181928 stimulates tubulin-dependent GTP hydrolysis. If analogs of this drug can be obtained, structure-function studies will be performed in an effort to obtain an agent which interacts with both tubulin and dihydrofolate reductase.

Efforts continued to perfect the separation of the α and β subunits of tubulin by hydrophobic chromatography, as well as to achieve this separation by other methods. Our future plans include refinement of the separation of the tubulin subunits; structural analysis of the subunits; and preparation of antibodies, including monoclonal antibodies, to the subunits, to assist in mapping of functional domains. We also plan to undertake reconstitution of activity from the subunits, and we anticipate that the study we have previously made of tubulin stabilization will benefit our efforts. When conditions for reconstitution are discovered, we plan to undertake an analysis of the structural requirements for the nonexchangeable nucleotide. This may reveal information about its function and permit development of antitubulin agents.

Efforts have also continued within the Office of the Chief to elucidate the mode(s) of resistance to the clinically important antitumor agent melphalan (L-PAM). The failure to observe a decreased intracellular accumulation or enhanced exodus of L-PAM in L-PAM resistant L1210 tumor cells prompted investigation to determine whether intracellular metabolism of L-PAM may contribute to resistance. The most likely possibility was considered to be conversion of L-PAM to its non-cytotoxic derivative 4-[bis(2-hydroxyethyl)amino]-L-phenylalanine, hereafter referred to as dihydroxy L-PAM. L1210/L-PAM$_1$ tumor cells dechlorinate 2-2.5 times more L-PAM than do sensitive tumor cells. Such dechlorination is accompanied by less intracellular L-PAM in resistant cells and less radioactivity, part of which may be drug bound to protein and nucleic acid, at the origin of the tlc plate. Identical intracellular concentrations of dihydroxy L-PAM were found in L1210 and L1210/L-PAM$_1$ 30 minutes following exposure of cells to labeled L-PAM. The amount of dihydroxy L-PAM in L-PAM resistant cells increased with time and reached a maximum after 3 hours of drug exposure. In contrast, the amount of dihydroxy L-PAM found in L-PAM sensitive cells decreased with time and, after 3 hours of L-PAM exposure, sensitive cells contained 2-fold less dihydroxy L-PAM than did L-PAM resistant cells. Although the total intracellular radioactivity was nearly identical in both sensitive and resistant tumor cells during the 5 hour duration of L-PAM exposure, L-PAM sensitive cells had higher concentrations of both L-PAM and origin associated radioactivity, especially during the first three hours of drug exposure. Decreases in intracellular L-PAM occurred with time in both sensitive and resistant tumor cells although the rate was slightly higher in L-PAM sensitive cells. This decrease in intracellular L-PAM was accompanied by a parallel increase in the amount of label associated with the origin in L-PAM sensitive cells.

The conversion of L-PAM to dihydroxy L-PAM was completely inhibited by a brief 5 minute treatment of tumor cells with the sulfhydryl reagent N-ethyl maleimide prior to L-PAM exposure. A positive correlation exists between the intracellular concentration of glutathione and the sensitivity of murine tumor cells to L-PAM and increases in the glutathione content of these cells are accompanied by linear increases in the LD_{37} values of L-PAM. A positive correlation also exists between the intracellular concentration of glutathione and dihydroxy L-PAM. The latter relationship indicates that increases in intracellular glutathione are accompanied by increased cellular content of dechlorinated L-PAM.

The positive correlation between the sensitivity of murine L1210 leukemia cells to L-PAM and the intracellular content of glutathione prompted studies to determine whether the latter could be reduced. Incubation of cells in growth medium with a reduced concentration of L-cystine resulted in a rapid decrease in the intracellular content of glutathione disulfide in both the L-PAM sensitive tumor and in the resistant tumor, L1210/L-PAM$_1$. After 6 hours of incubation the intracellular concentrations are equivalent. The cellular content of reduced glutathione also decreased, and after 18 hours incubation in medium with a reduced concentration of L-cystine, both the L-PAM sensitive tumor and L1210/L-PAM$_1$ have identical intracellular content of reduced glutathione. Attempts to reduce the cellular glutathione content by incubation of cells in medium with a reduced concentration of glycine or L-glutamic acid were not as successful.

L-PAM is equitoxic to L-PAM resistant cells incubated in medium with a reduced concentration of L-cystine for 24 hours to reduce the intracellular glutathione content and L1210 cells grown in medium with L-cystine. These results indicate that an L-PAM resistant tumor cell can be completely sensitized to L-PAM by reducing the intracellular concentration of glutathione.

The preceding outline summarizes the objectives of the Laboratory of Medicinal Chemistry and Biology, and describes some of the research carried out within the Laboratory during the year. The bibliography for the Laboratory as a whole is listed below, followed by the individual Section and Project Reports which describe this research in greater detail.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07102-07 LMCB

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Tubulin Structure and Microtubule Formation as Sites for Pharmacologic Attack

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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LAB/BRANCH
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The rational development of new antineoplastic agents directed against tubulin, a protein critical for cell division, requires greater understanding of the interactions between the polypeptide subunits of tubulin and its two tightly bound guanine nucleotides. Interactions of ribose-modified GDP and GTP analogs with tubulin were examined in both microtubule-associated protein (MAP) dependent and independent systems. These studies continue to indicate that GDP and GTP interact with tubulin by different mechanisms. The analogs 2',3'-dideoxyguanosine 5'-di- and triphosphate (ddGDP and ddGTP) in particular had unique properties: ddGTP supported polymerization without MAPs under conditions in which GTP and all other analogs were inert; and ddGDP, but no other diphosphate, supported vigorous microtubule formation in a reaction requiring MAPs. A clinically useful assay for taxol was developed, and preliminary pharmacokinetics were performed. NSC-181928, a methotrexate analog with antimitotic activity, was demonstrated to be an effective antitubulin agent. Studies on the ADP-ribosylation of tubulin by cholera toxin were initiated. Efforts to separate the α and β subunits of tubulin continued.

PHS-6040
(Rev. 2-81)

Objectives:

Microtubules are reversibly formed protein structures present in all eucaryotic cells. One of their most important functions is participation in cell division, as they form the framework of the mitotic spindle. Microtubule structure and function are sensitive to a number of antineoplastic drugs, including colchicine, the clinically established vinca alkaloids, and taxol, a new agent which should enter clinical trials by the end of 1982. It is the goal of this project to assist in the development of effective new anticancer agents interfering with microtubule function.

The major constituent of microtubules is an acidic protein known as tubulin, which consists of two different polypeptide chains. Tubulin tightly binds two mols of GTP per mol of protein. Although distinct, the tubulin subunits are highly similar in molecular weight, amino acid composition, and isoelectric point and have never been separated preparatively unless chemically modified.

One mol of GTP is nonexchangeable with free GTP, cannot be removed from tubulin without denaturing the protein, and is unaltered in microtubule assembly and disassembly. Its function is entirely unknown. The other mol of GTP is freely exchangeable with unbound GTP or GDP and can be removed from tubulin by treatment with charcoal or alkaline phosphatase. This exchangeable GTP is hydrolyzed in microtubule assembly.

The formation of the tubulin dimer from its subunits is an obvious potential locus for the action of antineoplastic agents. The rational development of such drugs would be greatly aided by a greater understanding of the interactions between the polypeptide chains of tubulin and the two bound GTP molecules. It is therefore a major goal of this project to develop a method of separating the two subunits of tubulin and then to reconstitute active protein from the separated subunits and guanine nucleotides.

The intimate involvement of GTP in the structure of tubulin and in the formation of microtubules suggests that guanine and guanosine analogs may interfere with microtubule function. A second major goal of this project is to explore this possibility.

Major Findings and Proposed Course:

1. Within the Office of the Chief, studies have continued on the interactions of GDP and GTP analogs at the exchangeable nucleotide binding site of tubulin by examining analog effects on tubulin polymerization. Studies with ribose-modified analogs in glutamate-induced polymerization were extended to arabinosylGDP (araGDP) and arabinosylGTP (araGTP), as well as to two sets of open ribose ring analogs, acycloGTP and acycloGDP (phosphorylated derivatives of acyclovir [9-(2-hydroxyethoxymethyl)guanine] synthesized in our laboratory) and periodate-oxidized and borohydride-reduced derivatives of GDP (ox-redGDP) and GTP (ox-redGTP). These compounds extended previous observations: araGTP was more active than GTP in supporting polymerization, acycloGTP was moderately less active and ox-redGTP substantially less active than GTP, and all three GDP analogs were poor inhibitors of polymerization compared to GDP. These observations were consistent with our earlier suggestions that GDP and GTP

bind to tubulin by different mechanisms, and that the ribose moiety of GTP is oriented toward the interior of the exchangeable site.

Nucleotide analog studies require protein preparations free of contaminating kinases and phosphatases. Our tubulin is free of these activities. While glutamate-induced polymerization of purified tubulin does allow direct evaluation of tubulin-nucleotide interactions and polymerization occurs at low nucleotide and tubulin concentrations, a significant limitation of this model system is that the polymer consists of sheets of parallel protofilaments rather than microtubules.

A heterogeneous group of proteins obtained from microtubules, which have been termed microtubule-associated proteins (MAPs), induce tubulin to polymerize into microtubules in 0.1 M 2-(N-morpholino)ethanesulfonate (Mes), a reaction condition in which tubulin will not otherwise polymerize. We have found that heat-treatment of isolated MAPs, which does not affect their ability to promote polymerization, destroys all contaminating kinase and phosphatase activity. The ribose-modified GDP and GTP analogs were therefore re-examined for their ability to affect microtubule formation with MAPs in 0.1 M Mes.

With one exception, the GTP analogs displayed the same relative ability to support tubulin polymerization with MAPs as with glutamate: deoxyGTP analogs and araGTP were more active than GTP, while ribose-substituted and open ribose ring analogs were less active. The exception was 2'-O-methylguanosine 5'-triphosphate (2'OMeGTP). With glutatmate, 2'OMeGTP was significantly less active than both GTP and acycloGTP in supporting polymerization, but with MAPs, it was equivalent to GTP. One analog, 2',3'-dideoxyguanosine 5'-triphosphate (ddGTP) was unique in also supporting polymerization without MAPs in 0.1 M Mes. Microtubules were formed in all cases except three: 1 & 2) with 2'OMeGTP and ox-redGTP bizarre, short tubules three times the diameter of microtubules were formed. These structures even appeared to lack protofilaments, but MAPs were required for their formation; and 3) without MAPs, ddGTP supported formation of a mixture of microtubules and open sheets.

The MAP-dependent GTP-supported reaction was significantly less sensitive to GDP inhibition than was the glutamate-induced reaction. Nevertheless, GDP was still more effective than any analog in inhibiting the reaction. MAPs, therefore, appear to have significant effects in modulating tubulin-nucleotide interactions, apparently affecting relative nucleotide affinities and polymer morphology.

Moreover, one GDP analog not only failed to inhibit polymerization, but was itself able to support polymerization both with MAPs in 0.1 M Mes (microtubules formed) and with glutamate (sheets formed). This analog, 2',3'-dideoxyguanosine 5'-diphosphate (ddGDP) was not hydrolyzed in the course of polymerization but was incorporated into the polymer. This observation is of potential importance since it unambiguously demonstrates that GTP hydrolysis is not essential for polymerization and that a molecule smaller than a nucleoside triphosphate can induce the reaction. The exchangeable site may therefore indeed be subject to pharmacological manipulation by appropriately designed drugs.

We are continuing our examination of these complex nucleotide-tubulin interactions with the ribose-modified analogs. Radiolabeled nucleotides are being prepared for more detailed studies. We also plan to explore the effects of modification of the guanine residue on tubulin-nucleotide interactions.

2. Based on earlier studies from this laboratory which demonstrated conditions in which tubulin-dependent GTP hydrolysis was totally dependent on taxol (NSC-125973), we were able to develop an assay for the drug sensitive to 0.1 μM. Conditions were established suitable for measuring serum concentrations, and a preliminary pharmacokinetic study was performed in rabbits. Although taxol appears to be protein-bound in serum, it was rapidly cleared with α-phase and β-phase half-lives of 2.7 and 42 min, respectively. This assay is suitable for human pharmacokinetic studies, and will be made available to clinical research groups conducting Phase I/II trials with this agent. When the drug enters clinical trials, such studies will be performed. In addition, we plan to examine penetration of taxol into the central nervous system.

3. Because of a report that the methotrexate analog, 5-amino-1,2-dihydro-3-[(N-methylanilino)methyl[pyrido[3,4-b]pyrazin-7-ylcarbamate (NSC-181928) had antitumor activity and caused mitotic arrest, we examined the drug for potential interactions with tubulin. We established that NSC-181928 was an antitubulin agent. Structurally, the drug most closely resembles the benzimidazole carbamates, such as nocodazole (an established antitubulin agent), and like them, inhibited colchicine binding to tubulin and tubulin polymerization. Like nocodazole and colchicine, but even more potently, NSC-181928 stimulates tubulin-dependent GTP hydrolysis. If analogs of this drug can be obtained, structure-function studies will be performed in an effort to obtain an agent which interacts with both tubulin and dihydrofolate reductase.

4. Preliminary studies have indicated that tubulin can be quantitatively ADP-ribosylated with cholera toxin, the ADP-ribose moiety being transferred from NAD^+ to the protein. This reaction appears to be significantly affected by a variety of agents which interact with tubulin, including guanine nucleotides, antimitotic drugs and calcium. We plan to find optimum conditions for this reaction and to determine whether ADP-ribosylated tubulin has been affected functionally.

5. Efforts continued to perfect the separation of the α and β subunits of tubulin by hydrophobic chromatography, as well as to achieve this separation by other methods. Our future plans include refinement of the separation of the tubulin subunits; structural analysis of the subunits; and preparation of antibodies, including monoclonal antibodies, to the subunits, to assist in mapping of functional domains. We also plan to undertake reconstitution of activity from the subunits, and we anticipate that the study we have previously made of tubulin stabilization will benefit our efforts. When conditions for reconstitution are discovered, we plan to undertake an analysis of the structural requirements for the nonexchangeable nucleotide. This may reveal information about its function and permit development of antitubulin agents.

Another approach to the nonexchangeable nucleotide is suggested by recent papers in the literature. These indicate that the nonexchangeable nucleotide may be removed by treating tubulin with charcoal or with lactoperoxidase. We shall attempt to explore these techniques as a potential method for substituting nucleotide analogs for GTP in the nonexchangeable site without dissociation of the protein.

6. Recent experiments have indicated that reduction of GTP, but not dGTP, levels in cells leads to a severe inhibition of DNA synthesis. Since GTP is an integral structural component of tubulin, we plan to study whether there is any link between tubulin levels and DNA synthesis.

Publications

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07104-07 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

L-Phenylalanine Mustard Cytotoxicity and Therapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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3	2.0	1.0

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A murine L1210 leukemia cell resistant to cis platinum and L-PAM contains elevated concentrations of the tri-peptide glutathione. Reduction in the glutathione content of these cells to levels characteristic of sensitive tumor cells confers complete sensitivity to L-PAM. This sensitization is accompanied by a corresponding reduction in dechlorinated L-PAM.

Objective:

This project is designed to investigate the mechanism(s) of resistance of neoplastic cells to alkylating agents and to implement pharmacological regimens to overcome such resistance.

Methods Employed:

A variant of the murine L1210 leukemia cell, developed at the Southern Research Institute, is utilized for this project. This variant is completely resistant to cis-platinum, markedly although not completely resistant to L-phenylalanine mustard, somewhat less resistant to cyclophosphamide and retains full sensitivity to the nitrosoureas BCNU, CCNU and MeCCNU.

A. Isolation of Cells of Varying Degrees of Resistance to L-Phenylalanine Mustard. Cellular aspirates from CDF_1 mice bearing the L-PAM resistant tumor are removed from the peritoneal cavity, washed in RPMI 1630 growth medium and seeded into Falcon tubes containing medium and 0.1% agar. L-Phenylalanine mustard is added in various concentrations and the cells incubated for 7-9 days at 37°C. Individual colonies, emanating from a single cell are removed from the soft-nutrient agar, dispersed in 1 ml of growth medium and seeded into small wells in multiwell plates. Cells are passaged at 2 day intervals and examined for L-PAM sensitivity when adequate numbers of cells have been attained for cytotoxicity studies.

B. Analysis of Cytotoxicity. Cells are exposed to the respective cytotoxic agent for 2 days in growth medium. Cell survival is then assessed following growth of the surviving cells in soft-nutrient agar for 2 weeks.

C. Cellular Transport Studies. Cells maintained either in vitro or in vivo are washed 3 times by centrifugation and exposed to labelled phenylalanine mustard. After appropriate times aliquots of the cell suspension are layered on Versilube F-50 silicone oil and centrifuged at 12,000 x g for one minute in an Eppendorf microcentrifuge to pellet the cells. Cell pellets are solubilized in sodium hydroxide and radioassayed by liquid scintillation spectrometry.

D. Dechlorination of L-Phenylalanine Mustard by Sensitive and -Resistant Tumor Cells. Sensitive and -resistant tumor cells, exposed to differentially cytotoxic concentrations of [^{14}C]-L-PAM for varying periods of time, are layered on Versilube F-50 silicone oil (10^7 cells in 40 ml of medium on 10 ml of oil) and pelleted by centrifugation thru silicone oil for 30 minutes at 12,000 x g. The radioactive supernatant and medium are removed by aspiration and the cell pellets lysed in 500 µl of distilled water. One-hundred µl aliquots are applied to MN 300 cellulose thin layer chromatographic plates and L-PAM (R_f = 0.93) and its dechlorinated counterpart, dihydroxy L-PAM (R_f = 0.5) are separated by thin layer chromatography in a solvent system consisting of isopropyl alcohol:formic acid:water (65:1:34 v/v).

E. Determination of Intracellular Reduced Glutathione and Glutathione Disulfide. Murine L1210 leukemia cells are harvested from experimental growth medium, washed twice in Dulbecco's phosphate buffered saline (pH 7.4) and lysed in 0.75 ml of distilled water. Cellular protein is precipitated by addition of

0.25 ml of 12% sulfosalicylic acid and removed by centrifugation at 12,000 x g for one minute. Total glutathione and glutathione disulfide in the supernatant are assayed by the method of Griffith with minor modifications. The incubation mixture for determination of total glutathione consists of 100 µl of the protein-free supernatant of the cell lysate, 100 µl of 1 M triethanolamine HCl buffer (pH 8.0), 700 µl of 0.3 mM NADPH, 100 µl of 6 mM DTNB and 0.48 units of glutathione reductase. All reagents are prepared in 125 mM sodium phosphate buffer containing 6.3 mM sodium EDTA (pH 7.5). The absorbance of 2-nitro-5-thiobenzoic acid at 412 nm is monitored on a Gilford Model 240 recording spectrophotometer. The concentration of glutathione disulfide is determined after derivatizing reduced glutathione with 2-vinylpyridine. Two hundred fifty microliters of the protein-free supernatant of the cell lysate, 250 µl of 1 M triethanolamine HCl buffer (pH 8.,0) and 5 µl of 2 M 2-vinylpyridine are mixed vigorously for 1 minute (final pH 7.0-7.5). The mixture is incubated at 25°C for one hour, and then 400 µl of 0.5 mM NADPH, 100 µl of 6 mM DTNB and 0.48 units of glutathione reductase are added. The formation of 2-nitro-5-thiobenzoic acid is determined as described above.

F. Reduction in Intracellular Glutathione. Cells are grown in RPMI 1630 medium with a reduced concentration of glycine, L-glutamic acid or L-cystine in an attempt to reduce the intracellular concentration of glutathione. Cells are harvested after varying periods of time in the respective medium and assayed for cellular content of reduced glutathione and glutathione disulfide as described above.

Major Findings:

A. Cytotoxicity of L-PAM Toward Sensitive and -Resistant Murine L1210 Leukemia Cells. The murine L1210 leukemia cells used in the present study exhibited substantial differences in their susceptibility to L-PAM. The cytotoxicity of L-PAM (LD_{37}) toward cells exposed to the drug in growth medium containing amino acids ranged from 0.8 µg/ml for the parent sensitive tumor cell to 4.0 µg/ml for L1210/L-PAM_1 and 8.2 µg/ml for L1210/L-PAM_2. The presence of amino acids in the exposure medium considerably reduced the cytotoxicity of L-PAM toward all 3 tumor cells although the differences in the degree of resistance did not change.

B. Transport and Exodus of Differentially Cytotoxic Concentrations of L-PAM by Sensitive and -Resistant Tumor Cells. In order to determine whether resistance to L-PAM is accompanied by decreased intracellular accumulation of the drug we examined the uptake of L-PAM at concentrations which were lethal to drug-sensitive cells and non- or minimally lethal to drug-resistant cells. Little difference was observed in the net uptake of a dose of L-PAM which is minimally cytotoxic in medium containing amino acids to L1210/L-PAM_1, non-cytotoxic to L1210/L-PAM_2 but which produces a 1.5 log decrease in the surviving fraction of L-PAM sensitive tumor cells.

The accumulation of a differentially cytotoxic concentration of L-PAM by L1210, L1210/L-PAM_1 and L1210/L-PAM_2 in medium devoid of amino acids was also similar and suggests that these 3 murine tumor cells, although varying substantially in their susceptibility to L-PAM, accumulate similar amounts of drug.

Similarly, no observable differences were observed in L-PAM exodus from sensitive and -resistant tumor cells preloaded with a differentially cytotoxic concentration of L-PAM.

C. Dechlorination of L-PAM by Sensitive and -Resistant Tumor Cells. The failure to observe a decreased intracellular accumulation or enhanced exodus of L-PAM in L-PAM resistant tumor cells prompted investigation to determine whether intracellular metabolism of L-PAM may contribute to resistance. The most likely possibility was considered to be conversion of L-PAM to its non-cytotoxic derivative 4-[bis(2-hydroxyethyl)amino]-L-phenylalanine, hereafter referred to as dihydroxy L-PAM. L1210/L-PAM$_1$ tumor cells dechlorinate 2-2.5 times more L-PAM than do sensitive tumor cells. Such dechlorination is accompanied by less intracellular L-PAM in resistant cells and less radioactivity, part of which may be drug bound to protein and nucleic acid, at the origin of the tlc plate. Identical intracellular concentrations of dihydroxy L-PAM were found in L1210 and L1210/L-PAM$_1$ 30 minutes following exposure of cells to labeled L-PAM. The amount of dihydroxy L-PAM in L-PAM resistant cells increased with time and reached a maximum after 3 hours of drug exposure. In contrast, the amount of dihydroxy L-PAM found in L-PAM sensitive cells decreased with time and, after 3 hours of L-PAM exposure, sensitive cells contained 2-fold less dihydroxy L-PAM than did L-PAM resistant cells. Although the total intracellular radioactivity was nearly identical in both sensitive and resistant tumor cells during the 5 hour duration of L-PAM exposure, L-PAM sensitive cells had higher concentrations of both L-PAM and origin associated radioactivity, especially during the first three hours of drug exposure. Decreases in intracellular L-PAM occurred with time in both sensitive and resistant tumor cells although the rate was slightly higher in L-PAM sensitive cells. This decrease in intracellular L-PAM was accompanied by a parallel increase in the amount of label associated with the origin in L-PAM sensitive cells.

The conversion of L-PAM to dihydroxy L-PAM was completely inhibited by a brief 5 minute treatment of tumor cells with the sulfhydryl reagent N-ethyl maleimide prior to L-PAM exposure. A positive correlation exists between the intracellular concentration of glutathione and the sensitivity of murine tumor cells to L-PAM (Table 1) and increases in the glutathione content of these cells are accompanied by linear increases in the LD_{37} values of L-PAM. A positive

TABLE 1. Reduced glutathione and glutathione disulfide content of murine L1210 leukemia cells sensitive and resistant to L-phenylalanine mustard

L1210 Variant	LD_{37} of L-Phenylalanine Mustard μg per ml	Reduced Glutathione nmole per 10^6 cells	Glutathione Disulfide pmole per 10^6 cells
L1210	1.0	2.9 ± 1.1*	74.6 ± 15.4*
L1210/L-PAM$_1$	4.8	5.8 ± 2.0	139.7 ± 34.6
L1210/L-PAM$_2$	8.0	11.2	208.3

*Mean ± standard deviation; n = 11.
**Mean ± standard deviation; n = 6.

correlation also exists between the intracellular concentration of glutathione and dihydroxy L-PAM. The latter relationship indicates that increases in intracellular glutathione are accompanied by increased cellular content of dechlorinated L-PAM.

D. Reduction of Intracellular Glutathione and Sensitization of the Resistant Tumor Cell to L-PAM. The positive correlation between the sensitivity of murine L1210 leukemia cells to L-PAM and the intracellular content of glutathione prompted studies to determine whether the latter could be reduced. Incubation of cells in growth medium with a reduced concentration of L-cystine resulted in a rapid decrease in the intracellular content of glutathione disulfide in both the L-PAM sensitive tumor and in the resistant tumor, L1210/L-PAM$_1$. After 6 hours of incubation the intracellular concentrations are equivalent. The cellular content of reduced glutathione also decreased, and after 18 hours incubation in medium with a reduced concentration of L-cystine, both the L-PAM sensitive tumor and L1210/L-PAM$_1$ have identical intracellular content of reduced glutathione. Attempts to reduce the cellular glutathione content by incubation of cells in medium with a reduced concentration of glycine or L-glutamic acid were not as successful.

L-PAM is equitoxic to L-PAM resistant cells incubated in medium with a reduced concentration of L-cystine for 24 hours to reduce the intracellular glutathione content and L1210 cells grown in medium with L-cystine. These results indicate that an L-PAM resistant tumor cell can be completely sensitized to L-PAM by reducing the intracellular concentration of glutathione.

Proposed Course:

These results clearly implicate the tripeptide glutathione as being the determinant factor in L-PAM resistance. In vitro observations will be extended to tumor-bearing animals and nutritional and pharmacological regimens will be implemented in an attempt to sensitize tumor cells in vivo which are resistant to alkylating agents.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07128-01 LMCB

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October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Illustration of the Intra-Arterial Delivery Advantage using a Model Compound

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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1.5	1.25	0.25

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies in a normal rat model were undertaken with ^{3}H and ^{14}C isotopes of 2-deoxyglucose (2DG) as a model anticancer agent to illustrate pharmacologic principles of the intra-arterial drug delivery advantage. In particular, the regional target tissue was defined as the right brain cortex and the regional artery the right common carotid artery. First, this model illustrates that the first pass delivery advantage to the target tissue (right brain cortex) exists for only a short time following arterial (intra-carotid) infusion. No advantage could be demonstrated subsequently. Second, the model illustrates that the intra-arterial advantage approaches the model's physiologic limits based on carotid blood flow and cardiac output. Third, tissue analysis demonstrates that there is increased delivery of drug to the target tissue (regional delivery advantage), and that less drug is delivered to other systemic organs (systemic protection advantage). Last, the model illustrates that the delivery advantage which would be predicted on the basis of the pharmacokinetic behavior of the model drug, 2DG, can be directly confirmed in the laboratory.

Objectives:

1. To develop a laboratory model which directly illustrates the pharmacologic principles governing the intra-arterial drug delivery advantage, and, in particular,
2. To define the intra-carotid advantage for drug delivery to the ipsilateral brain cortex.

Methods Employed:

1. Rat model. A normal rat is surgically prepared as follows:

   1.1 Under pentothal or halothane anesthesia the left and right femoral artery and vein are cannulated with PE 50 polyethylene tubing connected to separate heparin-containing syringes.

   1.2 The carotid artery and its branches are dissected such that a sampling catheter is placed in the external carotid artery and the major extracerebral branches of the carotid artery (occipital, pterygopalatine, and external carotid distal to cannula) are ligated.

   1.3 Previously calibrated Harvard syringe pumps are connected to the animal such that there is constant withdrawal from the carotid cannula and one of the femoral artery cannulas. An infusion syringe is connected to one femoral vein for the intravenous infusion, and to the common carotid artery for the intra-arterial infusion.

   1.4 Over a one minute period the infusion syringe pump infuses the model compound into both the femoral vein and carotid artery.

   The withdrawal syringe pump samples blood from the femoral artery (systemic arterial integral) and the external carotid artery (carotid arterial integral) for a defined period.

   1.5 At sacrifice the withdrawal catheters are separated from the animal and various tissues (brain, muscle, testis, liver, spleen, kidney, heart, and lung) are obtained and placed in scintillation vials.

   1.6 The blood samples obtained by the withdrawal syringes are also placed into scintillation vials.

2. Analytical Technique

   2.1 Important to this experiment is the double radionuclide label technique. One form of the compound (e.g., ^{3}H-2DG) is infused into the carotid artery (symbolized by A) and another form (e.g., ^{14}C-2DG) is infused into the femoral vein (symbolized by V). The amounts of A and V which are found in the arterial samples, therefore, represent the relative drug delivery obtained by intra-carotid (A) and intravenous (V) infusion. The amount of drug delivered by either A or V is determined by obtaining the arterial integral (i.e., arterial exposure, or area under the curve) for each (X_A and X_V, respectively).

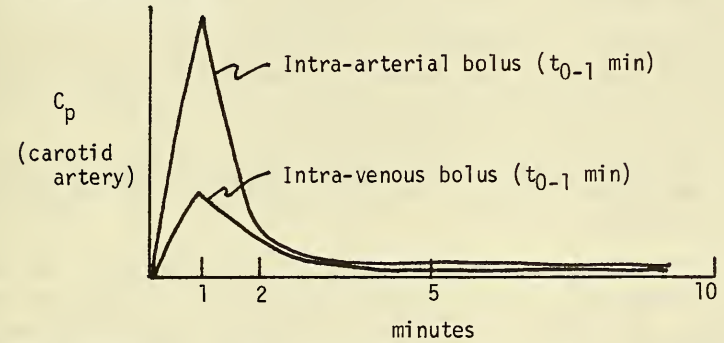
The intra-arterial advantage (R_d) is obtained from the carotid blood samples and is defined as the ratio of the integrals X_A/X_V,

$$R_d = \frac{X_A}{X_V}$$

This ratio will nearly always be greater than 1. The intra-arterial advantage of diminished systemic drug delivery is obtained from the femoral artery blood samples, and, again is defined by the ratio of X_A/X_V. This ratio will nearly always be less than 1.

2.2 Drug exposure (arterial integral) and drug tissue uptake are determined directly by liquid scintillation counting of the two labels (i.e., ^{3}H-2DG, and ^{14}C-2DG).

3. Experimental Design



The hypothetical curves illustrated above (Figure 1) represent the arterial integrals in the regional (carotid) artery for a one minute bolus infusion delivered simultaneously by an intra-arterial (intra-carotid) and an intravenous route. It is clear that the concentration of drug in the carotid artery (C_p) is initially greater when the drug is delivered intra-arterially compared to intravenously. In order to illustrate this in the rat, two experimental designs were employed.

3.1 To illustrate that the first pass advantage exists for only a short period of time following infusion, the two isotopes of 2-DG were infused (one isotope intra-carotid, one isotope intra-venous) from 0 to 1 minute, while the arterial integrals were obtained from 0 to 2 minutes. R_d was then calculated for both the carotid (intra-arterial delivery advantage) and the femoral (systemic protection advantage) arteries.

3.2 To demonstrate the absence of any advantage following the first passage of drug through the carotid artery, the two isotopes of 2-DG were again infused as described above. However, the arterial

integrals were obtained after the 2 minute mark, at a time when the recirculation integrals for the two isotopes should be equal.

Major Findings:

1. As predicted the intra-arterial delivery advantage (R_d) could be demonstrated only in the first experiment where the arterial integrals were determined from 0 to 2 minutes. This period of time is a convenient collection period and contains the first pass integrals as well as the initial recirculation integrals. The intra-arterial delivery advantage in this "first Pass" collection period was calculated to be 21, with a range of 8 to 32 in 6 animals. The theoretical first pass advantage based on the physiological limits of carotid artery blood flow (Q_i = 10 ml/min) and cardiac output (C.O. = 300 ml/min) in the rat (C.O./Q_i) is around 30.

2. No delivery advantage could be demonstrated in the second, "post-first pass" experiment when the arterial integrals were obtained for a period of 10 to 20 minutes following the 2 minute mark.

3. The tissue advantage for the target site, the right brain cortex, was 8.8, with a range of 2.7 to 16.6 in 7 animals. The actual ratio of right to left cortex in the 7 animals was 6.1 (range of 1.6 to 9.3). This data illustrates that in this surgical preparation the amount of model drug absorbed by the target tissue when delivered by the intra-arterial route was 9x that when delivered by the intra-venous route. Because of collateral circulation the actual target tissue to contralateral side advantage was slightly less, about 6x.

4. Also, as predicted, the systemic protection advantage (R_{d_s}) was demonstrated to be less than 1. The calculated R_{ds} was 0.72 (range 0.64 to 0.79 in 6 animals). The actual tissue advantage determined by tissue analysis was 0.81 (range 0.38 to 1.8 in 7 animals). This data illustrates that only about 70% of the drug is delivered to systemic organs when infused intra-arterially compared to an intravenous infusion, a reduction of 30% in the amount reaching systemic organs.

Proposed Course:

1. Additional laboratory studies should examine more fully the differences in tissue distribution obtained by intra-arterial vs. intra-venous infusion.

2. Drug development should proceed with the design of specific drugs whose pharmacokinetic properties make them more suitable for regional intra-arterial administration.

3. Clinical studies of intra-arterial infusion should carefully quantitate the regional delivery advantage based on the pharmacokinetic behavior of the drug and the estimated blood flow in the target tissues.

Applied Pharmacology Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1981 - September 30, 1982

The objectives of the Applied Pharmacology Section are to conduct biochemical and molecular pharmacological studies of anticancer drugs under development by the Division of Cancer Treatment, with emphasis on agents arising from within LMCB. The Section is presently engaged in biochemical and cytological studies of nucleoside analogs, anthracyclines and human fibroblast interferon (IFNβ).

The first project deals with the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF). This agent has shown activity in acute lymphoblastic leukemia, cutaneous T cell lymphomas (mycosis fungoides) and chronic lymphocytic leukemia (CLL). We have investigated the direct lymphotoxic properties of dCF in mitogen stimulated mouse splenocytes where changes in ribonucleoside mono- and triphosphates and the synthesis and methylation of nucleic acids have been characterized. We are also presently collaborating in a Phase II investigation of dCF in patients with CLL in order to assess the changes in ribonucleotide metabolism in leukemic lymphocytes following a single dose weekly schedule.

The second project entails detailed studies of the synthesis and methylation of RNA and DNA and the translational activity of mRNA in response to several nucleoside analogs. We have investigated 5-fluorouracil (5-FU), 5-fluorouridine (5-FUR), 5-azacytidine (AZC), dihydro-5-azacytidine (DHAZC), sangivamycin, and thiozdecarboxamide riboside (TR).

AZC and DHAZC-modified mRNA were isolated from Ehrlich ascites cells and assessed in vitro using a cell-free translation system. The coding capacity of drug-modified mRNA was not affected, but inhibition of rRNA synthesis was found to closely follow their relative antitumor potencies.

Studies of 5-FU and 5-FUR in a human colon carcinoma (HT-29) cell line refractory to nitrosoureas indicated that the lethal effects of these agents closely corresponded to their levels of incorporation into nuclear RNA. The concentration and time dependence of this effect should have important implications in the design of synergistic drug combinations employing 5-FU. These studies further suggest that 5-FUR may be a suitable drug for future clinical trials.

Ongoing studies with sangivamycin, a pyrrolopyrimidine antibiotic, have confirmed its time-dependent lethality in HT-29 cells in association with its marked incorporation into mRNA. Based on our early studies with mouse L1210 and S-180 cells and the present study with HT-29 cells, a new collaborative Phase I study is underway by Dr. Paul S. Ritch, Medical College of Wisconsin, wherein the drug will be administered by 5 day infusion to patients with colorectal cancer.

A collaborative study has been initiated with Drs. David Cooney and Hiremagalur Jayaram, Biochemistry Section, LMCB, on the mechanism of TR. We have defined its locus of action to inhibition of IMP dehydrogenase with the resulting

depletion of guanine nucleotides in sensitive, but not in resistant P388 leukemia. We are currently extending these studies to several human leukemia cell lines in order to examine the relationship between inhibition of cell growth and formation of the active inhibitory metabolite of TR, thiazole-carboxamide adenine dinucleotide.

We have also initiated studies with two new anthracyclines obtained from Dr. Ed Acton, Stanford Research Institute. 5-Iminodaunorubicin and 3'-deamino-3'-morpholinyldaunorubicin both possess a low capacity to spontaneously generate free radical intermediates and show a low level of myelotoxicity and cardiotoxicity. Their lethal effects, pharmacokinetics, inhibitory effects on RNA and DNA, and DNA strand breakage activities have been measured in HT-29 cells. The results thus far point to several novel properties of these drugs which may make them or their second generation analogs important candidates for clinical study.

A new project initiated this year has been a thorough biochemical and cytokinetic study of human fibroblast interferon (IFNβ) in several human tumor cell lines. We have determined whether the IFNβ mediated induction of (2'5')-oligo(A) synthetase, an enzyme which is unique to IFN-treated cells and is associated with antiviral activity, is related to the growth inhibitory properties of IFNβ and double-stranded RNA (poly(I·C);$I_n \cdot C_n$). A thorough study of the time and concentration dependence of (2',5')oligo(A) synthetase induction in association with the lethal and growth inhibitory properties of IFNβ and $I_n \cdot C_n$ used singly and in combination are underway. We also plan to measure the levels of (2',5')oligo(A) synthetase in patients with CLL treated with IFNβ as a measure of their responsiveness to treatment. The latter study is being implemented in collaboration with Dr. Paul S. Ritch, Medical College of Wisconsin.

The last project is concerned with the relationship between the phosphorylation of specific classes of nonhistone chromosomal proteins and the inhibitory activity of adenosine analogs on transcription. We are currently examining the high mobility group (HMG) class of nuclear proteins which are intimately associated with gene activity in the transcriptional structural unit, the nucleosome. We have discovered that two of the four HMG proteins (HMG 14 and 17) are highly phosphorylated, and that these phosphorylated species may be associated with transcriptionally active regions of chromatin. The phosphorylation of HMG 14 and 17, however, is not affected by several pyrrolopyrimidine analogs including sangivamycin despite their pronounced inhibitory effects on nuclear RNA synthesis. We have completed an investigation of the growth and cell cycle dependence of phosphorylated HMG 14 and 17 in several animal and human tumor cell lines and plan to extend these investigations to IFN-treated human tumor cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CM 06145-05 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Characterization of Adenosine Deaminase Inhibitors in Normal and Neoplastic Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Martin Earle, M.D.	Medical Staff Fellow	LMCB	NCI
	David G. Poplack, M.D.	Sr. Investigator	POB	NCI
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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of action of the direct lymphotoxic effect of 2'-deoxycoformycin is being determined in vitro in concanavalin A-stimulated mouse spleen lymphocytes. Measurements of ribonucleoside mono- and triphosphates, adenosine deaminase activity, methylation and synthesis of RNA and DNA, and the metabolism of 2'-deoxycoformycin will be correlated with the suppression of blastogenesis. Nucleotide levels and adenosine deaminase activity will be measured in leukemic lymphocytes in patients with chronic lymphocytic leukemia undergoing treatment with 2'-deoxycoformycin.

Objectives:

Adenosine deaminase plays a central, but passive role in maintaining the viability of cells. Suppression of its activity by inhibitors such as 2'deoxycoformycin (dCF) or by its genetic deletion in the severe combined immunodeficiency syndrome results in the inability of lymphocytes to function normally in their immune response. In the genetic syndrome, this is presumably due to the build up of adenosine metabolites such as ATP, dATP, cyclic AMP or s-adenosylhomocysteine, which are potent inhibitors of various metabolic processes if allowed to accumulate to inhibitory concentrations within the cell. In dCF-treated animals and patients, the mechanism for the pronounced lymphocytopenia is unknown. We are currently investigating the direct effect of dCF and its metabolite on the suppression of lymphocyte blastogenesis, nucleic acid synthesis, adenosine deaminase activity and ribonucleotide concentrations in vitro. In addition, pursuant to our previous collaboration with POB, DCT, NCI, on Phase I studies of dCF in acute leukemia, we have initiated a Phase II study of dCF in patients with CLL with Dr. Lyonel Israels in Winnipeg, Canada. We plan to measure ribonucleoside and ribonucleotide levels and adenosine deaminase activity leukemia lymphocytes from these patients.

Methods Employed:

Cultures of mouse spleen cells in RPMI 1640 medium supplemented with 10% newborn calf serum; measurement of blastogenesis after the addition of concanavalin A by the incorporation of [^{3}H]thymidine and [^{3}H]uridine, isolation of lymphocytes by Ficoll-Hypaque gradient centrifugation; separation of dCF metabolites and ribonucleoside mono- and triphosphates by anion-exchange hplc using a KH_2PO_4 gradient; isolation of DNA by phenol-detergent extraction and its separation into deoxyribonucleosides by reversed-phase hplc with 10mM KH_2PO_4(pH3.8):5% methanol following DNase I, snake venon phosphodiesterase and bacterial alkaline phosphatase digestions.

Major Findings:

1. In vitro studies of dCF on mouse spleen lymphocytes have been completed. Lymphoblastogenesis was measured 24 and 66 hours after concanavalin A stimulation by the incorporation of [^{3}H]thymidine and [^{3}H]uridine into DNA and RNA, respectively. When lymphocytes were incubated with 10^{-8} to 10^{-4} M dCF, a time-and dose-dependent inhibition of DNA and RNA synthesis occurred. The IC_{50} for DNA and RNA synthesis 66 hr after concanavalin A stimulation was 10^{-6} M and $3x10^{6}$ M dCF, respectively. Under identical conditions, the IC_{50} was $4x10^{-9}$ M dCF for the inhibition of adenosine deaminase activity. When lymphoblastogenesis was measured 24 hrs after the addition of concanavalin A, DNA synthesis was inhibited 10%, while RNA synthesis was inhibited 30% at 10^{-4} M dCF, despite the same marked inhibition of adenosine deaminase activity observed at 66 hr. Neither RNA nor DNA methylation was affected under these conditions. Measurement of nucleosides by hplc of perchloric acid-soluble

cell extracts obtained 24 and 66 hr after the addition of concanavalin A revealed a dose-dependent reduction in mono- and triphosphate nucleotides. In contrast, a 2 - 2.5-fold elevation in IMP at 10^{-6} M dCF and 50-100% reduction in IMP at 10^{-4} M dCF was observed at 24 and 66 hr after the addition of mitogen. A monophosphorylated metabolite of 2'-deoxycoformycin was also detected by high pressure liquid chromatography of perchloric acid-soluble extracts of concanavalin A-stimulated lymphocytes treated with 10^{-4} M dCF. These data suggest that 1) inhibition of adenosine deaminase is not dose-related to the inhibition of nucleic acid synthesis, 2) DNA and RNA methylation are unaffected by dCF, 3) dCF is metabolized to the monophosphate form, and 4) changes in nucleotide pools sizes may play a significant role in dCF-mediated lymphocytotoxicity.

2. dCF, a potent inhibitor of adenosine deaminase, has recently undergone Phase I clinical trials and has been found to be therapeutically active in acute lymphoblastic leukemia. In this study, levels of dCF in plasma, plasma concentrations of adenosine and deoxyadenosine, and urine levels of deoxyadenosine were measured in leukemic patients undergoing treatment with dCF during a Phase I clinical trial. dCF was administered i.v. at a dose of 0.25 to 1.0 mg/kg (7.5 to 30 mg/sq m) for 3 consecutive days. Plasma drug levels of 2 to 6 μM were observed following the third dose of dCF, and drug accumulation occurred only at the 1 mg/kg dosage. In this limited series of patients, the plasma concentrations of adenosine and deoxyadenosine did not show an obvious correlation with dCF dose, therapeutic response, or toxicity.

3. The extent to which systemically administered dCF crosses into the central nervous system was assessed in Rhesus monkeys administered i.v. boluses of dCF. Simultaneous blood and cerebrospinal fluid (CSF) samples were assayed for dCF levels at times ranging from 10 min to 6 hr after the drug was given. Average peak CSF drug levels of 5.5×10^{-8} M and 3×10^{-7} M were reached 1.5-2 hr following injections of 0.25 and 1.0 mg/kg, respectively. The ratio of peak CSF to simultaneous plasma levels was 1:10. Data obtained from a patient with acute lymphocytic leukemia who was administered i.v. 0.25 mg/kg dCF were comparable. Drug levels achieved within the CSF following this dose of dCF were similar to those previously demonstrated to inhibit adenosine deaminase. These results may be important both for understanding dCF-related CNS toxicity and for designing combination chemotherapy with dCF.

Proposed Course:

Phase II clinical studies are being initiated with Dr. Lyonel Israels, Winnipeg, Canada, to determine the relationship between inhibition of adenosine deaminase and changes in ribonucleoside and ribonucleotide levels in lymphocytes of patients with CLL. This population of patients has shown good responses to low (0.25 mg/kg) virtually non-toxic doses of dCF on a once weekly schedule.

Publications:

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Blatt, J., Venner, P.M. Riccardi, R., Cohen, L.F., Gangji, D., Glazer, R.I., and Poplack, D.G.: Cerebrospinal fluid levels of 2'-deoxycoformycin after systemic administration in monkey. J. Natl. Cancer Inst. 68:391-394, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07109-06 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Effect of Anticancer Drugs on Cell Lethality and The Synthesis and Function of RNA

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The influence of several anticancer drugs on the synthesis and methylation of nuclear and cytoplasmic RNA will be assessed in mouse and human tumor cell lines in vitro. 5-Fluorouracil, 5-fluorouridine, 5-azacytidine, dihydro-5-azacytidine, sangivamycin, formycin A, 8-azaadenosine and thiazole-carboxamide riboside will be examined. The influence of drug-modified messenger RNA on in vitro translation in a rabbit reticulocyte lysate will be studied following exposure of a human colon carcinoma (HT-29) cell line to 5-fluorouracil, 5-fluorouridine, sangivamycin and other nucleoside analogs. The anthracycline analog 5-iminodaunorubicin and 3'-deamino-3'-morpholino-daunorubicin will be examined in HT-29 cells with regard to cell viability, nucleic acid synthesis, drug uptake and efflux and DNA repair.

Objectives:

Many nucleoside anticancer drugs, as well as other classes of antitumor agents, act via inhibition of the synthesis and/or methylation of RNA. These alterations may directly result in cell death or alternatively, drug substitution into RNA may lead to changes in RNA function with similar consequences.

We have investigated the nucleoside analogs 5-azacytidine, dihydro-5-azacytidine, 5-fluorouracil, 5-fluorouridine, sangivamycin, formycin A, 8-azaadenosine and thiazolecarboxamide riboside (TR) with regard to RNA and DNA synthesis, drug substitution into these macromolecules and ribonuceloside triphosphate levels. These biochemical determinants have been related to the lethal effects of these drugs in most instances.

We have also initiated studies with two other classes of drugs: 1)the semisynthetic anthracycline analogs, 5-iminodaunorubicin and 3'-deamino-3'morpholinodaunorubicin and 2)human fibroblast interferon.

Methods Employed:

Isolation of nuclei from HT-29 cells by Triton X-100 cell lysis, labeling of RNA with [^{3}H]adenosine, [^{3}H]uridine or [^{3}H]methionine; RNA extraction by SDS-phenol procedures; poly(U)Sepharose affinity chromatography; agarose urea gel and polyacrylamide slab gel electrophoresis; hplc for determination of nucleoside triphosphate levels by isocratic elution with 0.4M KH_2PO_4 (pH3.8): 5% acetonitrile; in vitro translation of mRNA in a rabbit reticulocyte lysate assay; tissue culture and colony formation by soft agar cloning.

Major Findings:

1. The cytotoxic and biochemical activity of the pyrrolopyrimidine antibiotic, sangivamycin, was examined in a human colon carcinoma (HT-29) in tissue culture. Logarithmically growing cells were more sensitive than early plateau-phase cells to the lethal effects of the drug as determined by colony formation in soft agar. Cell lethality in both log- and plateau-phase cells became more pronounced when drug exposure time was increased from 2 hr to 24-48 hr. Reduced cell viability correlated with the inhibition of total RNA synthesis after 2 hr of drug exposure, and with reduced DNA synthesis and incorporation of drug into DNA and RNA after 24-48 hr of drug exposure. Fractionation of total RNA into nonpoly(A) containing and poly(A)-containing RNA indicated that inhibition of the synthesis of both RNA species occurred after 24 hr of exposure of log-phase cells to sangivamycin. Neither RNA fraction was affected in plateau-phase cells after 48 hr of drug exposure. In contrast, a close correlation was found between the incorporation of [^{3}H]sangivamycin into poly(A)RNA and cell lethality after 24-48 hr of exposure of both log and plateau-phase cells to sangivamycin. These results show that a human colon carcinoma is responsive to sangivamycin following prolonged drug exposure intervals and that the associated cytotoxicity correlates closely with the incorporation of drug into mRNA, as well as inhibition of DNA synthesis.

2. [^{3}H]Sangivamycin was preferentially incorporated into total cellular RNA in log and plateau phase sarcoma-180 cells in vitro. The incorporation of this drug into total poly(A)RNA showed the same time-dependency as its effects on cell viability and inhibition of nucleic acid synthesis.

3. The comparative effects of 5-azacytidine (AZC) and dihydro-5-azacytidine (DHAZC) on the synthesis of polysomal RNA, as well as on the translational activity of drug-modified poly(A)RNA were studied in Ehrlich ascites cells in vitro. AZC inhibited the incorporation of [^{3}H]adenosine into non-poly(A) RNA in a concentration dependent manner, but did not affect the synthesis of poly(A)RNA. In contrast, neither RNA fraction was significantly affected by equimolar concentrations of DHAZC except at 10^{-3} M, where 40% inhibition of the synthesis of only non-poly(A)RNA was achieved. Electrophoretic characterization of nonpoly(A)RNA revealed that the synthesis of 28S rRNA was inhibited to a greater extent than was 18S rRNA by 10^{-5} M AZC, but to equal extents at 10^{-3} M drug. Significant inhibition of 28S and 18S rRNA occurred only with 10^{-3} M DHAZC, where approximately 50% inhibition of both rRNA species occurred. Both cytidine analogues were ineffective in inhibiting tRNA synthesis. AZC and DHAZC were incorporated into all species of polysomal RNA, but the amount of drug substitution into a particular species of RNA was not proportional to its effect on the synthesis or function of that RNA. There was more analogue incorporated into poly(A)RNA than into nonpoly(A)RNA, but drug substituted poly(A)RNA showed neither an impairment of its coding efficiency nor qualitative changes in the translation products synthesized in a rabbit reticulocyte translation system in vitro. From these results, it appears that the relative differences in the inhibitory effects by the two cytidine analogues on rRNA synthesis, as well as their incorporation into rRNA, correlate closely with their relative potencies as antitumor agents.

4. The cytokinetic and biochemical effects of 5-fluorouracil (5-FU) and 5-fluorouridine (5-FUR) were examined in a human colon carcinoma cell line (HT29) in culture. Logarithmically growing cells were approximately 100 times more sensitive to the lethal effects of 5-FUR than 5-FU as measured by colony formation in soft agar medium. A 2-hr exposure of cells to 10^{-3} M 5-FU or 10^{-5} M 5-FUR produced a 2-log reduction in colony formation, a 31-33% inhibition of [^{3}H]adenosine incorporation into total RNA. Increasing the duration of drug exposure to 24 hr produced a proportional reduction in the drug concentration required to produce similar biochemical and cytocidal effects. However, cell lethality produced by either drug did not correlate quantitatively with inhibition of DNA or RNA synthesis. Examination of nuclear rRNA and 4 S RNA synthesis by agarose gel electrophoresis following 2-hr and 24-hr exposure to 5-FU or 5-FUR indicated that processing of rRNA was not impaired, rRNA synthesis was inhibited by 10-40%, and 4S RNA synthesis was unaffected. In contrast to these results, measurements of the incorporation of [^{3}H]5-FU or [^{3}H]5-FUR into nuclear RNA showed that a significant correspondence existed between the amount of drug incorporated into nuclear RNA and cell lethality. These results indicate that the primary determinant of cell lethality in HT-29 cells is the degree of fluoropyrimidine substitution in nuclear RNA and not inhibition of either DNA or RNA synthesis.

5. The semisynthetic anthracycline, 5-iminodaunorubicin (IM), was investigated to see whether modification of the benzoquinone moiety to produce a drug with low free radical potential would alter the cytotoxic and biochemical characteristics of this drug in comparison to adriamycin (ADR), an agent with high free radical potential. Cell viability was measured in human colon carcinoma (HT-29) cells by soft-agar cloning. Upon exposure of either log phase or early plateau phase cells for 2 hr to IM or ADR, a threshold exponential cell lethality curve was obtained. Prolonging drug exposure to 24 hr produced an exponential decline in cell survival and a marked reduction in viability of both log-phase and early plateau-phase cells. Inhibition of DNA synthesis in log-phase cells after 2 and 24 hr of exposure to IM and ADR paralleled the increased cell lethality produced by the drugs. In contrast, total RNA synthesis was not inhibited by IM, whereas ADR impaired both RNA and DNA synthesis. Nuclear rRNA synthesis was not significantly inhibited following 24 hr of exposure to 10^{-7} M ADR or IM but was inhibited by 85 and 35% at 10^{-6} M ADR or IM, respectively. The affinity of IM and ADR for HT-29 DNA was measured in vitro by displacement of acridine orange binding and was found to be similar for both analogs. These studies suggest that the cytotoxicity of IM and ADR results from the interactions of these drugs with DNA.

6. Studies on the mechanism of action of a new oncolytic nucleoside, thiazolecarboxamide riboside (TR), have been undertaken using P388 murine leukemia cells growing in culture. TR was cytotoxic at micromolar levels, but a number of simple substitutions of both the ring and sugar moieties nullified cytotoxicity. Cytofluorimetric analysis revealed that the drug arrests cells in the "S phase" of the cell cycle. At antiproliferative concentrations, the agent inhibited the synthesis of both RNA and DNA. The macromolecular incorporation of pyrimidines was inhibited by the drug, but among the purines, this effect extended only to members of the adenine family, and in fact, the utilization of guanine and its congeners was stimulated. Among the series of purines and pyrimidines tested to overcome the inhibition of thymidine incorporation produced by TR, guanine and its nucleoside and xanthosine were found to be active. Confirmation that the drug was producing a state of guanine deprivation was provided by hplc analysis of acid-soluble extracts: a time-dependent fall in the concentration of GMP and GTP ensued upon exposure to the drug; on the other hand, IMP concentrations increased by 15-fold. Although GMP synthetase was not inhibited in vitro or in culture following treatment with TR, the specific activity of IMP dehydrogenase underwent substantial reductions. Hplc analyses of extracts of cultures exposed to lethal concentrations of TR provided evidence of its anabolism to the 5'-monophosphate among other products. A chemically synthesized sample of TR-5'-monophosphate was 20 times more potent than the parent nucleoside in inhibiting IMP dehydrogenase in vitro and the inhibition was noncompetitive with IMP as the variable substrate.

7. Administration of TR to BDF_1 mice bearing s.c. P388 leukemia produced a sharp depression in the concentration of intratumoral guanine nucleotides and a correspondingly large expansion of the IMP pools. Measurements of IMP dehydrogenase in the tumors of treated mice revealed a dose-dependent inhibition of this enzyme, with ~50% inhibition produced by a dose of 25 mg/kg and ≥90% inhibition by all doses ≥100 mg/kg. The inhibition of enzyme activity seen after a dose of 250 mg/kg reached a maximum 2 hr after treatment and

subsided substantially 8 hr after dosing; by 24 hr, enzyme activity was fully restored. These results, coupled with the observation that the antitumor activity of the drug can be prevented in large part by the simultaneous administration of guanosine, support the conclusion that TR, after its anabolism, exerts its antineoplastic effects via a state of guanine nucleotide depletion. In extracts of the tumors of mice given parenteral injections of TR, a potent dialyzable inhibitor of IMP dehydrogenase was demonstrable and its concentration fluctuated in parallel with enzyme inhibition. Although the chemical identity of the proximate inhibitory species has yet to be unequivocally established, it is concluded on kinetic grounds that it is neither the native nucleoside nor its 5'monophosphate, but most probably the drug analog of NAD.

8. The pharmacological properties of the new anthracycline, 3'-deamino-3'(4-morpholinyl)daunorubicin (MD), and adriamycin (ADR) have been compared in human colon carcinoma (HT-29) cells in vitro. ADR was 10-fold more cytocidal than MD to log phase cells upon short (2 hr)- or long (24 hr)-term drug exposure. In plateau phase cells, 2 hr exposure to either ADR or MD produced equivalent reductions in colony formation but ADR was 3-fold more toxic than MD following 24 hr treatment. Although ADR produced greater cell lethality than MD, the latter drug was far more inhibitory to DNA and RNA synthesis at equitoxic concentrations. This disparity was related to pharmacodynamic differences between the two drugs. The cellular uptake of MD was rapid and reached a plateau after 1-2 hr, whereas ADR was taken up at a 15-fold slower rate. However, by 24 hrs after drug treatment, the intracellular concentration of MD decreased while ADR continued to accumulate until it reached one-half of the intracellular concentration of MD. When cells were incubated with 10^{-6} M drug for 24 hr, 65% of MD, but less than 10% of ADR were converted to alcohol metabolites. The fraction of metabolite was higher in the media than in the cells, demonstrating that it was less able to accumulate in the cells than the parent drug, and hence accounted for the decline in intracellular levels of MD at 24 hr. When placed in drug-free media the rate of efflux of MD was much greater ($t_{1/2}$ = 45 min) than that of ADR ($t_{1/2}$ = 6 hr). The strikingly different pharmacodynamic properties of these drugs related to the greater lipid solubility of MD vs. ADR and not their binding affinities to DNA which were similar. Therefore, the decreased cytotoxicity of MD in tissue culture appeared to be related to its extensive metabolism and rapid efflux from the cells.

9. Activities of 2',5'-phosphodiesterase (2',5'-PDE) and a double-stranded RNA (dsRNA)-dependent pppA(2'p5'A)$_n$ synthetase ((2',5')oligo(A) synthetase) were investigated in untreated, fibroblast interferon (IFNβ) and poly (I•C)(I_n•C_n) treated human tumor cells. Three T cell leukemia lines (CCRFCEM, MOLT-4 and HUT-78), two B cell leukemia lines (CCRF-SB and NALM-1) and one colon carcinoma cell line (HT-29) were studied. Measurement of the basal (2',5')oligo(A) synthetase activity in these cells indicated that CCRF-SB possessed high endogenous enzyme levels (58 units/mg), whereas other cell lines showed a lower range of basal enzyme activities (8-22 units/mg). High endogenous (2',5')oligo-(A) synthetase activity in CCRF-SB cells was attributable to constitutive production of low amounts of leukocyte IFN (4 IU/ml). Comparison of the basal (2',5')PDE activities in these cell lines revealed that MOLT-4 and NALM-1 possessed higher endogenous activities (36 units/mg) compared to HT-29, CCRF-SB and HUT-78 cells (1-2 units/mg). Upon treatment of the various cell lines with

100-1000 IU/ml of IFNβ, a 3-22-fold induction of (2',5')oligo(A) synthetase occurred, whereas activity in CCRF-SB was unaffected; however, IFNβ treatment did not induce (2',5')PDE activity in these cells. Treatment of tumor cells with 100 μg/ ml $I_n \cdot C_n$ induced (2',5')-oligo(A) synthetase activity (3-4 fold) in HT-29 cells. All other cell lines failed to show significant induction of (2',5') oligo(A) synthetase or (2',5')PDE activity with $I_n \cdot C_n$ treatment. Comparison of (2',5')oligo(A) synthetase induction in IFNβ-treated CCRF-CEM and HT29 cells at different stages of growth indicated that enzyme induction was greater in exponentially growing cells than in plateau phase cells. Based on these findings, cell lethality was examined in HT-29 cells following 1-3 day treatment with IFNβ and $I_n \cdot C_n$ used singly and in combination. Although each agent alone had slight toxicity after 3 day exposure the combination of IFNβ + $I_n \cdot C_n$ produced a 90% decrease in cell growth. A biochemical mechanism for the cytotoxic effect of IFNβ and $I_n \cdot C_n$ is being pursued.

Proposed Course:

Correlative studies examining the interrelationship between cell lethality and *in vitro* translational activity are in progress for 5-FU, 5-FUR and sangivamycin-modified mRNA from HT-29 cells. Analyses of this type are also planned for the anthracycline analogs as well as other nuceloside analogs under investigation.

Publications:

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Glazer, R.I. and Lloyd, L.S.: The effect of 8-azaadenosine and formycin on cell lethality and the synthesis and methylation of nucleic acids in human colon carcinoma cells in culture. Biochem. Pharmacol., in press.

Jayaram, H.N, Smith, A.L., Glazer, R.I., Johns, D.G. and Cooney, D.A.: Studies on the mechanism of action of 2-β-D-ribofuranosylthiazole-4carboxamide (NSC 286193). II. Relationship between dose level and biochemical effects in P388 leukemia in vivo. Biochem. Pharmacol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07111-06 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Action of Anticancer Drugs on the Phosphorylation and Activity of Nonhistone Chromosomal Proteins

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Mrunal S. Chapekar, Ph.D.	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Applied Pharmacology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The phosphorylation of high mobility group (HMG) nonhistone chromosomal proteins 14 and 17 is being investigated in log and plateau phase cells as well as in synchronized cells in vitro. The associate of [^{32}]HMG 14 and 17 with transcriptionally active regions of nucleosomal chromatin will be examined by limited digestion with DNase I and micrococcal nuclease. These studies will be extended to interferon-treated human tumor cells in vitro.

Objectives:

The phosphorylation of nonhistone chromsomal proteins (NHCP) is believed to exert a regulatory role on transcription in eukaryotic cells. This process is mediated via nuclear protein kinases, a heterogeneous group of enzymes. One objective of this project is to determine the effect of adenosine analogs upon the phosphorylation of a specific class of nuclear proteins known to be associated with transcriptionally active chromatin. The high mobility group (HMG) of NHCP are ideal for this study since they can be isolated relatively free of most histones and other NHCP by gel electrophoresis, and are known to be associated with the transcriptional unit of the cell, the nucleosome. In addition, little is known about the chemical modification of HMG proteins in the nucleus, and thus, investigations of their phosphorylation in transcriptionally active and inactive regions of chromatin will be carried out in both mouse and human tumor cell lines in culture under a variety of growth conditions.

Methods Employed:

Extraction of HMG proteins with 2% trichloroacetic acid; separation and quantitation of HMG proteins by SDS and acid-urea polyacrylamide gel electrophoresis and autoradiography; labeling of NHCP and HMG proteins by incubation of cells *in vitro* with ^{32}P.

Major Findings:

1. The phosphorylation of the high mobility group (HMG) proteins has been investigated in mouse Ehrlich ascites, L1210 and P388 leukemia cells, human colon carcinoma cells (HT-29), and Chinese hamster ovary (CHO) cells. HMG 14 and 17, but not HMG 1 and 2, were phosphorylated in the nuclei of all cell lines with a serine being the site of modification for both proteins in Ehrlich ascites cells. Phosphorylation of HMG 14 and 17 was greatly reduced in cultured cells at plateau phase in comparison to log phase cells suggesting that modification of HMG 14 and 17 is growth associated. However, phosphorylation was not linked to DNA synthesis since incorporation of ^{32}P did not vary through the G_1 and S phase in synchronized CHO cells. Treatment of HT-29 or Ehrlich ascites cells with sodium butyrate reduced HMG phosphorylation by 30 and 70 percent, respectively. The distribution of the phosphorylated HMG proteins in chromatin was examined using micrococcal nuclease and DNase I. [^{32}P]HMG 14 and 17 were preferentially associated with micrococcal nuclease sensitive regions as demonstrated by the release of a substantial fraction of the phosphorylated forms of these proteins under conditions which solubilized less than 3 percent of the DNA. Short digestions with DNase I did not show a marked release of [^{32}P]HMG 14 or 17.

Proposed Course:

The inter-relationship between phosphorylation of HMG 14 and 17 and cell growth will be investigated in HT-29 cells treated with interferon and $I_n \cdot C_n$. The ability of these biological response modifiers to perturb phosphorylation processes and produce growth inhibition will be assessed.

Publications:

Saffer, J.D. and Glazer, R.I.: The phosphorylation of high mobility group (HMG) proteins 14 and 17 and their distribution in chromatin. J. Biol. Chem. 257: 4655-4660, 1982.

Biochemistry Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1981 - September 30, 1982

Scope and General Summary of Accomplishments

The Biochemistry Section of the Laboratory of Medicinal Chemistry and Biology has continued to examine the mechanisms by which the high-priority C nucleoside, tiazofurin or riboxamide (2-β-D-ribofuranosylthiazole-4-carboxamide) produces its antitumor effects *versus* the P388 leukemia and the Lewis lung carcinoma. During the past year it was determined that these tumors, following treatment with the drug, generated a heat- and acid-stable small molecular weight inhibitor of IMP dehydrogenase (IMPD). The activity of this inhibitor could be abolished by incubation with phosphodiesterase from snake venom, but not by alkaline phosphatase. This suggested that the responsible principle contained a phosphodiester linkage and might be a dinucleotide of the pyridine nucleotide type. Confirmation of this supposition was provided by the finding that tumor cells incorporated both tritiated riboxamide and [^{14}C]adenine into the inhibitory species in equimolar amounts. Further confirmation was provided by the enzymic and chemical syntheses of a molecule with identical cytotoxic, enzyme-inhibitory and chromatographic properties *via* NAD-pyrophosphorylase and the Khorana condensation reaction respectively. NMR and mass spectral analyses of the synthetic principle from these two sources were wholly in accord with the postulated dinucleotide structure. By analogy to NAD, this inhibitory molecule has been abbreviated TAD, thiazole adenine dinucleotide. This compound inhibits a partially purified preparation of IMPD by ~50% at a concentration of 1×10^{-7} M; kinetic analyses reveal that the inhibition is formally competitive with NAD as the variable substrate. Further corroboration that TAD mediates the therapeutic activity of riboxamide was provided by studies of a line of the P388 leukemia resistant to the drug: this variant forms no TAD *in vitro* and only 1/10th as much of the dinucleotide *in vivo* as its sensitive counterpart, the parent P388 line. Moreover, the specific activity of the enzyme likely to be responsible for the synthesis of TAD, NAD pyrophosphorylase, is 6 to 10-fold lower in the resistant leukemia line.

Staff

The permanent staff, at present, consists of two senior scientists and two technicians.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07122-02 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies with Nucleosides and Bases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Cooney	Head, Biochemistry Section	LMCB	NCI
Other:	Hiremagalur N. Jayaram	Pharmacologist	LMCB	NCI
	Yvonne Wilson	Chemist	LMCB	NCI
	Robert Dion	Chemist	LMCB	NCI

COOPERATING UNITS (if any)

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Laboratory of Medicinal Chemistry and Biology

SECTION
Biochemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanisms by which the drug riboxamide kills tumor cells have been studied in mice bearing the P388 and Lewis lung transplantable tumors. Riboxamide-resistant variants of these ordinarily sensitive neoplasms have also been developed and the biochemical bases for resistance examined.

I. In Vitro Studies with Riboxamide

Studies on the mechanism of action of a new oncolytic nucleoside, 2-β-D-ribofuranosylthiazole-4-carboxamide (riboxamide), have been undertaken using P388 murine leukemia cells growing in culture. The title compound was cytotoxic at micromolar levels, but a number of simple substitutions of both the ring and sugar moieties nullified cytotoxicity. Cytofluorimetric analysis revealed that the drug arrests cells in the "S phase" of the cell cycle. At antiproliferative concentrations, the agent inhibited the synthesis both of RNA and DNA. The macromolecular incorporation of preformed pyrimidines, including thymidine, was inhibited by the drug, but among the purines, this effect extended only to members of the adenine family, and in fact, the utilization of guanine and its congeners was reproducibly stimulated.

When an examination was made of the ability of a comprehensive series of preformed purines and pyrimidines to overcome the inhibition of thymidine incorporation provoked by exposure to the thiazole nucleoside, the guanines were notably effective, but xanthosine also was shown to be an active antidote. Confirmation that the drug was producing a state of guanine deprivation was provided by HPLC analysis of acid soluble extracts: a time-dependent fall in the concentration of GMP and GTP ensued upon exposure to the drug; on the other hand, IMP concentrations increased ~15 fold. Pursuant to these findings, an examination was made of the enzymologic steps unique to the biosynthesis of guanine nucleotides in cells exposed to cytotoxic concentrations of the drug. No prominent inhibition of GMP synthetase could be demonstrated in vitro or in culture, but the specific activity of IMP dehydrogenase underwent substantial reductions in both of these cases. HPLC analyses of extracts of cultures exposed to supralethal concentrations of the title compound provided evidence of modest anabolism to the 5'-monophosphate among other products; in vitro a chemically synthesized sample of 2-β-D-ribofuranosylthiazole-4-carboxamide-5'-monophosphate was 20 times more potent than the parent nucleoside in inhibiting IMP dehydrogenase. On kinetic analysis, this inhibition was non-competitive with IMP as the variable substrate. Pursuant to these studies, a comparison of riboxamide with other established inhibitors of IMPD was also undertaken with the results tabulated below.

Table 1

Comparative Cytotoxicity of Inhibitors of IMPD to P388 Cells in Culture

NSC	Inhibitor	ID_{50}(μM)
4728	Aminothiadiazole	600
32074	6-Azauridine	4.0
95941	4-Amino-1H-pyrazolo[3,4-d]-3-carbonitrile	70
105826	4-(Methylthio)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d] pyrimidine	170
107519	7-β-D-Ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine	<1
111702	3,5-Dihydroxyl-6-[6-(methylthio)-9H-purin-9-yl]-p-dioxane-2-methanol	2.0
112521	1,2-Diaminoinosine	485
116072	Mycophenolic acid	0.8
261726	3-Deazaguanine	2.5
286193	Riboxamide	3.6
289637	Bredinin	2.0

Experiments were next conducted to determine whether the nucleoside-transport inhibitor, nitrobenzylthioinosine, would reduce or abrogate the cytotoxic effects of riboxamide on this line of cultured leukemia cells. However, at concentrations of 25 μM to 1000 μM, the inhibitor had no infuence on the antiproliferative effects of 16 μM riboxamide.

Cytotoxicity of Riboxamide to Human Cells In Vitro. A collaborative study on the cytotoxicity of riboxamide to human tumor cells was instituted with Dr. Bach Ardalan, U.S.C. For these studies, primary explants of tumor biopsies from patients are being analyzed for their responsiveness to pharmacologically meaningful concentrations of the drug. Preliminary results with explants from three patients with malignant melanoma have indicated that riboxamide exhibits potent cytotoxicity (IC_{50}'s: 1.8-3.2 μM). By contrast, elsewhere in this report, it has been documented that 5 lines of human lymphoblasts in culture exhibit diverse sensitivity to riboxamide with IC_{50}'s ranging from 5-38 μM. Similarly, in last year's report, the sensitivity of cultures of human small cell carcinoma also was found to be exceedingly varied, with IC_{50}'s ranging from 1 to >1000 μM.

II. In Vivo Studies with Riboxamide

A. Pharmacokinetic Studies. In a preliminary study, the pharmacokinetics of riboxamide was determined at two doses in mice. An i.v. bolus of ^{3}H-riboxamide was given at 250 mg/kg (0.96 mmole/kg) and 25 mg/kg (0.096 mmole/kg) to pentobarbital-anaesthetized CDF_1 mice. Blood samples were taken from the carotid artery starting at 5 min and continuing for 6 hr. Plasma and RBC's were separated and analyzed individually. Metabolism to the carboxylic acid derivative and phosphorylated products was determined by paper chromatography of plasma samples using ascending development on Whatman 3MM paper with ethanol:

1 M ammonium formate, 70/30, v/v, as developing solvent. This work was carried out in the laboratory of Dr. Richard Cysyk.

Initial half-lives were short (11-21 min), followed by a β-phase half-life of 260-310 min. The amount of circulating metabolites at either dose was small: five percent or less of the circulating radioactivity was found as the carboxylic acid and no significant amount was found as phosphorylated products. The integral of the plasma concentration was 110 mM x min for the 250 mg/kg dose and 8.3 mM x min for the 25 mg/kg dose. This indicates that the drug exposure is directly proportional to the dose over the range of 25-250 mg/kg. The steady state volume of distribution is very large in all cases, about 300 x the total estimated volume of the mouse, which suggests that most of the drug is bound, sequestered and/or metabolized.

2. Riboxamide Pharmacokinetics in the Rat: A preliminary evaluation of the pharmacokinetic behavior of riboxamide was undertaken in three male Sprague Dawley rats weighing 430, 410, and 405 grams. The drug ([5-^{3}H]thiazole-nucleoside) was administered as an intravenous bolus infusion into the awake, but cast-restrained, cannulated animals at doses of 123,133, and 135 mg/kg, respectively. One rat was sacrificed at 3 hours, and two at six hours. A whole blood aliquot was obtained for total radionuclide activity before separation and freezing of plasma and red cells. Plasma studies included total plasma radionuclide activity, as well as paper chromatographic separation of the parent compound and its carboxylated and phosphorylated metabolites. Tissue samples were obtained at sacrifice for total radionuclide activity. The total body clearance of the parent riboxamide was 2 ml/min (1.3, 2.6, 2.1), which is less than one-half normal renal plasma flow, or glomerular filtration rate. The volume of the central compartment was 24 ml (20.3, 23.4, 27.3), or 58 ml/kg, near the normal blood volume of the rat (64 ml/kg). The plasma arterial integral (i.e., drug exposure) for riboxamide over 6 hours was 55 mM x min (49.1, 61.9). This represents about 79% of the total plasma radioactivity, and 47% of the total blood activity for that period. The peak plasma concentration of riboxamide occurred between 20 and 30 seconds and was 6 mM (6, 6.6, 4.6). The logarithmic decline in plasma concentration of riboxamide when described as a 3-component curve obtained by a least squares fit of the data is characterized by an initial rapid fall ($t_{1/2}$ 30 sec), a slower intermediate phase ($t_{1/2}$ 4 min), and a prolonged terminal phase of at least 2.5 hours ($t_{1/2}$ 156 min). The terminal half-life is likely to be much longer, since the dominant exponent obtained by a standard peel-off method gave a terminal half-life greater than 6 hours. The plasma concentration at 6 hours was 69 μM (62.5, 75.5), still well above the cell culture minimum inhibitory concentration of 5 μM. At steady state, the volume of distribution (V_{dss}) was calculated to be 758 ml (497, 998, 779). This is nearly twice (1.8x) the total potential volume of the rat and implies binding of the drug in some compartment(s). Tissue/plasma ratios for total radioactivity suggest greater concentration relative to plasma at 3 hours in liver, spleen, kidney, lung and heart, and at 6 hours in skeletal muscle, liver, spleen, kidney, lung, heart, and bone marrow (Table 2). It is likely that this distribution can be accounted for on the basis of drug excretion (kidney and liver) and red cell sequestration (spleen, in particular). At 3 hours, the brain contained only 15% of plasma radioactivity, whereas at 6 hours it had increased to about 80%. The accumulation of radio-

activity was seen in all organs tested except the kidney, which is the major organ of excretion, and may be accounted for on the basis of an accumulation of radiolabeled metabolites.

Table 2

Tissue to Plasma Ratios of Total Radioactivity in Select Organs of Rats Given Tritiated Riboxamide

Tissue	3 hr	6 hr	6 hr
Eye	0.31	0.88	0.93
Cortex	0.16	0.77	0.85
Pons/Medulla	0.18	0.59	0.60
Temporalis	0.87	1.90	1.73
Liver	1.91	5.07	3.87
Spleen	3.31	10.97	6.88
Kidney	9.61	3.24	3.01
Lung	1.81	2.34	2.22
Heart	1.53	2.22	2.18
B. Marrow	-	2.12	2.24

This work was carried out in collaboration with Dr. Gregory R. Harper and Dr. Clifford S. Patlak.

B. Biochemical Pharmacology of Riboxamide

1. Perturbation of nucleotide pools and metabolism of riboxamide in P388 tumors. As was mentioned, administration of riboxamide to BDF_1 mice bearing subcutaneous P388 leukemia provokes a sharp depression in the concentration of intratumoral guanine nucleotides and a correspondingly large expansion of the IMP pools. Measurements of IMP dehydrogenase in the tumors of treated mice reveals that this enzyme is inhibited in a dose-responsive way with ~50% inhibition engendered by the administration of the drug at a dose of 25 mg/kg and >90% inhibition by all doses >100 mg/kg; this range of doses (given daily for 5 days) is therapeutically active versus this transplanted leukemia. The inhibition of enzyme activity seen after a dose of 250 mg/kg reaches a maximum 120 min after treatment and has subsided substantially 8 hr after dosing; by 24 hr, enzyme activity is fully restored. In extracts of the tumors of mice given parenteral injections of the thiazole nucleoside, a potent inhibitor of IMP dehydrogenase is demonstrable; its concentration fluctuates in parallel with enzyme inhibition. The compound responsible for this effect has been isolated from homogenates of the tumor by dialysis and ion-exchange HPLC, and its presence monitored by enzyme inhibition assay. Chromatographically, the inhibitory principle exhibits a moderately strong net negative charge at pH 3, and elutes in the general vicinity of the nucleoside-5'-diphosphates; its absorption maximum in aqueous solution (pH 7) lies at 252 nanometers. Exposure of the molecule to snake-venom phosphodiesterase or nucleotide pyrophosphatase

destroyed its inhibitory potency, whereas other phosphoesterases were either less effective or inert. Since these results suggested that the anabolite might be a dinucleotide with a phosphodiester linkage of the kind found in NAD, attempts were made to synthesize such an NAD analog from the 5'-monophosphate of thiazole nucleoside and ATP-Mg^{++}, using a purified preparation of NAD pyrophosphorylase; modest yields were obtained of a compound with chromatographic, spectrophotometric and enzyme-inhibitory properties identical to those of the material isolated from P388 tumor nodules. This enzyme-synthesized material was radioactive when [^{3}H]ATP was used as co-substrate, and yielded both AMP and thiazole nucleoside-5'monophosphate on treatment with phosphodiesterase. It resisted attack by NAD glycohydrolase. Mass spectral analysis and nuclear magnetic resonance studies were compatible with a dinucleotide structure in which the nicotinamide of NAD has been replaced by thiazole-4-carboxamide, i.e., adenine-D-ribose-phosphate-phosphate-D-ribose-thiazole-4-carboxamide. Versus IMP dehydrogenase, the dinucleotide exhibited a K_i of ca. 0.5 μM and was competitive with NAD as the variable substrate. This work was carried out in collaboration with Dr. Robert Glazer, LMCB.

2. Metabolism of Riboxamide in Representative Organs of the Mouse. In conjunction with the pharmacokinetic studies of riboxamide in mice (cf. supra), an examination was made of the metabolism of the drug in a panel of the principal organs of that species. The results of this study are tabulated below:

Table 3

Metabolism of TR by mouse tissues one hour after an intravenous dose of 100 mg/kg (10 μCi) [5-^{3}H]TR

	Concentration of metabolites expressed as nmoles/g tissue				
Tissue	TR	TR-MP	TAD	TR-DP	TR-TP
Brain	9.3 ± 0.6	8.0 ± 2.4	0.2 ± 0.1	0.3 ± 0.1	0.04 ± 0.0
Liver	19.4 ± 6.2	26.5 ± 7.0	4.4 ± 1.5	3.3 ± 0.8	3.6 ± 0.8
Lungs	10.5 ± 2.4	12.5 ± 1.6	0.4 ± 0.04	0.1 ± 0.04	0.2 ± 0.03
Heart	20.6 ± 7.0	17.6 ± 0.2	3.0 ± 0.3	0.6 ± 0.0	0.2 ± 0.03
Spleen	30.7 ± 11.0	16.6 ± 3.7	0.5 ± 0.1	0.4 ± 0.01	0.4 ± 0.1
Pancreas	7.8 ± 1.1	5.1 ± 1.2	0.5 ± 0.05	0.3 ± 0.04	0.3 ± 0.03
Stomach	6.3 ± 1.2	2.3 ± 0.2	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.01
Small Intestine	8.6 ± 1.9	3.6 ± 0.9	0.4 ± 0.05	0.2 ± 0.1	0.3 ± 0.8
Large Intestine	11.6 ± 2.7	5.3 ± 1.4	0.4 ± 0.1	0.2 ± 0.03	0.04 ± 0.01
Kidney	16.4 ± 7.1	8.1 ± 0.4	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.01
Muscle	9.1 ± 1.6	10.8 ± 0.5	2.2 ± 0.2	1.0 ± 0.4	4.6 ± 2.7

C. Therapeutic and Antidotal Studies with Riboxamide

1. Since the bulk of available evidence has established that riboxamide acts via the production of a state of guanosine nucleotide depression, attempts have now been mounted to establish this contention by demonstrating that guanosine is capable of abrogating the antitumor activity of the drug. (It will be recalled for last year's report that guanosine does not alter the cytotoxicity of TR to P388 cells in culture; moreover, this year it was found that guanosine produced only partial reversal of the cytotoxicity of riboxamide to Lewis lung cells in culture. This surprising inefficacy prompted studies in vivo.) Nicotinamide was also examined because of its obvious structural relationship to TAD, and, more importantly because of its capability of reversing both the cytotoxicity and antitumor effects of 2-aminothiadiazole, another inhibitor of IMPD which undergoes anabolism to a dinucleotide. Lastly, allopurinol was examined in order to gain some insight into the role that the vastly augmented roles of hypoxanthine nucleotides caused by riboxamide might play in the drug's activity and/or toxicity. The results of this study are tabulated below.

Table 4

The Influence of Treatment with Nicotinamide, Guanosine or Allopurinol on the Survival of Mice Bearing P388 Leukemia and Treated with Parenteral Riboxamide

Regimen	Treatment	Dose (mg/kg)	Survival Period (mean days ± s.d.)	% T/C
Q1Dx1	Saline	-	10.8 ± 1.4	-
	TR	25	10.8 ± 1.4	100
		100	12.1 ± 1.1	112
		250	12.3 ± 1.2	116
		500	12.3 ± 2.0	114
Q1Dx5	Saline	-	10.8 ± 1.4	-
	TR	100	14.7 ± 0.7[1]	131
		200	17.3 ± 2.7[1]	160
		400	20.5 ± 1.9[1]	190
Q1Dx5	Saline	-	10.8 ± 1.4	-
	TR	200	17.3 ± 2.7[1]	160
	Nicotinamide	500	10.0 ± 2.8	100
	TR +	200		
	Nicotinamide	500	17.6 ± 2.0[1]	168
	Guanosine	500	11.4 ± 1.0	105
	TR +	100		
	Guanosine	500	13.6 ± 3.2	126
	TR +	200		
	Guanosine	500	13.1 ± 1.7[1]	121
	TR +	400		
	Guanosine	500	13.8 ± 0.7[1]	128
Q1Dx5*	Saline	-	12.2 ± 1.0	100
	Allopurinol	50	12.2 ± 0.8	100
	TR	400	18.1 ± 1.4	146
	TR +	400		
	Allopurinol	50	17.8 ± 0.6	141

[1] $p < 0.01$.
* separate experiment

It can be seen that 1) single dose riboxamide was therapeutically ineffective; 2) five daily doses of the drug produced dose-responsive, criterion-meeting activity; 3) guanosine co-administered with TR, sharply diminished the drug's cytotoxicity; 4) nicotinamide failed to nullify the activity of TR; 5) allopurinol neither nullified nor augmented the antileukemia action of the drug.

2. Attempts to improve the therapeutic activity of riboxamide by means of continuous Alzet infusions. In collaboration with Dr. David Vistica and staff, attempts have been made to improve the therapeutic activity of riboxamide versus the P388 leukemia by infusing the drug continuously for 7 and 14 day periods. Towards this end ~30 g CDF mice were given subcutaneous implants of $7x10^6$ P388 cells. Twenty-four hours later, Alzet 7 and 14 day mini-pumps containing doses of 2 g/kg (310 mg/ml, sterile) or 4 g/kg (620 mg/ml, sterile) were implanted in the peritoneum. Both pumps have a capacity of 200 µl; however the 7 day pumps deliver at a rate of 1 µl/hour, while the 14 day pumps deliver at a rate of 0.5 µl/hour. One negative-control group received saline in lieu of drug; a second negative-control group received no pumps. Auxiliary positive control groups received 5 daily i.p. injections of riboxamide at doses of 400 and 800 mg/kg or single i.p. injections at 2 and 4 g/kg, 24 hours after inoculation. A last control group was given riboxamide (1%) in the drinking water beginning 1 day after transplantation of the tumors and for the duration of the study. The results of this experiment follow:

Table 5

Efficacy of Riboxamide Administered by Intraperitoneal Alzet Infusions

	Mean Survival (days)	T/C	Mean spleen weight at necropsy (mg)
P388 control	11.33	-	393*
P388 control, with saline pump	11.33	-	353*
4 g/kg I.P.	3.33	29	-
4 g/kg 7 day pump	8.67	76	100
4 g/kg 14 day pump	9.33	82	95
400 mg/kg I.P. daily x 5	16.33	144	480*
2 g/kg I.P.	16.33	144	460*
800 mg/kg I.P. daily x 5	17.66	156	445*
1% thiazole, drinking water	18.66	164	70
2 g/kg 7 day pump	20.00	177	Day 11, 90; Day 24 & 25, 550
2 g/kg 14 day pump	26.33	234	465*

*Heavy spleen weights are indicative of leukemic infiltration.

Quite clearly, riboxamide given by infusion in intraperitoneal Alzet pumpts is therapeutically superior to the five daily treatment schedule. Since, in previous studies at NCI and elsewhere, treatment for 9 days yielded results equivalent to those obtained in 5 day treatment regimens, it is the presumption that the T/C seen with the 7 day pumps (177) is genuinely superior to that realized from 5 daily injections (144). However, to examine this point directly, the 7 and 14 day pumps will be directly compared with 7 and 14 daily injections. This work was carried out in conjunction with Dr. David Vistica and his staff.

D. Mechanisms of Resistance to Riboxamide. In culture, a line of the P388 leukemia has been rendered resistant to riboxamide such that the IC_{50} concentration of the drug has risen from ~3.6 μM to 10 mM. This line shows no depression of GTP pools on exposure to riboxamide (up to 1 mM) and forms virtually no TAD from the drug. The variant can be propagated readily in vivo and maintains full resistance to riboxamide without selection pressure.

Paradoxically, this resistant line exhibits striking collateral sensitivity to a diverse group of cancer chemotherapeutic drugs. Thus, whereas agents such as methotrexate, thioguanine, actinomycin D, 5-fluorouracil, procarbazine, cytarabine and daunorubicin produce only modest ILS's in the parent line, they are, without exception, curative to the riboxamide-resistant variants at their optimal dose. This finding is suggestive of a radical genomic change in the P388/TR. Since the major enzymologic change in the P388/TR line has proven to be a virtual deletion of NAD pyrophosphorylase (cf. infra), it is tempting to speculate that an impaired ability to biosynthesize pyridine nucleotides is causative of the remarkable collateral sensitivity of this mutant line to cancer chemotherapeutic drugs. It also follows that effective inhibition of NAD pyrophosphorylase ought to unmask comparable drug sensitivity in even riboxamide-sensitive tumors. In other words, this enzyme would appear to be an important target for chemotherapeutic attack in its own right.

III. Chemical Studies with Riboxamide

A. Chemical Synthesis of TAD - Purification of Product. The chemical synthesis of TAD is presented elsewhere in this report from another section of the Laboratory of Medicinal Chemistry and Biology. The present resume will outline the steps taken by the Biochemistry Section to assist in the purification of this dinucleotide from heterogeneous reaction mixtures constituted according to the method of Moffet and Khorana. As a first step towards these purifications, the reaction mixtures were chromatographed on short columns of BAX4 resin using linear gradients of 0-1 and 0-2 M ammonium formate. Satisfactory separations were achieved, but the peaks were broad and occasionally skewed. Attempts were made, therefore, to partially repress the ionization of TAD, and therefore facilitate its elution by means of an acid eluent. Eventually, it was found that a linear gradient from H_2O to 2 M ammonium formate plus 2.6 M formic acid accelerated the elution of TAD, improved the resolution of it from co-eluting contaminants and sharpened peaks. This system, then, was deployed ultimately in the isolation of homogeneous TAD prepared by chemical synthesis exclusively. The properties of this synthetic molecule were, in all respects assessed, identical to those of TAD synthesized in vivo and, by enzymatic means, in vitro. As the more salient cases in point: the K_i's of enzymatically synthesized TAD = $\sim 1 \times 10^{-7}$ M; chemically synthesized TAD purified by the technique presented above, yielded an identical constant; in addition, the enzymically and chemically synthesized dinucleotides were essentially identically cytotoxic to P388 cells in culture with K_i's of ~4 μM in both cases.

B. Enzymatic Synthesis of Further Analogs of NAD Containing the Thiazole Nucleus. Incubation of NAD pyrophosphorylase at a fixed concentration of 3 mg/ml with 0.01 M riboxamide 5'monophosphate and either ITP·$MgCl_2$ or GTP·$MgCl_2$ leads to the generation of novel dinucleotides analogous to TAD but with correspondingly modified purine moieties. Following exhaustive hydrolysis of precursor

phosphoesters with alkaline phosphatase, the products have been purified essentially to homogeneity by ascending paper chromatography using ethanol: 1 M ammonium acetate (70/30, v/v) as the developing solvent; their chromatographic properites have also been examined on HPLC. Versus a partially purified preparation of IMPD, the hypoxanthine-containing dinucleotide (TIP) engendered 80% inhibition (NAD = 1 mM) at a concentration of 1 μM while the guanine-containing metabolite was ~10-fold less potent than this. Since the K_i of TAD itself is ~$1x10^{-7}$ M, it can be appreciated that these variant dinucleotides are inferior to it on kinetic grounds.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07124-02 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Methods Development

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In order to permit accurate measurements of select enzymes critical to the therapeutic activity of riboxamide, new analytical approaches to the assay of IMP dehydrogenase, 'riboxamide kinase' and 'TAD synthetase' (NAD pyrophosphorylase) have been undertaken.

Methods Employed:

A. Analysis of TR-Kinase Activity in Tumors. Groundwork is being done on the kinase responsible for the first step in the anabolism of riboxamide. Some preliminary information on this score has been accumulated: the drug is fully active versus murine leukemias deficient in adenosine kinase (data of Dr. R. Johnson); in addition, even at concentrations as high as 1 mM, riboxamide does not significantly inhibit a preparation of pyrimidine nucleoside kinase (data of Jean Karle and Robert Glazer). These results, viewed together with the structural similarity of the drug to the nucleoside of nicotinamide warrant the working hypothesis that it is phosphorylated by pyridine nucleoside kinase. In order to permit a test of this hypothesis, Dr. V. Marquez and staff are preparing nicotinamide riboxamide for use as a putative competitive inhibitor of the activation. A preliminary assay of this kinase has been carried out as follows.

Extracts of tumors were prepared by homogenizing freshly excised P388 tumor nodules with 0.1 M Tris-HCl buffer, pH 7.6 containing 0.5 mM EDTA and 1 mM dithiothreitol. The homogenate was centrifuged and the supernatant dialyzed against the homogenizing buffer for 3 hrs at 4° with constant stirring. The dialyzed enzyme was used for the assay of TR-kinase activity. The reaction mixture in a total volume of 20 µl contained: 10 µl of [5-^{3}H]TR, (7.2 nmoles, 4 µCi); 5 µl of ATP-$MgCl_2$ in 0.05 M Tris, pH 7.5 (100 nmoles each); and 5 µl of dialyzed enzyme. The reactants were mixed by a brief centrifugation at 12000 g for 10 sec and incubated at 37° for 10 or 20 min. The reaction was terminated by heating at 95° for 2 min, after which the vessels were spun at 12000 g for 2 min; 5 µl of the supernatant was spotted on Whatman 3MM paper, overspotted with 5 µl of TR and TR-MP (10 mM each) and subjected to ascending chromatography for 16 hr at room temperature (25°) using a 80% tetrahydrofuran: 20% water system. The chromatograms were dried and spots corresponding to TR-MP were analyzed for radioactivity by scintillation spectrometry. The P388 leukemia sensitive to TR (P388/S) had a specific activity of 0.74 nmoles/mg protein/hr, while the P388 leukemia - resistant to TR (P388/TR) exhibited a specific activity of 0.37 nmoles/mg protein/hr.

Pursuant to this result, TR5'MP levels were measured in vivo and in vitro in P388 cells sensitive and resistant to riboxamide. In accord with the specific activities of the kinase given above, it was found that P388/S accumulated 3 times as much of the monophosphate as P388/TR in vivo 1 hr following a dose of 100 mg/kg; [1.35 nmoles/g versus 0.43 nmoles/g]. However, in vitro after 2 hr exposure to 10 µM riboxamide, the resistant line paradoxically accumulated twice as much of TR5'MP as the parent (2.3 pmoles/10^6 cells versus 1.1 pmoles/10^6 cells).

B. Analysis of NAD Pyrophosphorylase in Liver and Tumors. NAD pyrophosphorylase, which catalyzes the synthesis of NAD from ATP·$MgCl_2$ and nicotinamide mononucleotide, NMN, is located principally in the nucleoplasm, often in tight association with the nucleolus or nuclear chromatin. Since this enzyme is probably responsible for the synthesis of TAD, measurements of its specific activity in the tumors of subjects given the drug were felt to be of predictive importance. For this reason, too, a straightforward and reliable assay was desirable. Initial attempts to measure NAD pyrophosphorylase utilized liver

Specific Activity of NAD Pyrophosphorylase in Cultured Mouse Cells S and R to Riboxamide

Line	specific activity (nanomoles/mg/hr) with NMN	with TR5'P
P388/S	5.2	2.1
P388/R	<0.1	<0.1
Lewis Lung/S	5.4	2.6
Lewis Lung/J	0.6	0.4

* Groups of mice bearing the appropriate s.c. tumors were injected IP with either NSC 286193, 400 mg/kg or normal saline. After four hours the mice were sacrificed, the tumors removed and homogenized in 0.01 M Tris buffer, pH 7.0, containing 1 mM dithiothreitol. Aliquots of whole homogenates were used for the measurement of NAD pyrophosphorylase in this experiment.

Quite plainly, both resistant lines exhibit markedly depressed capabilities of synthesizing NAD from NMN. It will be the task of future studies to determine whether NAD pyrophosphorylase is the sole or even principal catalyst for the formation of TAD from TMP; at the moment, the specific activities reported above are supportive of the notion that this enzyme plays a pivotal role in the anabolism of riboxamide.

C. Substantive Improvements in the Tritium Release Assay for IMP Dehydrogenase. In last year's annual report a tritium-release assay for IMP dehydrogenase was described using [2,8-^{3}H]IMP as substrate. The preponderance of the label of carbon-8 seriously diminished the sensitivity of the assay. For that reason, substrate with single-site tritium label has now been prepared from 2 bromo-hypoxanthine and the assay restructured as follows:

[2-^{3}H]Inosinic acid ([2-^{3}H]IMP) has been biosynthesized in good yield from [2-^{3}H]hypoxanthine via the action of a partially purified preparation of hypoxanthine/guanine phosphoribosyl transferase from mouse brain. The product was purified in one step by ascending paper chromatography, and used to assess the activity of IMP dehydrogenase. To conduct the assay, tritiated substrate is admixed with enzyme in a final volume of 10 µl; NAD is present to serve as co-factor for the reaction, uridine to retard the phosphorolysis of any inosine formed (by the phosphatasic decomposition of IMP) and allopurinol to inhibit the oxidation of any hypoxanthine generated. After an appropriate period of incubation, the $[^{3}H]_2O$ arising from the oxidation of tritiated IMP is isolated by quantitative microdistillation. Performed as described, the assay is facile, sensitive and accurate, with the capability of detecting the dehydrogenation of as little as 1 pmole of [^{3}H]IMP. Using it, measurements have been made of IMP dehydrogenase in a comprehensive panel of mouse organs: of these, pancreas contained the enzyme at the highest specific activity.

because the enzyme abounds in that organ. With whole homogenates, notable activity could be detected using a spectrophometric assay for pyridine nucleotides; however, this activity was non-linear with time and protein. HPLC measurements of substrates after typical periods of incubation revealed that both the ATP and NMN were destroyed, most likely by phosphomonoesterases and phosphodiesterases in the membrane fragments present in such homogenates. Attempts were next made to capitalize on the observation that salt solutions rapidly rupture the association of NAD pyrophosphorylase with chromatin. Homogenization of mouse liver in solutions of buffered NaCl of graduated molarities revealed that 0.25 M salt was effective in releasing >90% of the enzyme to a 105,000 g supernatant, while inhibiting the reaction only by ~30%; 0.5 and 1.0 M NaCl were strongly inhibitory under the analytical conditions used. Since nuclear isolation may not be practicable with frozen clinical specimens, the following approach has been adopted on the basis of the foregoing results: tissue is homogenized in 0.25 M glycyl glycine buffer containing 0.6 M nicotinamide (to inhibit NAD oxidation via glycohydrolases), 0.25 M NaCl and 0.06 M $MgCl_2$. The homogenate is centrifuged for 1 hr at 105000 g and the supernatant used for assay. If abundant activity is anticipated, the spectrophotometric assay for NAD is deployed. With most tumors, however, low activities are encountered, so that a radiometric assay has been devised: in a final volume of 25 µl are admixed 1 µCi of 8[^{14}C]ATP, S.A. ~39 µCi/µmole; 5 µl of 0.03 M NMN·$MgCl_2$ or of riboxamide monophosphate·$MgCl_2$; and to initiate the reaction, 5 µl of high-speed supernatant. After 10 or 20 minutes at 37°, the reaction is arrested by 1 min of heating at 95°. Protein is precipitated by a brief centrifugation at 12000 g and nucleotides (but not dinucleotides) are hydrolyzed overnight with alkaline phosphatase (AP) at a final concentration of 0.015 mg/ml and a final pH of 9.0. Replicate vessels receive 5 µl of phosphodiesterase from venom in addition to AP; these provide an absolute indication of the net amount of pyridine nucleotide synthesized. After 1 hr at room temperature, the vessels are recentrifuged and 50 to 100 µl of supernatant are spotted on Whatmann 3MM paper and subjected to ascending chromatography overnight in 500 ml of ethanol:1M ammonium acetate, 70/30, v/v. The pyridine or thiazole dinucleotides are identified under UV light, excised and counted by scintillation spectrometry. This radiometric assay responds in a proportional way to dilute supernatants and is linear with time for ~20 minutes with most extracts.

Using it, measurements have been made of NAD pyrophosphorylase in P388 tumors wholly resistant to riboxamide as well as in their native or sensitive counterparts:

D. A Radiometric Technique for Measuring Ribose-1-Phosphate and Deoxyribose-1-Phosphate. In the salvage of purine or pyrimidine bases, sugars can be re-acquired in one of two ways: via a phosphorolytic reaction catalyzed by nucleoside phosphorylase in which R-1-P(or dR-1-P) is the substrate and a nucleoside is the product; and, via a phosphotransferase reaction in which PRPP is the substrate and a complete nucleotide is the product. Not only are these reactions of great significance to intermediary metabolism, but they also play a role in the activation of a large number of chemotherapeutic drugs, most notably 5-fluorouracil.

It has recently been reported that transplantable tumors sensitive to this drug contain an elevated concentration of PRPP. Since this elevation occurs in that part of the substrate-saturation curve (i.e., in the vicinity of the K_m) where comparatively small changes in [S] can produce major changes in V, it might well influence the anabolism of 5-FU to its therapeutically active forms, FdUMP and/or FUTP.

By analogy to the case with PRPP, it seemed reasonable that the intratumoral concentrations of R-1-P and dR-1-P might also be important determinations of the anabolic route taken by 5-FU. For example, if the pools of dR-1-P were, or could be caused to be, expanded, then the DNA-directed toxicity of this drug would be promoted, via an enhanced generation of FdUMP. In order to test this hypothesis, then, we have developed an enzymatic technique for measuring both of the aforementioned pentoses, and used it to assess their concentrations in a select panel of tumors whose responsiveness to 5-FU is known.

In practice, the assay is carried out by reacting R-1-P and/or dR-1-P with [2-^{3}H]hypoxanthine through the catalytic action of crystalline purine nucleoside phosphorylase. After an appropriate period of incubation, unreacted [2-^{3}H]-hypoxanthine is quantitatively dissipated, as HO[^{3}H]↑, via the action of xanthine oxidase. The residual radioactivity can then be quantitated as such, to yield an estimate of the Σ R-1-P·dR-1-P, or resolved into its constituent elements, [^{3}H]1R and [^{3}H]1dR by ascending paper chromatography or Whatman 3MM paper using butanol:acetic acid:water, 2/1/1 as developing solvent. Since the specific activity of the tritiated hypoxanthine is 6200 μCi/μmole, the method offers sensitivity to the picomole level with the pure sugars. However, attempts to apply it to extracts of murine tumors have yielded values that are almost certainly spuriously high. This phenomenon is probably due to the fact that PNP catalyzes a freely reversible reaction, the equilibrium position of which will be strongly influened by endogenous nucleosides and deoxynucleosides. These latter intermediary metabolites, can moreover, enter into transglycosidations with HX. Therefore, efforts have been directed to the removal of interfering nucleosides by a preliminary chromatography of extracts from tumors on Dowex 50 or CM Sephadex.

Drug Design and Chemistry Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1981 - September 30, 1982

Scope

The objectives of the Drug Design and Chemistry Section of the Laboratory of Medicinal Chemistry and Biology are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, (4) the quantitative and qualitative analysis of anticancer drugs in physiological fluids and (5) the promotion of the interdisciplinary approach to the synthesis and evaluation of rationally designed drugs by means of collaboration between chemical and biological scientists.

Staff

The staff consisted of two senior scientists, one scientist, one NIH Visiting Scientist, one IPA, four postdoctoral fellows, and one secretary.

Summary of Accomplishments

Approximately seven man-years of effort were devoted to the intramural research described in Individual Project Reports ZO1-CM-03580 and ZO1-CM-03581 during the period covered. Several compounds developed somewhat earlier in this laboratory progressed toward clinical trial. AZQ (NSC-182986) continued Phase II trials at several institutions. This compound appeared to have some effectiveness against human brain tumors. Our research involvement has continued with this drug through collaboration in the clinical trial at NIH and the BCRP. Spirohydantoin mustard (NSC 172112) is scheduled to complete toxicology this year prior to a Phase I clinical study. Toxicology was completed on dihydro-5-azacytidine (NSC 264880) during the current fiscal year and an IND has been opened. A new compound from this Section, ara-AC (NSC 281272), achieved Decision Network 2A status this year. Once formulation research has been completed, this compound, which is active against all three xenograft tumor panel models, will begin toxicological evaluation prior to clinical trial.

Combination of what is currently the most potent cytidine deaminase inhibitor, a diazepinone riboside developed in this laboratory (NSC 322096), with ara-C produced increases in T/C values against murine L1210 leukemia comparable to those obtained with three times the dose of ara-C alone. The dose of the inhibitor was ten times lower than that of tetrahydrouridine required to produce a comparable increase in T/C values. The stereochemical disposition of the hydroxyl and amino groups in the transition state of cytidine deaminase was studied by using specific diazepinone nucleoside inhibitors bearing hydroxyl or amino groups with different stereochemical orientations. These compounds were used as probes to determine the preferred orientation of the hydroxyl and amino groups during the enzymatic reaction. Some novel 4-(2',3'-O-isopropylidene-β-D-ribofuranosyl)-O^5,5'-cyclo-7,7-dihalo-2,4-diazabicyclo[4.1.0]heptane-

3-one nucleosides were synthesized and studied for ring expansion to the corresponding diazepinone nucleosides. A new and efficient synthesis of 3-(β-D-ribofuranosyl)uracil (isouridine) was developed. The β-D-ribofuranosyl and β-D-arabinofuranosyl derivatives of antitumor active 2-methyl-3-hydroxy-4-pyridone and 3-hydroxy-2-pyridone were synthesized and shown to be cytotoxic in vitro against P-388 leukemia. The ribofuranosyl analog of 2-methyl-3-hydroxy-4-pyridone proved to be more potent than the parent base in this bioassay. The corresponding O-nucleoside of antitumor active 2-pyrimidinone riboside was synthesized and studied as a possible prodrug. The structural characterization and chemical synthesis of a fraudulent NAD analogue, which is an active metabolite of the antitumor agent 2-β-D-ribofuranosylthiazole-4-carboxamide, was accomplished. This dinucleotide, appropriately abbreviated TAD, contains thiazole-4-carboxamide in place of nicotinamide and acts as a potent inhibitor of IMP dehydrogenase. A new mass spectrometric technique for analysis of non-volatile compounds, fast atom bombardment (FAB) ionization and high resolution Fourier transform NMR were employed to help elucidate the structure of TAD.

A detailed study of the plasma and CSF pharmacokinetics of AZQ in conjunction with BCRP Phase I/II clinical trials was completed using previously developed HPLC techniques. Comparison of plasma and CSF areas under the entire concentration-time curves indicated a high degree of drug availability to the CSF. Indeed, when the extensive plasma protein binding (79 ± 1% at 1 μg/ml) was taken into account, free plasma AZQ was seen to be completely available to the CSF. The HPLC assay for AZQ was further refined to increase its efficiency and sensitivity. The improved assay was applied to study both infusion and post-infusion pharmacokinetics in high grade glioma patients in the NCI-MB Phase II trial. No evidence was found for a third elimination phase with an extended half-life after analysis of long post-infusion time samples in this or the BCRP study. Intact AZQ could also be measured in both brain tumor tissue and tumor cyst fluid of patients treated pre-operatively with the drug. The research area dealing with the quantitative aspects of structure-activity correlations has been transferred to the newly created Information Technology Branch.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03580-13 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Chemical Research in the Development of New Anticancer Drugs

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. The following topics are of current interest: (1) pyrimidine nucleosides as antitumor agents and transition-state enzyme inhibitors, especially inhibitors of cytidine deaminase and cytidine triphosphate synthetase, (2) pyridone nucleosides as antitumor agents, (3) diazepinone nucleosides as antitumor agents, (4) dinucleotide analogs structurally related to NAD as inhibitory agents of inosine monophosphate dehydrogenase, (5) N-chloroethyl derivatives of 2- and 4-pyridones as antitumor agents, and (6) antimelanoma agents based on catechols.

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:

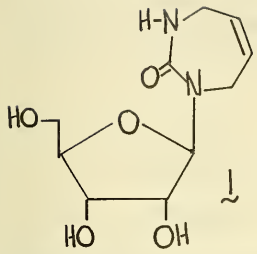
1. The design and synthesis of transition-state inhibitors of the enyzme cytidine deaminase.
2. The design and synthesis of transition-state inhibitors of the enzyme cytidine triphosphate synthetase.
3. The design and synthesis of pyridone and diazepinone nucleosides as anti-tumor agents.
4. The design and synthesis of dinucleotide analogs as inhibitors of the enzyme inosine monophosphate dehydrogenase.
5. The design and synthesis of pyrimidine O-nucleosides as potential prodrugs of active pyrimidines.
6. The design and synthesis of N-chloroethyl derivatives of 2- and 4-pyridones as active antitumor agents.
7. The design and synthesis of agents specific for melanoma.

Major Findings:

A. Nucleosides

1. Transition-State Inhibitors of Cytidine Deaminase:

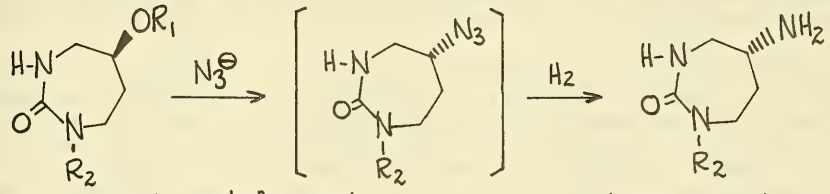
a. Ara-C Combination Studies (Dr. Marquez): As reported last year, nucleoside 1 selected for drug combination studies with ara-C showed no improvement over ara-C alone when studied in S-180 cells in culture despite its potent inhibition of cytidine deaminase (K_I = 2 x 10^{-8} M). In vivo studies were conducted in L1210 bearing mice with the idea that if the host's enzyme played a significant role in the degradation of ara-C, compound 1 would modify its pharmacokinetics in a favorable manner. In collaboration with Dr. Vistica (LMCB), a system to test this possibility was developed. First a dose-response curve was established for ara-C given ip daily for 9 days in doses ranging from 0.1 to 10 mg/kg. The ara-C dose selected for the drug combination studies was 3 mg/kg which produced a T/C of 136. The next higher dose of 10 mg/kg produced T/C values of 160. The results of the combination studies with either nucleoside 1 or tetrahydrouridine (THU) are shown on the following page.



ara-C (dose)	Inhibitor (dose)	T/C
3 mg/kg	-	136
10 mg/kg	-	160
3 mg/kg	THU (1 g/kg)	166
3 mg/kg	1 (100 mg/kg)	161

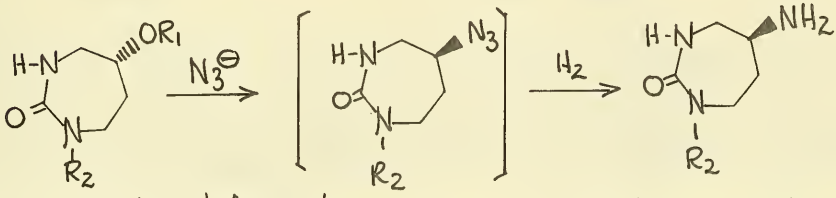
These results point out that the administration of ara-C at 3 mg/kg in the presence of either inhibitor is equivalent to the next higher dose of ara-C. Both 1 and THU are inactive as antitumor agents. The ten-fold difference between these two inhibitors in favor of 1 correlated well with the ten-fold difference of their K_i values against cytidine deaminase. New schemes of administration of ara-C + 1 are under development.

b. Mechanism of Action of Cytidine Deaminase (Dr. Marquez): In order to study the influence of the stereochemical disposition of the attacking hydroxyl and departing amino groups in the transition-state of cytidine deaminase, the amino counterparts of the very active hydroxy-1,3-diazepinone nucleoside inhibitors 2a and 3a were synthesized. Since the synthesis proceeded through a SN_2 displacement reaction of the mesylated alcohols by an azide nucleophile, an inversion of configuration occurred at the chiral center of the 1,3-diazepinone ring.



2a, R_1 = H ; R_2 = β-D-ribofuranosyl
b, R_1 = O_2SCH_3 ; R_2 = β-D-ribofuranosyl

4, R_2 = β-D-ribofuranosyl



3a, R_1 = H ; R_2 = β-D-ribofuranosyl
b, R_1 = O_2SCH_3 ; R_2 = β-D-ribofuranosyl

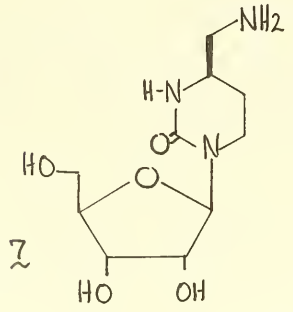
5, R_2 = β-D-ribofuranosyl

The results of the inhibitory assay against cytidine deaminase were as follows:

Compound	K_i (pH 7)	K_i (pH 9)
2a	1.6×10^{-8}M	-
3a	4×10^{-7}M	-
4	1.8×10^{-4}M	3.5×10^{-5}M
5	1.8×10^{-6}M	2×10^{-7}M

The amino compound 4, derived from the most active alcohol 2a, but with the inverted configuration at C-5, was the least active of the amino pair. On the contrary, compound 5, which originated from the less active alcohol 3a, produced the most potent amine inhibitor. Due to the increase in basicity of the amino analogs 4 and 5 in relation to cytidine, the assay was also conducted at a higher pH to insure that the amino groups would not be protonated. As it can be observed, the higher pH of the reaction was associated with a more powerful inhibition in the amino analogs 4 and 5. The results suggest that in the transition-state of the deamination reaction, the would-be "leaving group" (the amino group) is not as important for binding as the incoming hydroxyl group. If binding to the leaving group were very important, compound 4, having the amino group in the opposite configuration of the most active alcohol 2a, should have been the most active of the amino compounds. Keeping in mind the inherent differences in binding between hydroxyl and amino groups, the dominant factor for good activity appears to be the specific stereochemistry of the substituent (amino or hydroxy) at C-5. The differences between 2a and 5 simply revealed that the amino group is a poor isostere of the hydroxyl group in this instance.

As a by-product of this investigation, structures 7 and 8 were synthesized resulting from a newly discovered ring-contraction of diazepinone nucleosides. These compounds were generated during the azide displacement of the mesylated alcohols 2b and 3b via an intramolecular displacement of the C-5 mesylate by the N-3 nitrogen, followed by azide ring opening of the transiently formed aziridinium cation. The separation of these compounds from their seven-membered isomers was accomplished at the azide stage of the synthesis. Catalytic hydrogenation of all azides produced the corresponding amino compounds. Compounds 7 and 8 also possessed cytidine deaminase activity. The K_i values at pH 9 were 5×10^{-6}M and 2.5×10^{-6}M, respectively.



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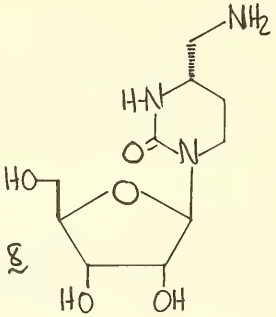

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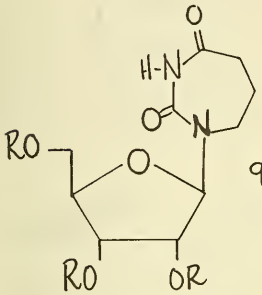
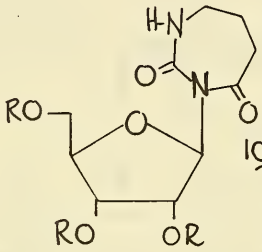
c. Other Compounds of Interest as Cytidine Deaminase Inhibitors (Dr. Marquez): Efforts towards the synthesis of compounds 9a and 10a continued. In an attempt to generate these compounds under mild conditions that would not cleave the highly reactive 4-keto-1,3-diazepinone ring, the per-acetylated precursors 9b and 10b were made and characterized. Unfortunately, the attempted deblocking



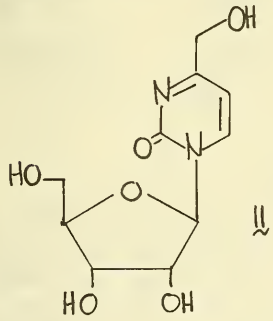
9a, R = H
b, R = $OCCH_3$
c, R = OCC_6H_5



10a, R = H
b, R = $OCCH_3$
c, R = OCC_6H_5

encountered the same fate as the previously reported benzoylated analogs 9c and 10c. Only deblocked ring-opened nucleosides were obtained even at low temperature.

2. 4-Hydroxymethyl and 4-Phosphonomethyl Pyrimidine Nucleosides as Potential Inhibitors of Cytidine Triphosphate Synthetase (Mr. Fuller and Dr. Marquez): Following the discovery of the ring contraction mechanism previously described (A.1.b.) which led to the synthesis of 7 and 8, several target compounds have been designed as transition-state inhibitors of cytidine triphosphate synthetase:



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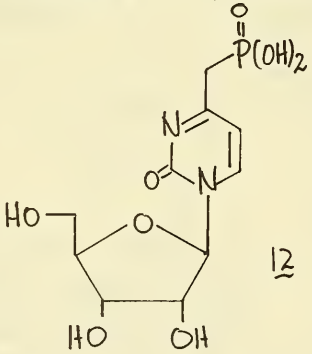
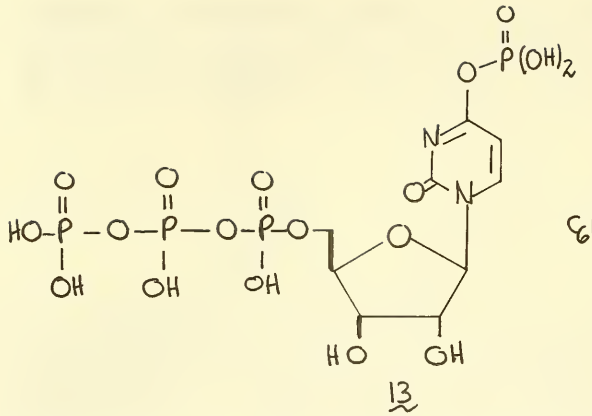


12

This enzyme appears to be crucial during malignancy and presents itself as a very attractive target for drug design. The antitumor compound 3-deazauridine once metabolized to the triphosphate level behaves as a good inhibitor of this enzyme. The target structures 11 and 12 are the results of a structural analysis of the postulated transition-state of this reaction (13) in which the 4-position of the pyrimidine ring of UTP is activated by ATP.



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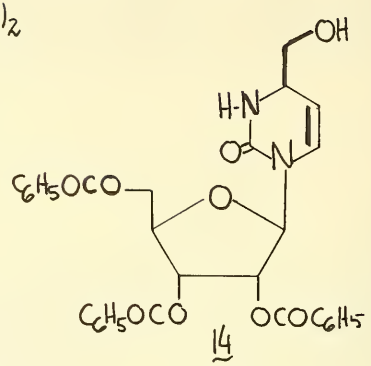
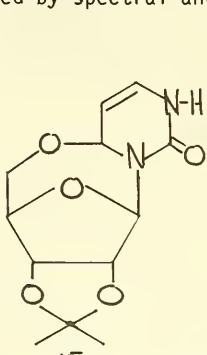


14

The present work has recently begun and efforts are underway towards the synthesis of our first key intermediate 14.

3. Novel Diazepinone Nucleosides via Ring Expansion Reactions (Drs. Rao and Marquez): Starting with the very versatile cyclonucleoside 15, a family of new compounds has been generated via a dihalocarbene insertion reaction. The yields obtained have been excellent and the products have been fully characterized by spectral and analytical means.



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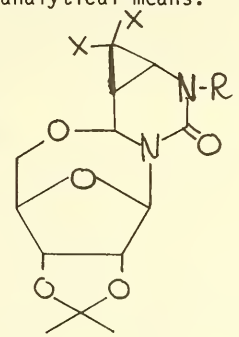
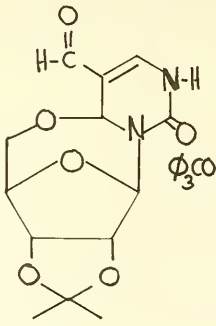


16a, R = $CH_2\emptyset$, X = Cl
b, R = $OC\emptyset$, X = Cl
c, R = H, X = Cl
d, R = $OC\emptyset$, X = Br
e, R = H, X = Br



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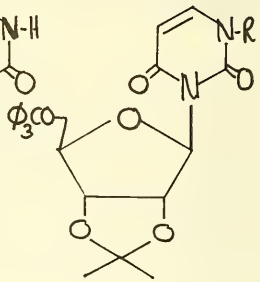
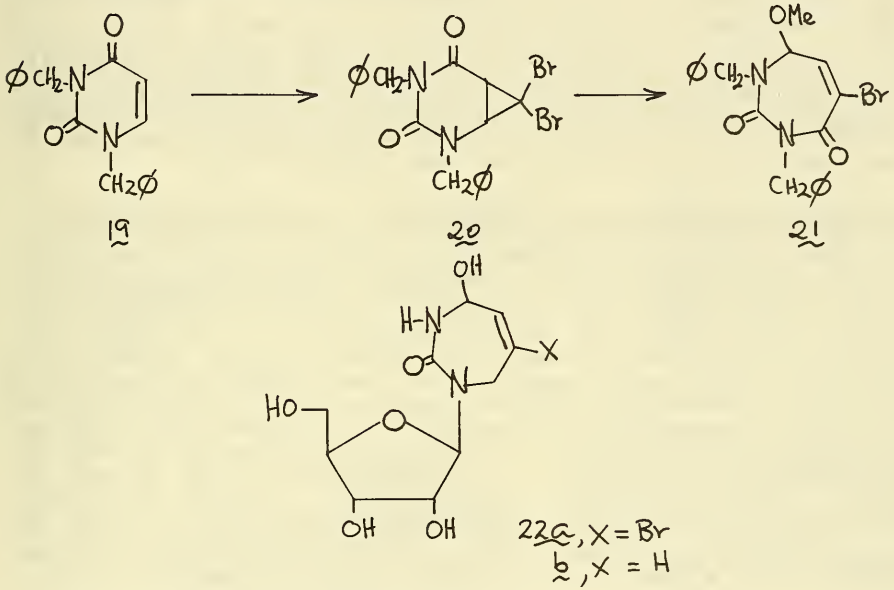


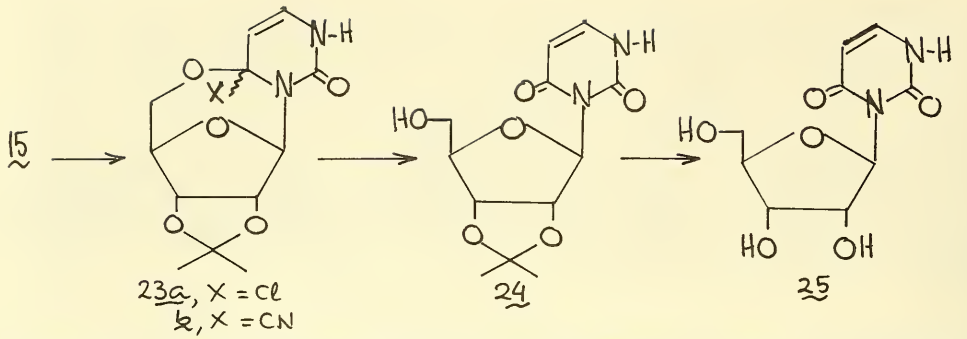
18a, R = $CH_2\emptyset$
b, R = $OCCH_3$

Compounds 16a-16e resisted all attempts to ring expand under a variety of conditions. When the nitrogen unprotected analogs 16c and 16e were treated with base (methoxide or guanidine) the dihalocyclopropyl ring opened to give the aldehyde 17 instead of the ring expanded product. The lack of success

of these reactions was attributed to the rigidity of the molecule imparted by the O^6,5'-cyclonucleoside structure of 16a-16e; therefore, compounds 18a and 18b were synthesized to be used as reactants for the dihalocarbene procedure. Inexplicably, 18a and 18b proved very unreactive toward dihalocarbenes and only starting material was recovered. In view of these results, it was decided first to prepare the ring-expanded aglycons and later to couple them with the desired sugar halides. Starting with protected uracil 19, the ring-expanded diazepinone 21 has been recently obtained. Efforts are underway to remove the protective benzyl groups by conventional methods. The resulting deprotected diazepinones will be coupled with the reactive sugar halides by a procedure developed earlier in this laboratory. Target compounds 22a and 22b are expected to be synthesized.

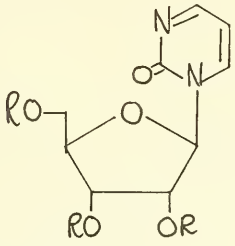


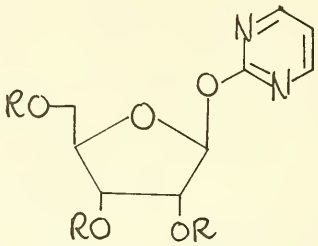
4. New Synthesis of 3-(β-D-Ribofuranosyl)uracil (Isouridine) (Drs. Rao and Marquez): Our previously mentioned versatile cyclonucleoside 15 (A.3.) presented to us the possibility of generating isouridine (25) in a regiospecific manner. Earlier syntheses of this compound invariably produced very low yields of isouridine at the expense of the more favorable isomer uridine. Other reported syntheses have made use of elaborate protective and deprotective steps. Isouridine was of interest to us for our ring-expansion project discussed previously where it served as the starting material for the preparation of compounds 18a and 18b. In addition, we noticed that isouridine had never been submitted for testing at the NCI. Our synthesis from 15 proceeded very well and in good yield. Additionally, two novel intermediates were generated (23a and 23b) which possessed unusual chemistry. It is expected that these compounds may also exhibit some interesting biological properties. Isouridine obtained by this method was identical in all respects to the material reported from earlier



syntheses. A sample was submitted for in vivo screening. However, cytotoxicity studies performed in collaboration with Drs. Cooney and Jayaram (LMCB) indicated that isouridine was devoid of any cytotoxicity against P388 cells at concentrations as high as 500 μM. Likewise, isouridine failed to show any substrate or inhibitory properties with crude uridine kinase from P388 cells.

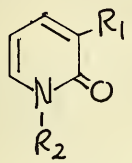
5. Synthesis of O-Nucleosides of 2-Pyrimidinones (Drs. Gebeyehu and Marquez): Compound 26a (NSC 309132), originally studied as a cytidine deaminase inhibitor, has been confirmed active against P388, L1210, B16 and MX-1 breast xenograft. It has been proposed (Votruba, Holy and Wightman, Biochim. Biophys. Acta 324: 14-23, 1973) that in bacteria, 26a is transported across the cell membrane where it undergoes cleavage to release the base intracellularly. Subsequently, the 2'-deoxynucleotide is formed and behaves as a potent inhibitor of thymidylate synthetase. It is not known, however, if the same pathway is operative in mammalian cells. Nevertheless, our past experience with the chemical transformation of O-nucleosides into N-nucleosides, via an intermolecular reaction with excess sugar halide, prompted us to investigate the use of O-nucleosides as carriers of the active aglycon. It was expected that inside the cell the O-nucleoside would react with active sugar phosphates (i.e. PRPP), as it reacted chemically with the sugar halides, to generate the corresponding nucleotide via a similar intermolecular reaction. In this fashion, the nucleotide would have been formed directly without the intervention of enzymatic kinases.



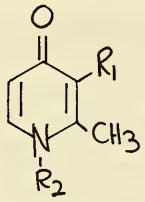


Choosing an active moiety of a cytotoxic N-nucleoside was an important prerequisite to test this idea. Consequently, compounds 27a and 27b were synthesized and tested for in vivo activity. The protected O-nucleoside 27b was of special interest because of its greater lipophilic character and because the 2'-O-acyl group was expected to facilitate cleavage of the O-nucleoside through an anchimerically assisted process. Results with 27a and 27b showed no in vivo activity. The possibility that the compounds are cleaved too rapidly before entering the cell or not cleaved at all is being investigated. This study will be extended to O-nucleosides of other active pyrimidines such as 5-FU and 5-fluoropyrimidinone.

6. Synthesis of 2- and 4-Pyridone Nucleosides (Drs. Mao, Marquez and Driscoll): Previous work in our laboratory demonstrated that 2- and 4-pyridone analogs 28a,b and 29a,b possessed substantial P388 activity. A program designed to study the corresponding ribo and arabinofuranosides of these active aglycons was initiated. The possibility that these nucleosides might provide advantageous transport properties for the aglycons and that the nucleosides themselves might possess activity as antimetabolites was considered very important at the onset of this investigation.



28a, $R_1 = OCOCH_3$, $R_2 = H$
b, $R_1 = OH$, $R_2 = H$
c, $R_1 = OH$, $R_2 =$ β-D-ribofuranosyl
d, $R_1 = OH$, $R_2 =$ β-D-arabinofuranosyl
e, $R_1 = OCOCH_3$, $R_2 =$ 2',3',5'-tri-O-acetyl-β-D-ribofuranosyl



29a, $R_1 = OCOCH_3$, $R_2 = H$
b, $R_1 = OH$, $R_2 = H$
c, $R_1 = OH$, $R_2 =$ β-D-ribofuranosyl
d, $R_1 = OH$, $R_2 =$ β-D-arabinofuranosyl
e, $R_1 = OCH_3$, $R_2 =$ β-D-ribofuranosyl

Initially, the intention was to prepare the nucleosides with the aglycon's hydroxyl group protected by an acetyl functionality. In the case of the free bases the acetyl group produced more active and more stable compounds (28a and 29a). However, the acetyl group on the nucleosides proved to be very labile and it was invariably removed during deblocking of the sugars or during simple workup. Therefore, the target compounds made and finally characterized include those with a hydroxyl group free on the aglycon ring (28c,d and 29c,d) and the fully or partially protected structures (28e and 29e). The compounds were initially tested for cytotoxicity against P388 cells

in culture and compared with the parent bases 28b and 29b. The results shown below were obtained as part of a collaborative effort with Drs. Jayaram and Cooney (LMCB).

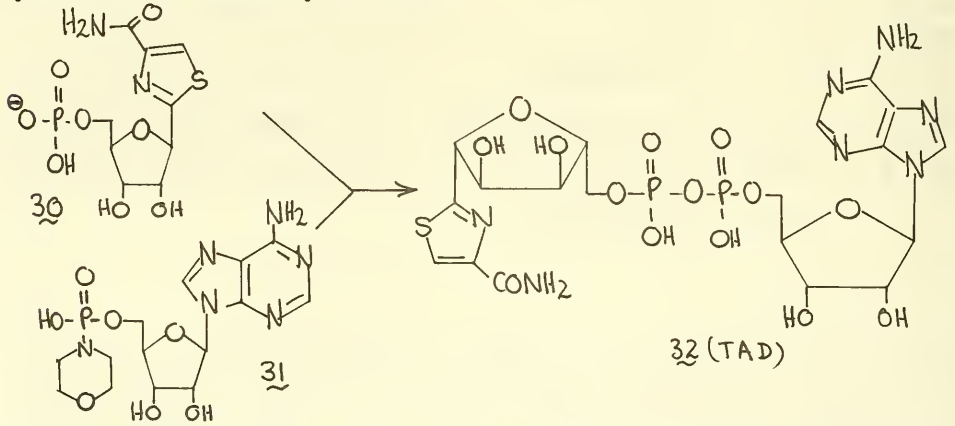
Compound	ID-50 (μM)
28b	300
29b	110
28c	60
29c	450
28d	inactive
29d	135

The results indicated that the 4-pyridone series was the most active and that the ribofuranosides were vastly superior to the corresponding arabinofuranosides.

All of these compounds have been submitted for in vivo testing. Preliminary results obtained with analogs 28e and 29e indicated lack of activity against P388 leukemia. Other in vivo results are pending.

B. Dinucleotide Analogs of NAD. Synthesis of a NAD analog containing 2-β-D-ribofuranosylthiazole-4-carboxamide (Drs. Gebeyehu and Marquez)

The active C-nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide (TR) has been shown to be metabolized to a fraudulent NAD analog (TAD) which causes strong inhibition of IMP dehydrogenase. Our laboratory was instrumental in the assignment of the chemical structure of TAD through the use of mass spectrometry and nuclear magnetic resonance. Subsequent to the structure-proof problem we have synthesized TAD chemically as shown:

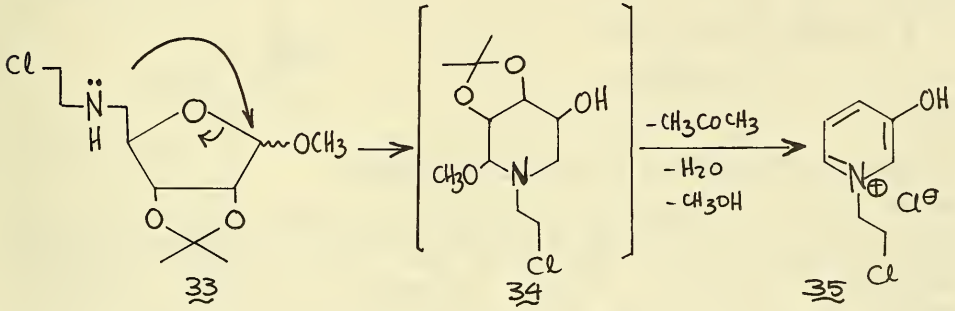


Following a laborious chromatographic workup TAD was isolated in 10% overall yield. Efforts to improve the yield and the purification procedures are under study. The synthetic material proved to be identical to biosynthetic TAD obtained previously by Drs. Cooney and Jayaram (LMCB) by reacting ATP and TR-5'-monophosphate in the presence of the enzyme NAD pyrophosphorylase.

In order to prove that the chemical reaction proceeded in the correct fashion, without the formation of esters between AMP and the 2' and 3' hydroxyl groups to TR, a reaction between the morpholidate of AMP and the isopropylidene analog of TR-5'-monophosphate was conducted. The final half-protected TAD analog was isolated and characterized. Future synthetic plans include changing the labile phosphodiester linkage by hydrolytically stable phosphonate groups.

C. Synthesis of 2- and 4-Pyridones as Potential Antitumor Agents (Drs. Mao and Driscoll)

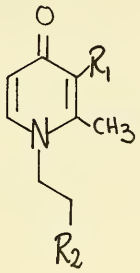
The one-arm mustard 33 was inactive in the murine L1210 leukemia system when first tested. Later it was discovered that a decomposed sample of 33 was highly active (T/C 200) in that system. Although the exact structure of the active intermediate has remained elusive, a final but inactive degradation product has been identified as structure 35. It was of interest, therefore, to synthesize a series of 2- and 4-pyridones chemically related to the possible intermediate of this decomposition reaction. As we have shown previously, 2- and 4-pyridone moieties have established antitumor activity against murine leukemias.



33 34 35

The following compounds, related to higher oxidation forms of the initial intermediate 34 have been synthesized, characterized and submitted for biological screening. The results of the tests are not yet available. The series comprises intermediates obtained throughout the synthesis as well as the final targets 36e and 37. Compound 36e has been recently prepared and efforts to synthesize 37 and related analogs are in progress.

36a, $R_1 = OCH_3$, $R_2 = OH$
b, $R_1 = OCH_2\phi$, $R_2 = OH$
c, $R_1 = OCH_2\phi$, $R_2 = Cl$
d, $R_1 = OCH_3$, $R_2 = Cl$
e, $R_1 = OH$, $R_2 = Cl$



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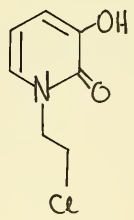
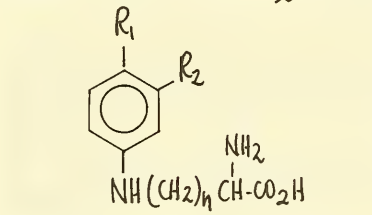
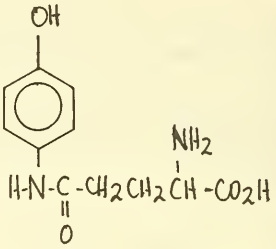


D. Design and Synthesis of Agents with Specific Activity Against Melanotic Melanoma (Dr. Lin)

A complete biological evaluation of compounds 38a-c revealed that although 38a and 38b were two-fold more active against B-16 melanotic melanoma than the amelanotic melanoma cell line in vitro, this difference is probably too small to correspond to the 27-fold difference in tyrosinase activity between the two cell lines. A non-specific oxidation of parent compounds 38a or 38b to the corresponding p-iminoquinone may be a more likely mechanism of action. Another closely related analog 39 was synthesized and submitted for in vivo testing.



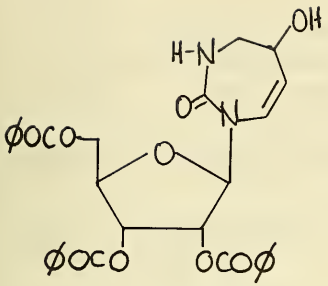
38a, $R_1 = OH$, $R_2 = H$, $n = 1$
b, $R_1 = OH$, $R_2 = H$, $n = 3$
c, $R_1 = H$, $R_2 = OH$, $n = 3$



39

E. Prep Lab (Mr. Fuller, Drs. Marquez and Driscoll)

The Drug Design and Chemistry Section devotes a one manyear effort in synthetic organic and HPLC analytical support devoted to requests for chemical assistance from various parts of DCT. Much of the work is in response to biochemical investigator's requests for specific non-commercially available molecules. Providing this service is in keeping with the Section's role in the interdisciplinary aspects of the LMCB. Since most of the requests are for compounds which are either unknown or inadequately described in the literature, considerable research usually is required before a target compound can be prepared and completely characterized. Among this category of compounds, structures 1 and 40-43 were typical examples of this type of work. Compounds 1 and 40 were required for cytidine deaminase studies. Compounds 41 and 43 were used for transport studies by Dr. Vistica (LMCB) and compound 42 was needed as a purine nucleoside phosphorylase inhibitor by Dr. Glazer (LMCB).



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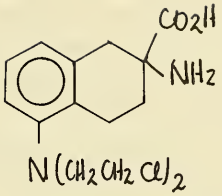
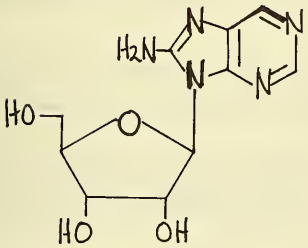
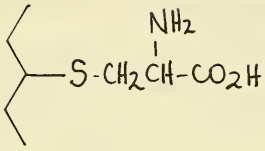


41



42



43

Publications:

Lin, A.J. and Kasina, S.: Synthesis of 3-substituted 7- or 8-(3,3-dimethyl-1-triazeno)-10-methylphenothiazines as potential antitumor agents. J. Hetero. Chem. 18: 759-761, 1981.

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Liu, P.S., Marquez, V.E., Driscoll, J.S., Fuller, R.W., and McCormack, J.J.: Cyclic urea nucleosides. Cytidine deaminase activity as a function of aglycon ring size. J. Med. Chem. 24: 662-666, 1981.

Marquez, V.E., Liu, P.S., Linevsky, J.K.: Mechanism of formation of cyclic urea nucleosides. Evidence for an O- to N-transglycosylation. J. Org. Chem. 47: 1712-1717, 1982.

Lin, A.J., Kelley, J.A., Breitman, T.R. and Driscoll, J.S.: Agents with potential specificity against melanotic melanoma. J. Med. Chem. 25: 501-505, 1982.

Marquez, V.E.: Antineoplastic Agents. In Hess, H-J. (Ed.): Annual Reports in Medicinal Chemistry, Vol. 17, Academic Press, N.Y., 1982, in press.

Cooney, D.A., Jayaram, H.N., Gebeyehu, G., Betts, C.R., Kelley, J.A., Marquez, V.E., and Johns, D.G.: The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP-dehydrogenase inhibitory properties. Biochem. Pharmacol., in press.

Patent:

Marquez, V.E., Liu, P.S., Driscoll, J.S.: Seven-membered ring compounds as inhibitors of cytidine deaminase. U. S. Patent 4,275,057, June 23, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03581-13 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Analytical Chemistry of New Anticancer Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. A. Kelley	Research Chemist	LMCB	NCI
Other:	J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI
	P. N. Huguenin	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Design and Chemistry Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	-

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is the research and development of analytical methods which are used to: (1) establish the structure and purity of new antitumor agents and their metabolites, (2) determine physical and chemical properties of new anticancer drugs, (3) quantitate drugs and their metabolites in biological samples to elucidate pharmacology and to determine pharmacokinetics, and (4) study reaction mechanisms of potentially useful synthetic transformations. Mass spectrometry, gas chromatography and high-performance liquid chromatography, either alone or in combination, are emphasized techniques. Compounds of current interest are aziridinylbenzoquinones, reduced pyrimidine nucleosides, pyrimidine nucleotides, modified amino acids, nitrogen mustards and cytidine deaminase inhibitors. The acid and base-catalyzed rearrangements of pyrimidine nucleosides continue under investigation.

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;
2. establish structures of metabolites of new antitumor agents;
3. determine important physical and chemical properties of new anticancer drugs;
4. study reaction mechanisms;
5. quantitate drugs and their metabolites in physiological samples.

Methods Employed:

Mass spectrometry (MS), gas chromatography (GC), high-performance liquid chromatography (HPLC) and the combination of these techniques (GC/MS, LC/MS) are emphasized. Other analytical methods such as NMR, UV and IR spectroscopy are also employed.

Major Findings:

I. Research Problems

A. Clinical Support and Pharmacology Studies

1. HPLC Analysis of AZQ in Biological Media (Dr. Kelley): The high-performance liquid chromatographic (HPLC) determination of AZQ was further refined for application to clinical samples. Both the speed and sensitivity of the assay were increased by adaptation to a Radial Compression Separation System using a 5 μm C_{18} reverse phase column. For a 2.0 ml plasma sample the limit of detection was less than 2 ng/ml and the analysis was completed in 12 rather than 20 min. The sample workup procedure was also modified to allow determination of AZQ in human tissue. After addition of the 2,5-diamino-3,6-dichloro-1,4-benzoquinone internal standard, the tissue sample was homogenized in pH 6.5 0.1 M phosphate buffer and the homogenate extracted with a single volume of chloroform. These new modifications were applied to the analysis of the clinical samples generated in the NCI MB-COP Phase II study of AZQ in patients with malignant gliomas in order to resolve several pharmacological questions (vide infra).

2. AZQ Plasma and CSF Pharmacokinetics (Drs. Kelley, Bachur, Collins and Van Echo, DRS and BCRP): Detailed pharmacokinetic studies of AZQ were conducted on five informed Phase I/II patients who already had indwelling Ommaya reservoirs which had been placed for therapy of meningeal leukemia or at the time of glioma resection. The two leukemia patients received the drug as a 30 min iv infusion on a daily x 7 schedule, while the three patients with malignant glioma were treated with 17.5 mg/m^2 AZQ on day 1 and day 8 every 4 weeks. Serial post-infusion plasma and CSF samples were analyzed for AZQ by using the

HPLC assay developed in this laboratory. The following table summarizes the pharmacokinetic data obtained in this study:

Patient	Dose	Plasma $t_{1/2}(\alpha)$ (min)	Plasma $t_{1/2}(\beta)$ (min)	CSF $t_{1/2}$ (min)	CSF $[AZQ]_{max}$ (ng/ml)	AUC Ratio CSF:Plasma
S.S.	16 mg/m^2 x 7	3.8	25	89	89	0.22
M.W.	24 mg/m^2 x 7	5.8	33	80	170	0.42
L.Q.	17.5 mg/m^2 d 1 & 8	2.4	30	53	253	0.27
J.H.	17.5 mg/m^2 d 1 & 8	2.1	32	-	54	-
J.L.	17.5 mg/m^2 d 1 & 8	3.7	28	-	-	-

The AZQ plasma disappearance curves were fitted to a two-compartment open model with all calculated pharmacokinetic parameters falling within the range of the previous NCI Medicine Branch Phase I study. Maximum CSF levels were attained 45-60 min after the end of infusion and they equalled or exceeded the corresponding plasma concentrations in the first three patients. Patient J.H. had a lower peak AZQ value for the CSF, but this concentration persisted for several hours before gradually declining. The AZQ in the CSF of J.L. could not be measured because of sampling difficulties.

The measured AZQ plasma concentrations at times in excess of two hours did not support the existence of a third plasma elimination phase with an extended half-life. In addition, AZQ was below the 5 ng/ml limit of quantitation of the HPLC assay in preinfusion sample of a patient on the 7th day of daily drug therapy, so the parent compound does not accumulate in the plasma. Area under the infusion and post-infusion concentration-time curve for CSF was 22-42% of the corresponding plasma area, indicating a high degree of drug availability to the CSF. Total body clearance of AZQ was relatively low, 115-368 ml/min/m^2. This combination of rapid disappearance, short half-life and low clearance is due to the small volume of distribution for AZQ of 2-10 l/m^2. Plasma protein binding of AZQ was 79 ± 1% at drug concentrations of 1 µg/ml with the extent of binding appearing to be dependent on drug concentration. When the effect of plasma protein binding is considered, the distribution volumes become more plausible and it appears that free plasma AZQ is completely available to the CSF.

3. AZQ Phase II Clinical Trial (Drs. Kelley, Curt and Collins, MB-COP and DRS): Seventeen patients with high grade gliomas, who had documented evidence of tumor regrowth, were treated with 20 mg/m^2 of AZQ given iv on days 1 and 8 of a 28 day cycle. The modified HPLC assay described above was used to measure AZQ in the plasma, urine or brain tumor tissue of 7 of these patients. Detailed infusion and post-infusion AZQ plasma pharmacokinetics could be obtained in 6 different patients who were given the drug as a constant rate iv infusion over 35-78 min. Post-infusion plasma clearance occurred rapidly and showed the two phase behavior observed in earlier studies. Careful analysis of plasma samples from post-infusion times greater than 3 hr likewise indicated no third phase of elimination with an extended half-life. The measured area under the concentration-time curve for the infusion period closely approximated that

estimated by the pharmacokinetic model. Further evidence was also obtained for penetration of AZQ into the central nervous system since measurable amounts of the drug could be found in either brain tumor cyst fluid or brain tumor tissue of two patients treated pre-operatively. These findings are detailed in the table below:

Patient	Dose (mg/m^2)	Sample	Time min post-infusion	AZQ ng/ml or ng/gm	[AZQ] plasma ng/ml
N.M.	20	cyst fluid	60	28	61
		peripheral tumor	106	605	20[b]
		central tumor	120	- [a]	14[b]
N.J.	20	cyst fluid	45	127	157[b]
		tumor	67	- [a]	106
		tumor	80	- [a]	64
		tumor	100	- [a]	47

a. AZQ was not detectable, but tissue samples were small.
b. Calculated from pharmacokinetic curve.

Parent drug could not be detected in any of the post-infusion urine samples.

B. Analytical Methods Development (Drs. Huguenin and Kelley):

Dihydro-5-azacytidine (DHAC, NSC 264880), 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC 286193) and 5-azacytosine arabinoside (NSC 281272) are new candidate antitumor agents scheduled to undergo Phase I clinical trial in the near future. Development of analytical methods using HPLC to measure these compounds in biological fluids was initiated. Use of affinity chromatography employing a boronate gel to isolate ribosides preferentially from plasma and urine was tested for DHAC. At plasma concentrations of 2.5 μM, the upper limit of expected therapeutic drug levels, 89% of the DHAC could be recovered from the entire extraction and chromatography process. Investigation and refinement of these procedures to isolate important ribosides from biological matrices continues.

II. Synthetic and Collaborative Project Support (Dr. Kelley)

1. Structure Determination of TAD: The new antitumor agent, 2-β-D-ribofuranosylthiazole-4-carboxamide, is intracellularly converted to a thiazole carboxamide analogue of NAD (TAD) which strongly inhibits IMP dehydrogenase, a key step in purine nucleotide biosynthesis. Fast atom bombardment mass spectrometry of the underivatized substance was used to elucidate the structure of this dinucleotide. Both the positive and negative ion mass spectra convincingly supported a dinucleotide structure of MW 669 in which the nicotinamide of NAD had been replaced by thiazole-4-carboxamide. In addition to strong molecular ion peaks, the mass spectral fragmentation patterns provided information about the identity of the nucleotide bases and the phosphate ester linkage.

2. Nω-Hydroxyphenyl Amino Acids: A series of amino acid derivatives, designed to exploit the high tyrosinase activity in melanotic melanoma by metabolic activation to toxic metabolites, were synthesized. The structure and purity of β-[(p-hydroxyphenyl)amino]alanine, N^{δ}-(p-hydroxyphenyl)ornithine and N^{δ}-(m-hydroxyphenyl)ornithine were determined by combined GC/MS after derivatization. Accurate analysis of N^{δ}(m-hydroxyphenyl)ornithine required room temperature trimethylsilylation in order to prevent intramolecular cyclization. This rearrangement was studied using accurate mass measurement and deuterated derivatizing reagents during GC/MS analysis of the reaction mixture.

3. Miscellaneous: Numerous samples which cannot be categorized as coming from one of the above project areas were also analyzed by the appropriate mass spectral and chromatographic techniques on an individual basis. Included in this group were anhydronucleosides, pyridone ribosides, nucleosides of cyclic and ring-opened ureas, O-nucleosides, 2-aminotetrahydronaphthoic acid mustards, an unsymmetrical piperazine mustard, tricyclo[4.2.2.$0^{2,5}$]dec-9-ene-3,4,7,8-tetracarbocyclic acid diimide (NSC 284356) and its aqueous decomposition products and isouridine and its derivatives.

Publications:

Kelley, J.A. and Siu Chong, E.D.: Reverse phase HPLC determination of AZQ in biological fluids. J. Liquid Chromatogr. 4: 1855-1867, 1981.

Schilsky, R.L., Kelley, J.A., Ihde, D.C., Howser, D.M., Cordes, R.S. and Young, R.C.: Phase I trial and pharmacokinetics of aziridinylbenzoquinone (NSC 182986) in humans. Cancer Res. 42: 1582-1586, 1982.

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Flora, K.P., Cradock, J.C. and Kelley, J.A.: The hydrolysis of spirohydantoin mustard. J. Pharm. Sci., in press.

Cooney, D.A., Jayaram, H.N., Gebeyehu, G., Betts, C.R., Kelley, J.A., Marquez, V.E. and Johns, D.G.: The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP dehydrogenase-inhibitory properties. Biochem. Pharmacol., in press.

Drug Interactions Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1981 - September 30, 1982

Objectives

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of factors. For example, the distribution of a drug within an organism depends upon relative organ perfusion, affinity of the drug for binding sites within different organs, extent of binding of the drug to plasma proteins, and the rate of drug excretion into the urine, bile, and exhaled air. Moreover, the metabolic clearance of many drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, tissue distribution, binding, metabolism and excretion determine the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known either to diminish or to intensify biologic responses, the need to assess these parameters quantitatively is of obvious therapeutic significance. Studies have been continued in the Drug Interactions Section to investigate these factors in a variety of animal species with respect to xenobiotics in general, and oncolytic agents in particular. A large body of evidence has linked drug metabolism to drug disposition and the duration of drug action. Only recently have data accumulated which emphasize the important role of drug metabolism in drug-induced toxicity. Recent findings have demonstrated that many relatively inactive foreign chemical compounds can be enzymatically activated *in vivo* to what might be termed ultimate toxicants.

Because of the diversity of interests of members of the Section, a large variety of research projects continue to be conducted. For organizational purposes, these projects may be grouped under three general headings which are described in detail in subsequent pages of this report.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07119-03 LMCB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Interactions of Antineoplastic Agents in Biological and Chemical Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore E. Gram, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Charles L. Litterst, Ph.D.	Pharmacologist	LMCB	NCI
	Edward G. Mimnaugh, B.S.	Chemist	LMCB	NCI
	Michael A. Trush, Ph.D.	Research Associate	LMCB	NCI
	Yoichiro Hirokata, M.D., Ph.D.	Visiting Fellow	LMCB	NCI
	Erika Ginsburg, B.S.	Biologist	LMCB	NCI
	Samuel Tong, Ph.D.	Visiting Fellow	LMCB	NCI
	Federico Bertolero	Visiting Fellow	LMCB	NCI
	Vanessa Schweitzer, Ph.D.	Anatomist	U. Michigan	

COOPERATING UNITS (if any)

Kresge Hearing Research Institute, University of Michigan.

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Interactions Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	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 (200 words or less - underline keywords)

Studies reported in this section relate to: 1) cis platinum; its intracellular binding, pharmacokinetics, and its alteration by changes in acid-base balance, and the microdistribution of platinum within specific aural structures of the guinea pig; 2) A comparison of the pulmonary toxicity and anti-tumor activity of bleomycin and two analogues, pepleomycin and bleomycin BAPP.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. 1. Interaction Between Cis-Platinum and Possible Tissue Receptors. In a continuing study of the interaction between cisplatinum (cis-Pt) and potential tissue receptors, lysosomes were investigated as a possible site of platinum interaction. Other investigators have reported apparent morphologic interaction between platinum and lysosomes. If platinum localizes in the lysosomes, it is reasonable to question whether there is any change in lysosomal enzyme activity following cisplatinum administration. If platinum alters membrane stability of lysosomes the release of proteolytic enzymes into the cells of the proximal tubule might be involved in the renal tubular degeneration that is observed. Rats were given a single non-lethal i.p. dose of cis-Pt and a lysosome-enriched fraction prepared from liver and kidneys at various times from 1-12 days after treatment. The fraction was analyzed for the activity of six different lysosomal enzymes. In addition, a portion of tissue homogenate was incubated with Triton X-100 to study labile enzyme activity. Results were enzyme and tissue specific, but in general, enzyme activity was elevated by approximately two-fold, with the kidney showing a peak effect three days after treatment. Effects on the liver were quantitatively less obvious and did not demonstrate any temporal relationship. Whether this effect represents a primary result of cis-Pt administration or is secondary to other effects is unknown.

2. Interactions Between Intracellular Proteins and Platinum. A study was conducted to determine the interaction between intracellular proteins and platinum following administration of cisPt. The drug, labeled with ^{195}Pt, was administered i.v. to rabbits and 1,8,24 and 96 hr later animals were killed by exsanguination. Distribution of radioactivity between RBC cytoplasm and ghosts, and in subcellular fractions of lung, liver and kidney was studied. Plasma and cytosol of liver and kidney were chromatographed on Sephacryl S-200 and Sephadex G-75 columns in order to study the in vivo association between ^{195}Pt and intracellular proteins. Although there was more total platinum in RBC cytoplasm than in ghosts, there was 3-5 times more platinum per mg protein in ghosts. Platinum content in liver and lung cytosol and microsomes decreased from 1 to 24 hr after treatment while platinum content of kidney cytosol and microsomes increased.

The elution profile of plasma was unremarkable, with radioactivity present mainly in high molecular weight regions. In both liver and kidney cytosols, radioactivity eluted mainly in the region corresponding to high molecular weight components, but with peaks in the low molecular weight region also. One low molecular weight peak, with a relative elution volume of 2.4 and an estimated molecular weight of less than 13000, was present in liver and kidney at all times studied. The platinum content of this component significantly decreased in liver between 8 and 24 hr, but increased in the kidney during the same time. Binding of platinum to this peak increased in rats that had been pretreated with $CdCl_2$, but not in rats pretreated with cis-Pt. This component thus, has some of the chromatographic characteristics of metallothionein (MT). Platinum was also found associated with a chromatographic peak that was positive when

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07120-03 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

The Role of Drug Metabolism and Tissue Distribution in Modulating Pharmacological and Toxicological Responses.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Theodore E. Gram, Ph.D.	Pharmacologist	LMCB	NCI
Other:	Charles L. Litterst, Ph.D.	Pharmacologist	LMCB	NCI
	Edward G. Mimnaugh, B.S.	Chemist	LMCB	NCI
	Michael A. Trush, Ph.D.	Research Associate	LMCB	NCI
	Yoichiro Hirokata, M.D., Ph.D.	Visiting Fellow	LMCB	NCI
	Erika Ginsburg, B.S.	Biologist	LMCB	NCI
	Samuel Tong, Ph.D.	Visiting Fellow	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Interactions Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two projects are described in this section, whose only connection is that they deal with selective concentration (uptake) or selective toxicity of xenobiotics in the lung. Naphthalene, administered once to mice produced a significant (30-70%) and prolonged (8-15 days) impairment of pulmonary monooxygenase without altering these activities in liver microsomes. Inhibition of pulmonary enzymes was accompanied by selective necrosis of pulmonary nonciliated bronchiolar (Clara) cells with no evidence of morphologic damage to other pulmonary cell types. No morphologic changes were observed in sections of livers from these animals. In an extension of earlier work, ^{14}C-imipramine and its major metabolite desmethylimipramine (DMI) were found to be selectively concentrated in lung relative to other tissues. Tissue/plasma (T/P) ratios for rat lung exceeded other tissues by 5-10 fold. Lungs of female rats concentrated more total drug, and more metabolite (DMI) than male lungs.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. Damage to Clara Cells and Inhibition of Pulmonary Mixed-Function Oxidation by Naphthalene. Injection of a single dose of naphthalene into C57BL/6J (255 mg/kg, ip) produced a significant (30-70%) and prolonged (8-15 days) impairment in pulmonary microsomal monooxygenase activities without altering these activities in liver microsomes. The time course of naphthalene-induced morphologic damage to bronchiolar epithelium paralleled compromises in pulmonary monooxygenase activity. No concomitant alterations in hepatic morphology were observed. Five microsomal enzymes were studied: benzphetamine N-demethylase, aryl hydrocarbon hydroxylase, NADPH cytochrome c reductase, 7-ethoxyresorufin O-deethylase (a cytochrome P-448-dependent enzyme), and styrene epoxide hydrolase (a cytochrome P-450-independent enzyme). In general, the time course of the inhibition of these pulmonary enzymes was similar but the magnitude of the inhibition varied somewhat. Maximum inhibition of enzyme activity occurred about 3 days after naphthalene administration; 7-ethoxyresorufin O-deethylase activity was reduced to about 30% of control values whereas benzphetamine N-demethylase declined to about 70% of control. The remaining enzymes clustered midway between these extremes at about 50% of control values. Inhibited activities remained at relatively constant levels between days 3 and 8 and by day 15, there was a clear trend returning toward controls. Despite this trend, 3 of the 6 pulmonary enzyme activities examined remained significantly below control levels 15 days after a single dose of the hydrocarbon. Histologically, the pulmonary non-ciliated bronchiolar epithelial (Clara) cell was the primary target of naphthalene toxicity. At early time points and at low magnifications, it appeared as if the entire bronchiolar epithelium was undergoing necrosis and sloughing into the lumen. However, higher magnifications revealed residual ciliated epithelium. The distribution of Clara cell damage appeared to vary considerably. One could find bronchioles that appeared completely denuded of epithelium and others in the same section whose Clara cells had been spared or, alternatively, were in the process of regeneration.

B. Sex Differences in the Uptake and Retention of Imipramine and Desmethylimipramine in the Rat Lung. ^{14}C-Imipramine was administered to male and female Sprague-Dawley rats and animals were sacrificed at 4, 8, 12, and 20 hours later. At all time points, total radioactivity in female lung was several-fold higher than in males. In addition, female lungs had a higher concentration of desmethylimipramine (DMI) as compared to imipramine than did male rat lungs. This was reflected by a higher conversion of imipramine to DMI by hepatic and pulmonary microsomes from female rats. Finally, male rats cleared both imipramine and DMI from their lungs at a slower rate than did female rats.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 CM 07121-03 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Investigations on the Involvement or Reactive Forms of Oxygen in Drug-Induced Pulmonary Toxicity

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	Edward G. Mimnaugh, B.S.	Chemist	LMCB	NCI
	Erika Ginsburg, B.S.	Biologist	LMCB	NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Interactions Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	0.7	1.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The drug-induced generation of reactive forms of oxygen (superoxide anion, hydroxyl radical and singlet oxygen) can contribute to drug cytotoxicity through attack of reactive oxygen species on intracellular targets (nucleic acids, lipids, proteins) and/or through reactive oxygen-mediated activation of the drug to an active intermediate. There are an increasing number of reports describing the pulmonary toxicity of antineoplastic agents. Reactive forms of oxygen have been implicated in the action of several of these agents. The present projects were designed to evaluate this hypothesis in order to better understand the possible biochemical and molecular mechanisms which contribute to this pulmonary toxicity.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. Reactive Oxygen-Dependent Mitochondrial Lipid Peroxidation Stimulated Adriamycin. Ultrastructural examination of adriamycin-damaged hearts reveal, among other alterations, intramitochondrial swelling, condensation, and frank degeneration including dissolution of cristae and the formation of myelin figures. Since enzymatic activation of adriamycin to a free radical and subsequent enhanced reactive oxygen generation may play a role in adriamycin cardiomyopathy, we investigated the possibility that the morphologic changes in mitochondria could result from adriamycin-stimulated lipid peroxidation. Using purified rat liver mitochondria as a model biological system, we found that NADPH-dependent lipid peroxidation, measured by the 2-thiobarbituric acid method was stimulated as much as 4-fold by adriamycin. Marker enzyme analysis indicated that mitochondria were substantially free of contaminating microsomes. NADPH substituted for NADH as electron donor to mitochondrial NADH-dehydrogenase and supported adriamycin-stimulated mitochondrial lipid peroxidation; however, when reducing equivalents were provided by ascorbate, adriamycin potently inhibited peroxidation. Superoxide dismutase, catalase, 1,3-dimethylurea, reduced glutathione, EDTA, and α-tocopherol inhibited adriamycin-augmented mitochondrial lipid peroxidation, suggesting that multiple forms of reactive oxygen and possibly trace amounts of endogenous iron participated in the peroxidation reactions. Thus, it is possible that the in vivo effects of adriamycin on mitochondrial morphology and function are mediated by (adriamycin) drug-enhanced reactive oxygen mediated lipid peroxidation.

B. Superoxide Anion (O_2)-Dependency of NADPH-Dependent Rat Liver Microsomal Lipid Peroxidation (LP) as Demonstrated by the Inhibition of Peroxidation by Superoxide Dismutase (SOD). Several reports have questioned the involvement of O_2 in the peroxidation of microsomal lipids, mainly because LP could not be inhibited by relatively low concentrations of SOD (10-20 μg/ml). In the present studies when incubations were conducted in the absence of exogenous iron salts, NADPH-dependent rat liver microsomal LP measured by the 2-thiobarbituric acid method was inhibited in a concentration-dependent manner by bovine erythrocyte CuZn SOD, indicating the superoxide dependency of LP. In the presence of $4x10^{-5}$ M SOD, which is approximately twice the rat liver cytosolic concentration of the enzyme, LP was inhibited >90%. Heat-inactivated SOD or an equivalent amount of BSA failed to inhibit LP. When O_2-generation was enhanced by adding the redox cycling drug, adriamycin, LP was stimulated 4-fold, yet remained SOD inhibitable. Exogenous Fe^{2+} (0.5 μM) added to microsomal incubations doubled the extent of LP measured at 30 min, while 5 μM Fe^{2+} increased endogenous LP by more than 20-fold, also at 30 min. SOD was capable of inhibiting LP promoted by 0.5 μM Fe^{2+}, however, no inhibition of LP by SOD was observed at 5 μM Fe^{2+}. Preliminary experimental results suggest that SOD is inactivated - as indicated by the inability to inhibit the xanthine:xanthine oxidase-dependent reduction of cytochrome c - during Fe^{2+}-promoted NADPH-dependent microsomal LP, possibly by H_2O_2 or $^{\bullet}OH$ resulting from the Fe^{2+}-catalyzed interaction of O_2 and H_2O_2. These observations in three peroxidation systems clearly demonstrate a primary role for O_2 in NADPH-dependent microsomal LP.

C. The Effects of Adriamycin in Vitro on Hepatic Microsomal Drug-Metabolizing Enzymes: Role of Microsomal Lipid Peroxidation. The quinone-containing anticancer drug adriamycin augmented the reduction of dioxygen to reactive oxygen species and thereby stimulated (sixfold) NADPH-dependent microsomal lipid peroxidation. In vitro the extensive adriamycin-promoted peroxidation depleted (85%) rat liver microsomal cytochrome P-450, severely inhibited cytochrome P-450-dependent monooxygenation (70%), and glucose-6-phosphatase activity (80%), and activated (450%) UDP-glucuronyltransferase activity. When lipid peroxidation was blocked by EDTA, adriamycin selectively decreased cytochrome P-450 and aminopyrine N-demethylase activity; NADPH-cytochrome c reductase, UDP-glucuronyltransferase, and glucose-6-phosphatase activities were unchanged. Washing and resedimenting peroxidized microsomes to remove adriamycin and soluble lipid peroxidation products failed to restore enzyme activities to control values. Adriamycin administered subacutely (5 mg/kg x three doses) to rats significantly decreased hepatic microsomal cytochrome P-450 content and reduced aminopyrine N-demethylase and NADPH-cytochrome c reductase activities compared to saline-treated controls. Microsomal lipid peroxidation was increased following the adriamycin treatment. Thus, these data suggested that adriamycin was capable of impairing hepatic drug metabolism in vitro by stimulating membrane lipid peroxidation in a manner similar to carbon tetrachloride; the mechanism by which adriamycin treatment in vivo decreased the activity of the drug monooxygenase system remains unclear.

D. Life-threatening pulmonary toxicity as a result of anticancer drug therapy is becoming increasingly recognized. It is also becoming apparent that because of the inherent molecular properties of some antineoplastic agents, reactive oxygen may be involved in the cytotoxic reaction(s) to lung cells. Two possible reactions are microsomal lipid peroxidation and DNA damage.

1. Study of the Ability of Misonidazole and 4-Nitroquinoline N-Oxide to Stimulate Reactive Oxygen-Dependent Lipid Peroxidation in Mouse Lung Microsomes. Misonidazole is a nitro containing drug currently being utilized to increase the sensitivity of hypoxic tumor cells to ionizing radiation. 4-Nitroquinoline N-oxide (4NQO) administration induces pulmonary tumors and leukemia in rodents. Both of these compounds are capable of being metabolically activated to a nitro anion radical intermediate which, under aerobic conditions, autoxides and generates reactive oxygen species capable of damaging biomolecules such as lipids and DNA. This project examines the ability of these compounds to enhance lipid peroxidation in microsomes isolated from mouse lung. A significant enhancement in lipid peroxidation was observed at 10 μM 4-NQO whereas 5 mM misonidazole was required in order to significantly enhance this reaction. The augmentation of lipid peroxidation by these two compounds was inhibited by superoxide dismutase, demonstrating the reactive oxygen dependency of this reaction. Thus, it is possible that reactive oxygen metabolites and/or toxic products of lipid peroxidation are involved in the induction of pulmonary tumors by 4-NQO. On the other hand, it is unlikely that pulmonary toxicity will be a significant consequence of misonidazole administration.

E. Aspects of the Biochemical and Molecular Pharmacology of Bleomycin

1. Studies on the Interaction of Bleomycin A_2 with Rat Lung Microsomes: Effect of Exogenous Iron on Bleomycin-Mediated DNA Chain Breakage. The interaction of bleomycin A_2 with rat lung microsomes results in bleomycin-mediated DNA chain breakage due to the mixed-function oxidase catalyzed activation of bleomycin. This study demonstrates that the addition of exogenous Fe^{3+} significantly enhances the bleomycin-mediated cleavage of DNA deoxyribose, that the enhancing effect of Fe^{3+} is maximum when a 1:1 ratio of bleomycin to Fe^{3+} is achieved and that either NADPH or NADH can serve as pyridine cofactors for this reaction. Since the activation of bleomycin is facilitated by iron in the Fe^{2+} form, these observations affirm the hypothesis that the mixed-function oxidase system serves to maintain either adventitious or exogenous iron in the Fe^{2+} form. In the absence of DNA, the interaction of bleomycin with rat lung microsomes results in the self-inactivation of bleomycin, a reaction which is also enhanced by the addition of exogenous Fe^{3+}. Thus, the microsomal mixed-function oxidase oxidase system represents an efficient biological system for the "activation-inactivation" of bleomycin.

2. Mechanisms of the Inhibition of Microsomal Lipid Peroxidation by Bleomycin. To ascertain if the inhibition of microsomal lipid peroxidation by bleomycin can be attributed to its sequestering of adventitious iron, the effects of bleomycin A_2 and bleomycin A_2 complexed with copper (1:1) (NSC 260620) were compared. Bleomycin A_2 significantly inhibited rat liver microsomal lipid peroxidation in a concentration-dependent fashion, 5 μM bleomycin inhibited lipid peroxidation by 35% while 25 μM bleomycin was nearly 100% inhibitory. In contrast, the bleomycin A_2-Cu^{2+} complex had no significant effect on microsomal lipid peroxidation at these low drug concentrations. Thus, the results of these experiments demonstrate that bleomycin inhibits microsomal lipid peroxidation by sequestering the adventitious iron necessary to catalyze the initiation of microsomal lipid peroxidation. However, this inhibition by bleomycin is only temporary since as a result of the channeling of electron flow from the microsomal mixed-function oxidase system to the bleomycin-iron-oxygen complex, bleomycin undergoes an activation-inactivation process resulting in a metabolite which apparently can no longer bind iron. Thus, the iron previously sequestered by bleomycin is available to participate in the generation of reactive oxygen species involved in the initiation of microsomal lipid peroxidation. This suggests that one of the manifestations of the interaction of bleomycin with cells may be the subcellular relocalization of iron.

3. Factors which Affect the Microsomal "Activation-Inactivation" of Bleomycin: The Role of Superoxide Anion. Incubation of bleomycin with rat lung microsomes in the absence of DNA results in a time-dependent decline in the ability of bleomycin to inhibit lipid peroxidation and mediate DNA damage. This microsome-catalyzed inactivation of bleomycin was found to be dependent on both oxygen and NADPH, suggesting that a similar mechanism may be involved in bleomycin-mediated DNA and bleomycin inactivation. Based on this conclusion, the hypothesis was examined that any factor which increases bleomycin-mediated DNA damage should similarly increase bleomycin inactivation. As anticipated, the addition of Fe^{3+}, ascorbic acid and the redox cycling compound, paraquat, increased both bleomycin-mediated DNA damage and bleomycin inactivation. The enhanced inactivation of bleomycin by these agents is oxygen dependent. Superoxide

dismutase and EDTA inhibited the inactivation of bleomycin, suggesting the involvement of adventitious iron and superoxide anion in this process. These results suggest that O_2 probably derived from microsomal NADPH cytochrome P-450 reductase, mediates the reduction of bleomycin-bound Fe^{3+} to Fe^{2+} which, in turn, facilitates the activation of BLM to a DNA damaging species.

4. Characterization of the Interaction of Bleomycin with Microsomes Isolated from Lewis Lung and B16 Melanoma. DNA is generally considered the cellular target by which bleomycin elicits cytotoxicity and exerts its antiproliferative activity. In order for bleomycin to damage DNA it is necessary for it to be activated. Since the mixed-function oxidase system represents an efficient biological mechanism for the activation of bleomycin, we examined the ability of microsomes isolated from the Lewis lung carcinoma and the B16 melanoma to catalyze bleomycin-mediated DNA deoxyribose cleavage. Microsomes from both tumors were able to catalyze this reaction although those from the B16 melanoma exhibited three times the activity as microsomes isolated from the Lewis lung carcinoma. The addition of ascorbic acid, Fe^{3+} or the redox cycling chemical, paraquat, significantly enhanced this reaction whereas EDTA, superoxide dismutase and glutathione inhibited the cleavage of deoxyribose by bleomycin. In addition to bleomycin A_2, bleomycin PYP (NSC 276381), bleomycin BAPP (NSC 294979), bleomycin PEP (NSC 276382) and tallysomycin A (NSC 279496) were activated to a DNA damaging species by microsomes from the B16 melanoma and the Lewis lung carcinoma. It is hoped that future studies planned on the in vitro interaction of bleomycin with tumor microsomes will lead to a better understanding of mechanisms by which to increase the therapeutic effectiveness of this drug.

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Molecular Biology and Methods Development Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1981 - September 30, 1982

The Section of Molecular Biology and Methods Development is concerned with the development of strategies and regimens for the potentiation of cancer chemotherapeutic activities. Its staff consists of a Section Head, a Visiting Fellow and a senior technician.

Mercaptoethanol is an essential growth-promoting additive for a wide range of human tumors in primary culture, and has become a standard medium component to insure their growth. It is also essential for the growth of the in vivo line of murine leukemia L1210 in primary culture but not for our L1210 established culture line. In general, it is required neither for growth of normal tissue in primary culture nor for growth of tumors in established culture.

Mercaptoethanol is not a natural metabolite, and the reason for its requirement by tumor cells in primary culture has remained undefined. Since an understanding of its mechanism of action could furnish a basis for chemotherapy, we initiated an exploratory study of its role in primary culture. A portion of this study involved the possible substitution for mercaptoethanol of compounds of known reactivity. One such compound, 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline-disulfonic acid, a water soluble copper chelating agent, could promote growth or our L1210 line in primary culture, indicating that the mercaptoethanol requirement is related to copper-mediated cytotoxicity. Our attention has therefore been directed to the application of copper-mediated cytotoxicity as a basis for chemotherapy.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07129-01 LMCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Copper and Its Chelates in Cytotoxicity and Chemotherapy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marco Rabinovitz, Ph.D.	Head, MBMDS	LMCB	NCI
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COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION
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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

2,9-Dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid supported the growth of mercaptoethanol requiring L1210 cells in primary culture. This specific copper chelating agent removes trace copper in tissue culture media, indicating that the mercaptoethanol requirement is associated with susceptibility to copper-mediated cytotoxicity. A survey of congeners of the 1,10-phenanthroline series indicated that only the 2,9-dimethyl derivative is cytotoxic in the presence of copper. The 1,10-phenanthroline ligand, its other dimethyl derivatives and 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline are moderately cytotoxic in tissue culture, but added copper reduces their cytotoxicity. 2,9-Dimethyl-1,10-phenanthroline plus copper was minimally effective against the L1210 leukemia in vivo but gave a significant increase in life span in mice bearing the P388 leukemia.

Objectives

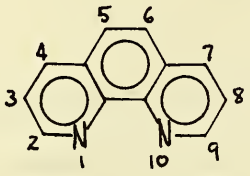
Our studies are directed toward understanding the basis of copper-mediated cytotoxicity and the sensitivity to copper (or some of its chelates) by tumor cells. This information is applied to the design of regimens for chemotherapy.

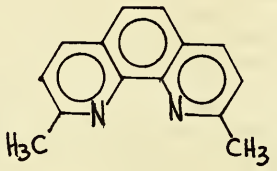
Methods Employed:

The principal methods employed involve tissue culture techniques such as primary and established cell culture, viability by clonal growth, and cell size distribution as well as chemotherapeutic evaluation with tumor bearing mice. Studies with isotopically labeled components are also carried out in experiments designed to ascertain mechanisms of action.

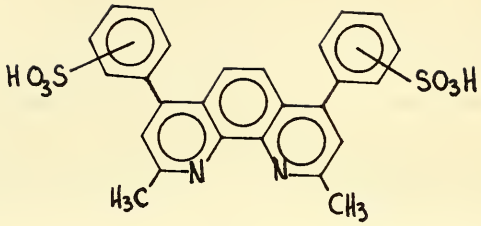
Major Findings:

1. Structure-Activity Relationships of Copper Chelates in the 1,10-Phenanthroline Series. Some of the characteristics of cytotoxicity to L1210 cells by 1,10-phenanthroline and its 2,9-methylated derivatives are shown below.





	1,10-Phenanthroline	2,9-Dimethyl-1,10-phenanthroline
Common Name	ortho-phenanthroline	neocuproine
Abbreviation	phen	NC
Toxicity in L1210 culture	at 5 μM	at 1 μM
Effect of Cu^{++} on toxicity	abolishes toxicity	promotes toxicity



2,9-Dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid

Common Name	bathocuproinedisulfonic acid
Abbreviation	BCS
Toxicity in L1210 culture	not toxic at 50 μM
Effect of Cu^{++} on toxicity	abolishes toxicity of NC

A most striking feature of structure-activity relationships in tissue culture is the observation that copper abolishes the cytotoxicity of 1,10-phenanthroline but promotes the cytotoxicity of its 2,9-dimethyl derivative. The 4,7-dimethyl- and 5,6-dimethyl derivatives behave as the unmethylated compound. The 2,9-dimethyl derivative forms a bidentate chelate with cupric ion which has an unusually high redox potential due to the steric hindrance encountered in the formation of a planar chelate.

BCS, as indicated in the introduction, can replace mercaptoethanol as a growth promoter in primary culture of L1210 cells. It also abolishes the toxicity of members of three classes of ligands which depend upon their copper chelates for cytotoxicity. These include 2,9-dimethyl-1,10-phenanthroline, diethyldithiocarbamic acid and the bis-thiosemicarbazone of 3-ethoxy-2-oxobutyraldehyde. Its low toxicity, water solubility, and compatibility with tissue culture media make it an ideal indicator for evaluating possible copper associated toxicity of compounds which can act as copper chelating agents.

2. Mechanism of Action

a. Uptake. The uptake of tritiated 2,9-dimethyl-1,10-phenanthroline is markedly promoted by copper ion. Maximum uptake occurs at one hour, and then a rapid release of label takes place at a rate equivalent to that of uptake. No major membrane damage, such as lysis, was observed, although there is some cell swelling and about 20% of the cells stain with trypan blue.

b. Intercalation. Members of the phenanthroline series are good intercalators of DNA, and the possibility exists that such reactivity may play a role in its mechanism of action. Support for such a mechanism is the observation that the copper chelate of 2,9-dimethyl-4,7-diphenylphenanthroline is not cytotoxic. Addition of phenyl groups destroys the planarity of the phenanthroline ring and abolishes intercalation. However, using alkaline gel electrophoresis, there was no evidence of DNA strand breaks in the nuclei of L1210 cells which had been incubated with high levels of neocuproine and copper (cooperative study with Applied Pharmacology Section, LMCB).

3. Chemotherapeutic Evaluation. Since copper ion is toxic to mice upon i.p. injection, we have utilized nitrilotricarboxylic acid, a copper specific ligand, to reduce its toxicity. This ligand has been reported to be of value in copper administration for the therapy of Menkes Syndrome. Optimal therapeutic efficacy was observed when it was administered i.p. at 20 μmoles/kg with 10 μmoles/kg copper sulfate, followed immediately by 20 μmoles/kg neocuproine, on days 1, 2, 4, 5. For L1210 cells this gave borderline activity 115-120% ILS while for P388 cells a significant 184% (mean) 144% (median) ILS was observed. Dose limiting host toxicity appeared to be neurotoxicity at acute doses and peritoneal irritation with intestinal toxicity on multiple dose schedules. At this dose level, neocuproine in the absence of added copper was well tolerated by the mice.

Proposed Course

A. Attempts to Reduce Host Toxicity. A principal tenet in the application of metal chelates to biological systems is the requirement for a high order of stability of the chelate. Otherwise the metal dissociates from the ligand and reassociates with binding agents in the biological milieu. This limitation cannot be applied to the copper-neocuproine system, since both the cupric and cuprous chelates are easily dissociated. Instead an approach will be used involving prior uptake and retention of one component, e.g. copper, by the tumor cell followed by uptake of the other component, e.g. neocuproine. Thus the active cytotoxic agent, the chelate of copper with neocuproine, would be synthesized within the cell. This should eliminate the toxicities due to their combined presence in the extracellular milieu. Principal candidates for directing copper uptake by the tumor cells are members of the bis-thiosemicarbazone group, some of whose members have chemotherapeutic activity. Cells surviving their cytotoxic action should still bear a non-toxic copper burden making them susceptible to neocuproine. This approach should be particularly applicable to the slow growing tumors and will be applied to the B-16 melanoma and the Lewis lung carcinoma.

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ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 to September 30, 1982

The LMP is engaged in 2 general areas of investigation: (1) studies of the effects of DNA-reactive drugs in mammalian cells in relation to cytotoxic mechanisms, and (2) studies of nuclear proteins and chromatin in relation to the control of cell proliferation and differentiation. In both areas, new methodology has previously been developed in this Laboratory and is being applied to several problems.

Studies of DNA Crosslinking Agents:

The effects of drugs on DNA in mammalian cells are being studied by means of filter techniques, developed in this Laboratory, for the measurement of several types of DNA lesions.

Our previous work had shown that a particular DNA repair defect affecting removal of O^6-alkyl-guanine lesions confers sensitivity of cells to chloroethyl-nitrosoureas. Some human tumor cells have this defect, whereas others do not. Repair-defective cells (designated Mer- phenotype), when treated with chloroethyl nitrosoureas, produce increased amounts of interstrand crosslinks. We had previously found this repair defect to be specific for nitrosoureas, and not to apply to other classes of interstrand crosslinking drugs. During the current year, we have examined 13 cell strains derived from surgical specimens from glioblastoma patients. The cell strains were provided by the Surgical Neurology Branch, NINCDS. These cell strains were found to vary widely in the extent of interstrand crosslinking produced by chloroethylnitrosoureas. DNA-protein crosslinking varied relatively little among these cell strains and served to eliminate factors, such as drug uptake and intracellular drug inactivation. Our working hypothesis is that the differences among the cell strains derives from differences in ability to repair DNA-chloroethyl monoadducts prior to their conversion to interstrand crosslinks. The cell strains were also tested for interstrand crosslinking following treatment with cis-Pt(II). A range of crosslinking potentials was observed among the cell strains, but the potential for crosslinking by cis-Pt(II) and by chloroethylnitrosourea were independent of each other. A further difference between the drugs was that, whereas Pt(II) generated a uniform distribution of crosslink frequencies among the cell strains, chloroethylnitrosourea gave a bimodal distribution in that some strains showed substantial crosslinking while others showed almost no detectable crosslinking. The production of interstrand crosslinks in glioblastoma cells thus is governed by different and potentially predictable factors. The possibility of predicting clinical response will be tested in cooperation with the Surgical Neurology Branch, NINCDS.

Since PCNU has recently been studied as a potentially superior nitrosourea for the treatment of brain tumors, we compared PCNU with BCNU in regard to their effects on Mer+ and Mer- cells. We had previously found in cytotoxicity assays that Mer- (guanine-O^6-alkyltransferase-deficient) cells are more sensitive

than Mer+ cells to several nitrosoureas. The magnitude of the differential however was greater for non-carbamoylating than for carbamoylating nitrosoureas. The carbamoylation reaction appears to interfere with a DNA repair step required for optimum survival of Mer+ cells. PCNU is intermediate in ability to carbamoylate. It was therefore of considerable interest to find that PCNU produced the greatest differential cytotoxicity between Mer+ and Mer- cells that we have seen. PCNU also appeared to give somewhat more interstrand crosslinking in the Mer- cells than did BCNU. Because differential cytotoxicity is the probable basis for anti-cancer actions by these drugs, attempts should be continued to elicit an improved clinical response using PCNU.

Studies were conducted on the effects of aziridinylquinone (AZQ) on several cell types. This compound is both a bifunctional alkylating agent and a quinone which is potentially capable of undergoing oxidation-reduction reactions that may generate free radicals. In accord with these potentials, we found this compound to produce both DNA crosslinks (interstrand as well as DNA-protein) and DNA strand breaks. The relative magnitude of the strand breaks compared to the crosslinks was very different in different cell types, indicating that different mechanisms are involved.

Work was begun on a project to study the formation and removal of specific DNA adducts in nitrosourea-treated cells. The major questions addressed are how the distribution of different adducts compares in cells relative to reactions with pure DNA, and and how removal of particular adducts is affected by the presence of DNA repair deficiencies, such as Mer-. The initial phase of this work is being carried out in collaboration with Dr. John Groopman at Boston University and with Dr. David Ludlum at Albany Medical College. Preliminary studies have shown that current HPLC techniques can disclose a number of adducts, some of which have been identified, in DNA from cells treated with [^{14}C-ethyl]-PCNU. The adducts observed in treated cells appears to be a sub-set of the adducts obtained when pure DNA is reacted.

We plan to set up an assay for guanine-O^6-alkyltransferase activity which might be applicable to tissue samples, and to correlate this activity with chloroethyl-nitrosourea-induced DNA crosslinking. Studies have been initiated of the possibility of inducing or inhibiting this activity. Studies have also been initiated on the mechanism of interstrand crosslink repair. If resources become available, the DNA repair studies will be carried to the gene and enzyme levels.

Studies of DNA Intercalating Agents.

Our previous work had identified a new type of DNA alteration in mammalian cells treated with DNA intercalating agents. Several classes of intercalating agents were shown to produce DNA strand breaks associated with a protein which is probably linked covalently to one terminus of the strand break. During the current year, this work was extended in several respects. It was found that the characteristic protein-associated strand breaks can be generated in isolated nuclei treated with the intercalators, m-AMSA and 5-iminodaunarubicin and ellipticine. The strand breaks and DNA-protein crosslinks were produced in approximately equal quantities, as was the case in intact cells. Also as in the case of intact cells, the breaks and crosslinks appeared with a temperature-dependent half-time (about 15 min at 37°) and disappeared rapidly after drug removal. Thus the intercalator-induced DNA alteration does not require an exogenous source of high-energy compounds or other co-factors.

It will now be possible to characterize the intercalator-induced effect in a subcellular system and to study the enzyme(s) which are presumed to be responsible for the effect.

The intercalator-induced strand breakage has been observed by alkaline elution and by alkaline sedimentation methods. We have now found that these breaks are not observed in the nucleoid sedimentation method which does not involve exposure to alkali. The breaks are however brought out in this procedure by means of a mild protease treatment. This study gives information on the conditions under which the topoisomerase-like protein, which we believe to be responsible for the protein-associated breaks, allows the strands to swivel and under what conditions it keeps the strand topology fixed.

DNA strand breaks produced by a variety of DNA damaging agents are known to stimulate the synthesis of poly(ADP-ribose). It was of interest to test whether the intercalator-induced strand breaks would stimulate this synthesis, in view of our hypothesis that the intercalator-induced DNA alterations do not constitute DNA damage in the usual sense. In accord with our hypothesis, we found that high frequencies of m-AMSA-induced strand breaks fail to stimulate poly-ADPR synthesis, whereas similar frequencies of x-ray-induced breaks produced a clear stimulation.

Furthermore, we found that ataxia-telangiectasia cells, which are known to have enhanced sensitivity to x-ray and to bleomycin due to the presence of a DNA repair defect, do not exhibit any change in sensitivity or protein-associated break production by m-AMSA.

The relationship between the intercalator-induced DNA alterations and cytotoxicity was explored in mouse leukemia L1210 cells. In order to make this type of study possible, it was necessary that the DNA effects of the drugs be reversible. This is not the case with all intercalators (e.g. adriamycin), when the drug is not easily washed out of the cells. We found 3 compounds, representing different chemical classes of intercalators, to be suitable for this study: m-AMSA, 5-iminodaunarubicin and 9-hydroxy-2-methyl-ellipticinium. All 3 produced readily reversible protein-associated DNA strand breaks. When inhibition of colony-forming ability was compared relative to frequency of strand breaks, wide disparities were observed between the 3 compounds. This was true whether the comparison was made relative to single-strand or relative to double-strand breaks. The 3 compounds differed widely in the ratios of single-strand to double-strand breaks produced. These results indicate that the protein-associated single or double-strand breaks are not generally responsible for intercalator-induced cytotoxicity. We hypothesize that these DNA alterations are a cell physiological response to DNA torsion, and that these alterations might in fact help cell survival.

The physiologic significance of these DNA alterations was further explored in MCF-7 human breast cancer cells which are responsive to estradiol. Pre-treatment of the cells with estradiol was found to enhance the production of protein-associated strand breaks by m-AMSA and by 5-iminodaunarubicin. The enhancement was dependent on the presence of estrogen receptor, and was not attributable to any differences in drug uptake. Another means of altering the physiologic state of cells that was examined was pretreatment with 5-azacytidine. This pretreatment led to confirmed hypo-methylation of DNA, which is known to enhance gene expression and is thought to play a role in cell differentiation. This

pretreatment also was found to enhance the formation protein-associated strand breaks by intercalators. Thus the formation of the intercalator-induced DNA alterations appears to be sensitive to the structural and functional state of chromatin, as might be expected if the intercalator-induced DNA alterations are due to a cell physiologic response.

In the course of the intercalator studies, a new uptake phenomenon was discovered and characterized for m-AMSA. This compound exhibited an anomalous uptake as its concentration was raised. The anomaly was characterized by cooperativity, saturability, hysteresis, temperature-dependence and lack of interaction with DNA. This phenomenon was attributed to a sequestration of drug in a cell compartment from which the drug does not access the DNA and does not produce major cytotoxicity.

The kinetics of the formation and reversal of the DNA alterations produced by m-AMSA, observed in our experiments, may have pharmacologic implications. The frequency of these alterations rises rapidly to a steady state level and then remains constant until drug is removed, whereupon the alterations rapidly disappear. This suggested that the drug could be considered to behave pharmacodynamically in a manner associated with some antimetabolites rather than with DNA-damaging agents. In accord with this idea, a clinical protocol was designed in which m-AMSA is delivered as a prolonged continuous infusion. A phase I-II trial has been completed, and a new trial on non-lymphocytic leukemia has been initiated.

Future work in this area will focus on the isolation and characterization of the enzyme that we presume to be responsible for the intercalator-induced DNA alterations. For this purpose, we will utilize a cell-free assay system, the essential feasibility of which we have already demonstrated. The hormone stimulation effect will be pursued by determining whether the DNA alterations selectively affect hormone-stimulated genes.

Other DNA Repair Studies.

There have been conflicting reports on the relationship between DNA repair and poly(ADP-ribose) synthesis. In a detailed kinetic study, we have now shown that inhibition of poly-ADPR synthesis by means of 3-aminobenzamide or 5-methylnicotinamide slows, but does not stop, the repair of x-ray-induced single-strand breaks. It was recently reported that ataxia-telangiectasia (AT) cells are deficient in their response to x-ray-induced poly(ADPR) synthesis. We have found that this is not invariably the case. We are currently determining whether this difference might depend on cell type, since the reported study used lymphoblastoid cells whereas we used fibroblasts.

We are studying the repair of x-ray induced strand breaks by the concurrent use of alkaline elution and nucleoid sedimentation methods. Alkaline elution measures the resealing of strand breaks whereas nucleoid sedimentation depends also on the recovery the DNA winding topology that normally exists in chromatin. Thus we can observationally separate the repair into 2 phases. The strand rejoining phase was specifically blocked by the inhibitor of poly(ADPR) synthesis, 3-aminobenzamide, whereas the winding phase was specifically blocked by the topoisomerase inhibitor, novobiocin. These results will now permit studies of the rewinding phase of DNA repair.

Studies of the Metabolism of Nuclear Proteins in Relation to Cell Proliferation and Differentiation.

Using methodology developed in this Laboratory to resolve and characterize variant species of histone proteins, the patterns of histone synthesis during different cell behavioral states is being analyzed.

It had generally been assumed that histone synthesis is closely linked to DNA synthesis. While true for the major histone variant species, we had found that certain minor variants, discovered in this Laboratory and called H2A.X and H2A.Z, are synthesized throughout the cell cycle and are not suppressed as the major histone species are when DNA synthesis is inhibited with hydroxyurea. The same was true also for the previously known variant species, H3.3. During the current year, this work was extended to quiescent (G_0) cells. It was found that quiescent cells also continue to synthesize histones at a reduced but significant rate. The pattern of histone variant synthesis in quiescent cells was found to be different from that of either S-phase or G_1-phase cells. The main difference between the G_0 and G_1 patterns is that that the major variants, H2A.1 and H2A.2, continue to be synthesized at a slow rate in G_0, but are totally suppressed in G_1; this slow continued synthesis is not attributable to the presence of a small number of S-phase cells because it is not blocked by hydroxyurea. Thus 3 different patterns of histone synthesis have been defined which are characteristic of of the S, G_1 and G_0 states.

These characteristics were found to hold for several human and rodent cell types, including normal human lymphocytes and normal human embryo cells. The histones synthesized in G_0 or G_1 were stably incorporated into nucleosomes of chromatin.

These results raise the possibility of histone turnover. This question is now under study, as is the possibility of histone turnover in association with DNA repair synthesis.

The studies of histone variant synthesis patterns is now being extended to the mRNA level, utilizing cell-free translation, in order to determine whether the synthesis controls are at the transcriptional or post-transcriptional stage. We plan then to carry the study of these controls to the gene level.

The study of the newly discovered histone variant, H2A.Z, is also being pursued from an evolutionary viewpoint. This was stimulated by our finding during the current year that the amino acid sequence of H2A.Z is conserved between mouse and sea urchin to a much greater extent than is the sequence of the other H2A's. Another finding that points to a critical role for this variant species is that its synthesis continues throughout sea urchin development, whereas each of the other H2A variants is synthesized only during a particular time period. When processes are phylogenetically conserved in this manner, we believe that studies of distant species can be very useful in understanding the processes occurring in human cells. Hence related studies are also under way using the acellular slime mold, Physarum.

Another area of current investigation is the physiologic significance of histone modification by acetylation, phosphorylation, methylation, poly-ADP-ribosylation and ubiquitination. Progress in this field, which is crucial for an understanding of histone function, is limited by a lack of adequate separation

techniques that can be practicably applied in function studies. Significant improvements in these separation techniques have already been accomplished in our Laboratory and further studies to this end are in progress. In functional studies, we found that changes in the ion content or pH of the growth media can lead to greatly increased phosphorylation of H2A species. This may become a useful tool for studying how phosphorylation alters chromatin structure and function, and how chromatin may react to environmental influences.

Future studies in this area will focus on the fate and function of histones in relation to cell proliferation control, and on the mechanisms that regulate histone synthesis patterns.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06112-12 LMPH

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Mechanism of Action of DNA Reactive Chemotherapeutic Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard C. Erickson	Cancer Expert	LMPH NCI
	Leszek Szmigiero	Visiting Fellow	LMPH NCI
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LAB/BRANCH
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SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 4.5	PROFESSIONAL: 4.0	OTHER: 0.5

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☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relationship of DNA damage to differential cytotoxicity is being determined in human cells exposed to chloroethylnitrosoureas, platinum complexes, and AZQ. Alkaline elution filter techniques are being used to measure DNA single strand breaks, DNA protein crosslinks and DNA interstrand crosslinks produced by these agents. DNA interstrand crosslinking has been found to correlate with decreased survival of cells unable to repair chloroethyl DNA monoadducts which subsequently form interstrand crosslinks in DNA in a delayed reaction. The ability to block DNA interstrand crosslinking may be related to O^6 MeG repair systems present in normal and some tumor cells. This O^6 MeG repair system may be missing in some tumor cells rendering these cells more susceptible to chloroethylnitrosourea therapy. This repair system does not appear to remove cis-platinum monoadducts, thus cis-Pt may be a useful alternative for chemotherapy of tumor cells resistant to chloroethylnitrosoureas.

Project Description:

Objectives:

(1) To apply filter elution techniques developed in this laboratory to the study of DNA damage produced by chloroethylnitrosoureas in human normal cells and cell lines established from malignant glioma patients by the Surgical Neurology Branch of NINCDS.

(2) To determine the ability of these cell lines to repair DNA chloroethyl monoadducts which are capable of producing DNA interstrand crosslinks.

(3) In cell lines resistant to chloroethylnitrosoureas, to determine whether these cell lines would be sensitive to cis-platinum exposure.

(4) To determine the mechanisms of action of the new antitumor agents AZQ, and PCNU, by studying the DNA damage produced in normal and SV-40 transformed human cells, and in mouse L1210 leukemia cells.

(5) From the results of 1-4 above, to develop improved chemotherapeutic strategies, particularly in regard to selecting the most appropriate antitumor agent for an individual patient's tumor.

(6) To develop new applications of the alkaline elution technique, particularly in the study of DNA alkali labile sites (ALS).

Methods:

(1) Alkaline elution filter techniques developed in this laboratory, to measure DNA strand breaks, DNA alkali-labile sites, DNA protein crosslinking, and DNA interstrand crosslinks.

(2) Human cell tissue culture systems including colony formation assays without soft agar, and a differential cytotoxicity assay which compares the survival of cell lines exposed to chemothrapeutic agents to untreated control cells which complete 3 or more population doublings.

(3) Identification of drug-DNA adducts by high perfromance liquid chromatography (HPLC) in a collaboration with Dr. John Groopman of the Boston University School of Public Health.

Major Findings:

Chloroethylnitrosoureas in Malignant Glioma Cell Lines

In the two previous annual reports we have demonstrated: (1) normal human cells are capable of blocking DNA interstrand crosslink formation, probably by removing DNA monoadducts before the delayed second step of crosslink formation. (2) SV-40 transformed human fibroblasts (VA-13) and a variety of human tumor cells are apparently deficient in this repair process, and thus DNA interstrand crosslinks accumulate in these cells. (3) Survival is increased in cells capable of repairing DNA monoadducts. (4) Those cells capable of repairing

MNNG damaged Adeno 5 virus (Mer+ cells) are also capable of removing O^6-methyl-Guanine from their DNA, and are capable of avoiding DNA interstrand crosslink formation. Mer- cells do not repair MNNG damaged cells, do not remove O^6-MeG, and do not block chloroethylnitrosourea interstrand crosslinking.

Human malignant gliomas are brain tumors clinically treated by surgial resection, high dose radiation, and chemotherapy with chloroethylnitrosoureas. Response rates with nitrosoureas have been limited and in the range of 30-40%. In the past year we studied 13 human glioma cell lines provided by the Surgical Neurology Branch of NINCDS. Following exposure in vitro to chloroethylnitrosourea it was found that in 7 cell lines DNA interstrand crosslinking was absent. Two cell lines had intermediate levels of interstrand crosslinks, and 4 cell lines had DNA crosslinking levels expected for sensitive cell lines. These data suggest that a large proportion of malignant gliomas may be resistant to nitrosourea chemotherapy because they possess an active DNA monoadduct repair sytem which protects the tumor cells from the formation of a critical cytotoxic DNA lesion. Thus treatment with this chemotherapeutic agent may be unsuccessful.

Cis-Platinum in Malignant Glioma Cell Lines

Twelve of the same malignant glioma lines were also examined for DNA crosslinking following exposure to cis-platinum, a drug which has recently shown promise in the treatment of brain tumors. Unlike the results with chloroethylnitrosourea no cell lines were found in which the DNA interstrand crosslinking was totally absent. However, the levels of crosslinking varied by almost an order of magnitude from the lowest to highest. There was no correlation found between the cell lines with little or no chloroethylnitrosourea crosslinking, and low cis-platinum crosslinking. Thus it appears that the two types of DNA damage may be repaired by separate repair systems. Therefore cis-platinum may be a useful alternative to nitrosourea therapy in tumors which might be resistant to nitrosourea treatment.

As a result of these studies a clinical trial is being designed in collaboration with the Surgical Neurology Branch, NINCDS, to test malignant glioma patient's cells following surgery, and before the initiation of chemotherapy. The goal would be to select the chemotherapy that appears most appropriate from in vitro DNA crosslinking assays.

Comparison of PCNU and BCNU for DNA Damaging Properties, and Differential Cytotoxicity

PCNU is a new chloroethylnitrosourea which is undergoing clinical trials in this country and Japan. This compound was designed for optimum lipophilicity, and is a candidate for chemotherapy of tumors of the brain. PCNU was compared to BCNU in normal human fibroblasts (IMR-90), an SV-40 transformed human fibroblast (VA-13) and 6 malignant glioma cell lines. In differential toxicity studies PCNU was found to be more toxic to VA-13 cells than BCNU, and less toxic to IMR-90 cells than was BCNU. Survival of IMR-90 was approximately 7 times that of VA-13 when compared on a molar concentration producing similar cell kills. In the case of BCNU, this measure of differential cytotoxicity was only 3. DNA interstrand crosslinking levels were found to be higher for PCNU than for BCNU in cell lines in which crosslinking was observed.

Studies on the Mechanisms of Action of AZQ

AZQ is a new antitumor agent currently undergoing clinical trials. Studies on the mechanisms of action of AZQ were in IMR-90, VA-13 and mouse leukemia L1210 cells. Colony formation assays demonstrated that the cytotoxicity of AZQ was: VA-13 > IMR-90 > L1210. AZQ was found to produce DNA single strand breaks (SSB) in all cell lines with the SSB frequencies being VA13 > IMR-90 > L1210. AZQ was also found to produce DNA protein and DNA interstrand crosslinking with the ranking of VA-13 > L1210 > IMR-90. Glycerol, a free radical scavenger, protects the cells from the formation of some of the SSB's and some of the interstrand crosslinking. AZQ therefore appears to have a complex mechanism of action which produces a variety of DNA lesions. The ultimate cytotoxicity of AZQ in a cell may be due to the combination of all of these lesions.

Identification of Drug Induced DNA Adducts by HPLC

In a collaboration with Dr. John Groopman of the Boston University School of Public Health, we have begun to study chloroethylnitrosourea induced adducts in the DNA of L1210 cells exposed to PCNU in vitro. Preliminary experiments have identified the presence of 3 adducts: 7-OH-ethyl-guanine, 7-chloroethyl-guanine, and O^6-chloroethyl-guanine. Dr. Groopman is currently developing the methodology for the routine assay of these DNA adducts by HPLC of DNA from a variety of tumor types. This study will document the DNA adduct repair capabilities of these tumor cells.

Development of an Alkali-labile Site Elution Assay

In collaboration with Dr. Don Slagel of the University of Kentucky Medical School, during a 3 month sabatical visit to the LMP, we have developed an application of the alkaline elution methodology for the measurement of alkali labile sites (ALS). DNA from drug treated cells is eluted at pH 12.1 (the standard pH) and 12.8, a pH high enough to cleave most ALS. DNA elution rates at the two pH levels are then compared. Using this methodology it was found that the highly mutagenic methylating agent MNU produces high frequencies of ALS, whereas MMS, another methylating agent, does not. Work from other laboratories has shown that MNU produces primarily DNA phosphotriesters, whereas MMS produces primarily alkylated bases. Our data suggest, that the phosphotriester may be the lesion cleaved by the pH 12.8 treatment. We are currently studying the repair of these lesions over time.

Proposed Course:

1. To determine whether in vitro measurements of DNA crosslinking can be used to select the most favorable chemotherapeutic agent for treatment of patient's individual tumors. A clinical trial is currently being designed with Drs. Paul Kornblith and Barry Smith of NINCDS.

2. To develop an assay for guanine O^6-alkyl-transferase (GOAT) to measure protein acceptor levels in various Mer+ and Mer- cell lines. To determine if the presence of GOAT correlates with observed DNA crosslinking levels.

3. An HPLC system has been purchased by the Laboratory of Molecular Pharmacology. This instrument will be used to measure the presence, and repair, of various DNA adducts, to directly correlate the repair capacity with survival, and DNA crosslink formation.

4. Studies are planned to develop a cell isolation procedure to purify viable cells directly from tumor specimens to be analyzed for GOAT levels, and DNA crosslinking utilizing the fluorometric alkaline elution assay developed in this laboratory. This would circumvent the necessity for establishing a cell culture of each tumor to predict chemotherapeutic response.

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11. Thorgeirsson, S.S., Erickson, L.C., Smith, C.L. and Glowinski, I.B.: Genotoxicity of N-acetyl-arylamines in the Salmonella hepatocyte system. In: Proceedings of the 2nd International Conference on Carcinogenic and Mutagenic N-substitued Aryl Compounds. Hot Springs, Arkansas, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06140-06 LMPH

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Protein Interactions in Chromosomes; Cell Cycle and Cell Proliferation Controls

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: William Bonner Sr. Investigator 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 MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 Go). 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 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 but a discrete state or cycle.

Project Description

Introduction:

This year we found that when dividing cells cease division and become quiescent, they continue to synthesize histones at a reduced but significant rate. The pattern of variant synthesis in quiescent cells differs from that in S-phase; therefore, this synthesis cannot be attributed to the presence of S-phase cells in the culture.

Specifically, the synthesis of histone 3 variants .1 and .2 is turned off in quiescent cells. Cell lines IMR-90, 3T3, and CHO as well as normal human lymphocytes all show this change between cycling and quiescent states, indicating that this is a general phenomenon.

The histone synthesis in quiescent cells is unaffected by treatment with inhibitors of DNA synthesis such as hydroxyurea, indicating that this synthesis is not linked to DNA synthesis.

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.

HISTONE VARIANTS SYNTHESIZED IN VARIOUS CELL STATES

S-phase	Quiescent	Basal (G1, G2)
H4	H4	H4
H2B	H2B	H2B
H2A.1	H2A.1	---
H2A.2	H2A.2	---
H2A.X	H2A.X	H2A.X
H2A.Z	H2A.Z	H2A.Z
H3.1	---	---
H3.2	---	---
H3.3	H3.3	H3.3

Objectives:

A. To study the function of quiescent and basal histone synthesis. This includes investigating the fate of the histones synthesized in quiescent and G1 cells and the possible involvement of these histones in such processes as transcription, cell cycle timing, maintenance of chromatin, and DNA repair.

B. To use the patterns of histone synthesis to study the relationship of different cell behavioral states, particularly those other than S-phase.

C. To study the differential controls on histone synthesis at the mRNA and gene levels in order to help elucidate the factors that control cell cycling and proliferation behavior.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional eletrophoresis. (Methods developed in this laboratory).

(2) Peptide analyses on acrylamide gels to determine the relationship of proteins to each other. (Method developed in this laboratory).

(3) Synchronization of cell lines, particularly human Hela cells and Chinese hamster ovary cells for studies on cell cycle.

(4) Maintenance of cells in 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.

(6) Cell free translation of mRNA.

(7) Preparations to screen for genes of histone variants.

Specific Projects:

A. Function of Histones Synthesized in Non-S-Phase Cells

1. Fate

This study includes the fate of these newly synthesized histones. Basal histones synthesized in G1 are stable and present several generations later; they are found in mononucleosomes within 0.5 hr. Histones synthesized in quiescent cells are also stable and are rapidly incorporated into mononucleosomes.

2. Turnover

The results just discussed raise the possibility of histone turnover. Preliminary experiments comparing histone variant content of whole tissues with that of cells grown in vitro suggest that there may be histone turnover in quiescent cells. Experiments to document this process are in progress.

3. DNA Repair and Chromatin Maintenance

In collaboration with Dr. Len Erickson, we have studied the effect of stimulating DNA repair by UV on quiescent histone synthesis. Stimulating DNA repair synthesis does not stimulate the synthesis of either quiescent or S-phase histone variants. Because there is already significant histone synthesis in these cells, interpretation of these results will depend on the results of turnover studies.

4. Linkage of Quiescent, and S-Phase Histone Synthesis to DNA Synthesis

Many studies have shown that inhibition of DNA synthesis immediately leads to a similar inhibition of histone synthesis even though total protein synthesis is not significantly inhibited. Our studies with hydroxyurea, a classical inhibitor of DNA synthesis, show that basal and S phase histone synthesis are inhibited to different extents when DNA synthesis is inhibited. While hydroxyurea inhibited the synthesis of S-phase specific variants to less than 10% of their control levels the basal variants still maintained 26% to 34% of their control levels. This approximate threefold difference is also found in other cell types including Hela, a continuous human line; IMR90, a normal human embryonic fibroblast line; BE, a human colon tumor line, as well as L1210, a mouse line, and Rueber, a rat hepatoma line. The differential inhibition of basal and S-phase histone synthesis could be due to a differential linkage of these two types of synthesis to DNA sythesis or to another effect of the hydroxyurea. Comparing several types of DNA synthesis inhibitors and antitumor antimetabolites could enable us to distinguish between these possibilities. We are presently doing such a study with Dr. Leonard Erickson.

Quiescent histone synthesis is also resistant to inhibition of DNA synthesis by HU, this evidence supports other evidence including the DNA repair studies that quiescent histone synthesis is not linked to DNA synthesis.

B. Use of Histone Synthesis Patterns to Study the Relationship of Various States of Cell Behavior

1. Quiescence and G1

As discussed in the Introduction, quiescent histone synthesis and basal (G1, G2) histones synthesis do not exhibit the same variant pattern. This data was presented at the GWU Spring Symposium (May 20, 1982).

This finding indicates that quiescent cells are not simply arrested at some point in G1, but are in a discrete state or cycle. Using cells in appropriate states we are investigating when the decision to enter or exit quiescence is made. Results of histone variant synthesis patterns indicate that quiescent cells do not pass through G1 before entering S, but enter S from the quiescent state after an appropriate lag.

2. Other States. Studies are being initiated using defined media to study factors which influence cells to cease or initiate proliferation. This is an extremely broad area, but presently our objectives are limited to studying whether several kinds of quiescent states may exist.

C. Cell Proliferation and Cell Cycle Controls

1. mRNA Studies

mRNA from cells in various phases of the cell cycle and states of cell proliferation have been isolated and translated in cell free lysates from red cells. Experiments are being initiated to study the linkage of various kinds of histone synthesis to DNA synthesis at the mRNA level. Since inhibition of DNA synthesis

shuts down types of histone synthesis and not others, differences in mRNA or polysome structure are possible explanations.

D. Histone Protein Studies

1. Comparison of Sea Urchin Histones with Mouse Histones

In collaboration with Prof. David Nishioka at Georgetown, we have found that the sea urchin contains a protein which co-migrates with mouse H2A.Z. Comparison of the tryptic peptides of these two proteins shows that the sequence of H2A.Z is conserved between mouse and sea urchin to a much greater extent than is the sequence of the other H2A's. Another difference is that the synthesis of H2A.Z continues throughout sea urchin development from fertilization at least through gastrulation, while each of the other H2A variants is synthesized for a particular time period only.

The comparative studies of sea urchin and mammals have suggested that H2A.Z is important in a basic cellular process among multicellular organisms.

2. Acellular Slime Mold, Physarum

In a collaborative study with Professor Morton Bradbury, Head of the Department of Biological Chemistry, School of Medicine in Davis, California, we are doing a similar study with an acellular slime mold, Physarum. The preliminary results indicate that Physarum does contain a histone which migrates with or at least very close to mammalian H2A.Z. We hope to be able to compare the fingerprints of these proteins this summer.

3. Histone Variants in Evolution

This spring we were awarded a NATO Collaborative Fellowship to study Histone Variants in Evolution with Dr. H. Pataryas, Professor of Biology at University of Athens, Greece. Dr. Pataryas visited this laboratory in summer 1981, at which time he proved the feasibility of this approach. The fellowship will be used to set up the study in his laboratory in Athens.

E. Histone Modification

Histones are modified by acetylation, phosphorylation, methylation, poly-ADP ribosylation, and ubiquitination of various amino acid side chains. Our purpose here is to develop more rigorous procedures for separating and quantitating the various forms in the complex mixtures of modified histones normally present in living cells, and to study whether some insight can be gained as to the functional roles of these modifications. It should be noted that up to now it has only rarely been possible to quantitate various types of histone modifications.

Just before he left, Dr. Pantazis discovered that altering the ion content of the grow media can lead to greatly increased phosphorylation of H2A. This promises to be a useful tool for studying not only how phosphorylation alters chromatin structure and function, but also how the chromatin may react to enviromental influences. Lowering the pH of the growth media has a similar but not identical

effect.

F. H4 Expression in an SV40-Mouse H4 Gene Hybrid

Dr. Ajit Kumar of the George Washington University Medical School and Dr. Dean Hamer of the Laboratory of Biochemistry, DCBD have constructed a hybrid between SV40 DNA and mouse H4 gene. They have shown that the H4 region is transcribed using the late SV40 promoter. The question we are concerned with is whether or not the resulting mRNA is translated to give mouse histone 4. Since mouse H4 and monkey H4 (the host for SV40) are identical proteins, the answer must be a quantitative one rather than a qualitative one. Therefore we compared the ratios of the four nucleosomal histones in Green monkey kidney cells infected with wild-type SV40, a hybrid between SV40 and globin DNA, and the SV40-H4 hybrid. When given a 10 minute pulse of ^{14}C-arigine, the first two infections showed equimolar synthesis of the four nucleosomal histones. The third infection with the SV40-H4 hybrid showed equimolar synthesis of the H3, H2A and H2B with a 3 to 4 fold excess synthesis of H4. These results are a strong indication that H4 is translated from the mRNA transcribed from the SV40-H4 genome.

Significance to Biomedical Research and Program of the Institute:

Cancer at one level is the inappropriate multiplication of cells. Our findings during the last year have suggested that analysis of histone variant synthesis and the histone variant genes may yield some insight into the relationship of different cell states including neoplastic states.

Proposed Course:

1. To study the fate and function of histones synthesized in quiescent, G1, and G2 cells.

2. To gain insight into the relationship of different cell states through their pattern of histone synthesis.

3. To study the differential controls on histone synthesis at the mRNA and gene levels in order to help elucidate the factors that control cell cycling and proliferation behavior.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06150-1 LMPH

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cellular Effects of DNA Intercalating Agents

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard A. Zwelling	Cancer Expert	LMPH NCI
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	Kurt W. Kohn	Chief	LMPH NCI

COOPERATING UNITS (if any)

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Laboratory of Molecular Carcinogenesis, National Cancer Institute
Laboratory of Chemical Pharmacology, National Cancer Institute

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.9	3.2	1.7

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism by which DNA intercalating agents produce protein-associated DNA strand breaks in mammalian cells and the significance of these effects were studied. Various cell and subcellular targets were examined for their ability to demonstrate this reversible DNA effect. It appears to differ from other forms of DNA scission in its inability to stimulate cellular responses commonly elicited by DNA breaking agents, its dissociation from drug-induced cytotoxicity, its equivalent production in repair-proficient and repair-deficient human cells, and its enhanced production in the DNA from cells with actively transcribing chromatin, or with hypomethylated DNA. Aspects of the cytotoxicity, transport and DNA breaking potency of m-AMSA suggested a clinical trial testing its utility as a continuous infusion. This trial has been completed.

Project Description:

Objectives:

(1) Study the biologic effects in cells and subcellular systems of active, antineoplastic DNA intercalating agents.

(2) Study the relationship between these biologic effects and drug cytotoxicity.

(3) Relate the DNA effects of intercalating agents to those produced by other cytocidal agents through their effects on human cells derived from patients with known hypersensitivity to the cytotoxic actions of these other agents.

(4) To use these drugs as probes for the alterations in chromatin structure which accompany transcription and gene expression.

(5) To use the principles elucidated in the laboratory to design clinical trials using intercalating agents in a previously untried manner so as to increase their therapeutic index.

Methods Employed:

(1) Cell culture of various rodent and human cell lines -- treatment with chemical agents in vitro; radioactive labeling of macromolecules.

(2) Isolation of cell nuclei

(3) Nucleoid sedimentation

(4) Cell permeabilization

(5) X-irradiation of cells and subcellular components.

(6) DNA break and crosslink assays on membrane filters as developed in the laboratory.

(7) Synthesis of poly-(adenosine-diphosphoribose) in a permeabilized cell system.

(8) Soft agar colony formation of rodent cells.

(9) Colony formation assays of human cell lines.

(10) Radioactive drug uptake and egress

Major Findings:

(1) Previous work in this laboratory had elucidated the existence of a new effect of DNA intercalating agents in cells, the protein-associated DNA strand break. Detailed studies with a new, effective antileukemic agent m-AMSA showed that the most likely etiology of these breaks was that of an enzymatic, cellular

response to intercalator-induced DNA distortion. The anatomic location of this DNA breaking-rejoining response, its biochemical characteristics, the potential cofactors needed for this apparently enzymatically-mediated process, its relation to other forms of DNA scission and its relationship to cytotoxicity remained unknown. During this year we have made progress in all of the areas listed above.

(2) Basic Approaches to the Biochemistry and Cell Biology of Intercalating Agents.

a) Isolated Cell Nuclei

The protein-associated DNA strand breaks produced by m-AMSA could be reproduced in L1210 cell nuclei isolated by means of non-ionic detergent. DNA strand breaks and protein-crosslinks produced by m-AMSA formed and disappeared stoichiometrically and cotemporally in a manner identical to that initially described in whole cells. This localized the effect to the cell nucleus as well as demonstrating the effect could occur without cytoplasmic drug metabolism and without exogenously supplied energy sources, nucleotide triphosphates, or cofactors. However, the frequency of m-AMSA-induced scission in nuclei was less than that seen in whole cells indicating optimal conditions for m-AMSA action in nuclei had not yet been achieved. 5-iminodaunorubicin, an anthracycline previously studied in whole cells (vide infra) also produced DNA strand breaks and protein crosslinks with approximately equal frequency in nuclei. By contrast, 2-methyl-9-hydroxyellipticinium, another agent which, like m-AMSA and 5-iminodaunorubicin, had also produced protein-associated DNA breaks in cells, could not reproduce these effects in nuclei.

Examination of the conditions needed to optimize the individual drug effects in nuclei is now underway. The result with ellipticinium suggests the possibility that a necessary component for its action in cells is absent in isolated nuclei.

b) Nucleoids

Nucleoids are the nuclear components from cells which have been gently lysed with non-ionic detergent and then sedimented in high salt (1.9 M), neutral sucrose gradients. These conditions dissociate many nuclear proteins, including histones, from nuclear DNA leading to an alteration in the native, supercoiled DNA structure. This technique is very sensitive for detecting DNA breaks and DNA supercoil relaxation, both of which tend to retard the sedimentation of nucleoids. Coupling this technique with concurrent assays for strand breaks (alkaline elution) can allow study of the relationship of 2-dimensional DNA structural alterations to that of 3-dimensional alterations (altered supercoiling). We were interested in studying whether intercalators produced effects in this system comparable to those found in the alkaline elution system.

The nucleoids from m-AMSA-treated L1210 cells sedimented identically to those from untreated cells, indicating no breaks or supercoil alterations could be detected following m-AMSA treatment. This was at variance with the strand breaks detected in elution studies except that protein had to be enzymatically digested from cell lysates in the elution assays prior to break detection. A similar approach had not heretofore been accomplished in nucleoids. Limited

proteinase digestion of nucleoid cell lysates prior to centrifugation did unmask DNA breaks in nucleoids from m-AMSA-treated cells indicating that the breaks were present in vivo and were not simply artifacts of the detergent or alkaline conditions used in alkaline sedimentation or filter elution assays. This result, however, does raise the possibility that the protein associated with the DNA break does, in fact, bridge the break. One bond of the protein-DNA bridge can be dissociated by alkali without enzymatic digestion (in alkaline sedimentation studies) while the other is of a covalent nature. This system could potentially be used as a preparative technique to obtain a source for the isolation of this intercalator-stimulated DNA breaking protein.

c) Poly-(Adenosine-Diphosphoribose) Synthesis

The synthesis of poly-(ADP-R) is thought to be a prerequisite to DNA repair. The major stimulis to the synthesis of this polymer is DNA scission. The protein-associated nature of intercalator-induced DNA scission and its possible enzymatic origin distinguishes these drug-induced breaks from those associated with other cytocidal agents like x-rays. We therefore wished to see whether comparable frequencies of DNA breaks produced by m-AMSA vs. x-radiation would similarly stimulate poly-(ADP-R) synthesis using the permeabilized cell system of Berger. m-AMSA did not stimulate polymer synthesis despite the production of DNA breaks in excess of those produced by 4000 R of x-ray. Further the DNA breaks produced by m-AMSA could seal under conditions wherein DNA synthesis could not occur. This further substantiated the unique nature of the DNA effect produced by intercalating agents.

d) m-AMSA Uptake

For various studies it became necessary to dissociate the effects of m-AMSA on DNA scission from those resulting from altered m-AMSA uptake and egress. Using radioactive m-AMSA the transport of the drug was thoroughly examined. A new uptake phenomenon was described which was characterized by cooperativity, hysteresis, irreversibility, saturability, slowness, and temperature dependence. This cooperative sequestration in an extranuclear cell compartment had not been previously defined. Its importance rests in the fact that at or above the m-AMSA concentrations at which this process occurs, the sequestered drug has no access to nuclear DNA and appears not to contribute to cytotoxicity. This finding, along with our previous work, led to a clinical protocol which seeks to examine the toxicity and efficacy of infusional m-AMSA.

(3) The Relationship Between Intercalator-induced DNA Scission and Cytotoxicity.

a) 5-iminodaunorubicin

In previous work we sought to compare the DNA effects and cytotoxicity produced in L1210 cells by m-AMSA and adriamycin. The rapid reversibility of m-AMSA's effect were in sharp contrast to the persistent nature of adriamycin's effects. This difference precluded a meaningful comparison between these intercalators from 2 chemical classes. However, examination of the DNA effects produced by the adriamycin analog, 5-iminodaunorubicin, revealed that they reversed with a kinetics similar to the rapid reversal of m-AMSA kinetics. This allowed a

meaningful comparison. Neither the single-strand breaks (as measured by alkaline elution) nor the double-strand breaks (as measured by pH 9.6 elution) predicted the relative cytotoxicity of 5-iminodaunorubicin (vide supra - Section 3b).

b) 2-methyl-9-hydroxyellipticinium

Previous work had demonstrated that although ellipticine like other intercalators, could produce protein-associated DNA breaks, the breaks persisted following drug treatment thus precluding a comparison of ellipticine with intercalators from other chemical classes. 2-methyl-9-hydroxyellpiticinium is always cationic in aqueous solution and is far less lipophilic than its parent compound. Like ellipticine, it produced protein-associated DNA scission, but unlike ellipticine's breaks, those produced by the cationic compound rapidly reversed following cell treatment.

A meaningful comparison between intercalators from the acridine class (m-AMSA), anthracycline class (5-iminodaunorubicin), and the ellipticine class (2-methyl-9-hydroxyellipticinium) could now be made as to their relative DNA and cytotoxic effects (as measured by soft agar colony formation).

DSB/SSB: 2-methyl-9-hydroxyellipticinium > 5-iminodaunorubicin > m-AMSA
$t_{1/2}$ SSB Resealing: 2-methyl-9-hydroxyellipticinium > 5-iminodaunorubicin > m-AMSA
Cytotoxicity per SSB: 5-iminodaunorubicin $\geq$ 2-methyl-9-hydroxyellipticinium > m-AMSA
Cytotoxicity per DSB: 5-iminodaunorubicin > 2-methyl-9-hydroxyellipticinium > m-AMSA

These DNA breaks are not uniformly cytotoxic in these cells.

c) Effects of DMSO

Dimethyl sulfoxide is an active free-radical scavenging agent. As others had suggested a free-radically-mediated reaction might account for the DNA breaking and cytotoxic potential of adriamycin, we examined the effects of DMSO on intercalator-induced DNA effects and cytotoxicity. DMSO was able to proportionally abrogate a portion of the DNA breaking and cytotoxic effects of x-rays. However, whereas DMSO partially blocked x-ray breaks, it enhanced adriamycin-induced-DNA breaks and did so without altering the cellular uptake of adriamycin. Thiourea, another radical scavenger, was able to decrease adriamycin DNA scission and also did not alter drug uptake. The effects of DMSO and thiourea on adriamycin cytotoxicity were not quantitatively similar to their effects on DNA scission. Further, nucleoids from DMSO or thiourea-treated cells displayed altered DNA structure indicating the possibility that DMSO and thiourea altered DNA 3-dimensional structure in such a way as to change the target for DNA intercalators. This work further dissociates the intercalator-induced strand break from the lethal lesion produced by these drugs as well as from the mechanism of x-ray break production.

(4) The Use of Human Cells to Study the Etiology of the Intercalator-induced DNA Break.

a) Repair-deficient Fibroblasts

Several DNA-reactive agents are known to be more cytotoxic to repair-deficient human cells than to normal cells. These agents have in common the producton of DNA breaks in normal cells either directly or through the action of repair endonucleases. 2 of these deficient cell types are Xeroderma pigmentosum (XP) which are abnormally sensitive to ultraviolet light and ataxia-telangiectasia (A-T) which are abnormally sensitive to x-rays or bleomycin. We compared the cytotoxicity, DNA strand break production and drug uptake of m-AMSA in XP and A-T fibroblasts with normal fibroblasts and found no differences among the cell types. m-AMSA, and presumably other intercalators producing protein-associated breaks, are the first agents described which produce DNA strand breaks without an accompanying enhanced cytotoxic effect in XP or A-T cells. This also differentiates the intercalator-induced cellular response from that which is defective in these repair-deficient lines.

b) Hormone-responsive Human Breast Cancer Cells

MCF-7 cells are human breast cancer cells which possess estrogen receptor. Following estrogen treatment, this cytoplasmic receptor binds to the hormone, and the receptor-hormone complex translocalizes to the nucleus and interacts with cellular chromatin in a manner which results in the synthesis of specific gene products. The structural and biochemical events associated with gene expression are not well understood, but certainly an alteration in the chromatin structure must accompany the conversion of chromatin from a transcriptionally inactive to an active state. We theorized that this transcriptionally active chromatin state might display an altered response to DNA intercalation which is thought to depend on 3-dimensional DNA structure. MCF-7 cells which had been treated with 10^{-9} M estradiol for 19-24 hr display an enhancement in the protein-associated DNA scission produced by m-AMSA or 5-iminodaunorubicin. This does not result from altered drug uptake. Further, breast cancer cells lacking estrogen receptors do not display this enhancement. The enhancement of intercalator-induced DNA scission increases with increasing time of exposure of the cells to estrogen, but not with increasing or decreasing estrogen dose. The enhancement of DNA scission is only 20-60%. These results are consistent with a hormornally-dependent alteration in chromatin structure which either allows enhanced drug intercalation or an enhanced response to intercalation either through an upward modulation of the DNA distortion produced by each intercalated molecule or through the estrogen-stimulated synthesis of the products responsible for intercalator-induced DNA scission.

(5) 5-Azacytidine

Among the structural characteristics of DNA from cells with actively transcribing chromatin is a decrease in the amount of 5-methyl cytosine present when compared to DNA from non-transcribing cells. The hypomethylated state can be induced in cells by 5-azacytidine and this drug has been associated with the production of cellular differentiation at doses producing decreased methylation. The mechanism by which altered DNA methylation triggers gene expression is not known, but could result from local alteration in the 3-dimensional structure around sites of altered methylation which act as switches for gene expression. Recent findings that conversion of DNA from the B to the Z state can also

depend on cytosine methylation could eventually explain such switching. We have demonstrated that the DNA from 5-azacytidine treated cells will display more m-AMSA-induced, protein-associated DNA strand breaks than untreated cells. This effect is most clearly demonstrable 24 hr following 5-azacytidine treatment as would be expected if cell division and DNA synthesis on a hypomethylated template were required prior to the cellular DNA acquiring its new more readily scissioned configuration. This work, along with that done using MCF-7 cells, suggests that intercalators may serve as probes for cells with actively transcribing chromatin and/or the DNA structural correlates of gene expression.

(6) Clinical Trial of Continuous Infusion m-AMSA.

m-AMSA-induced DNA breaks formed rapidly in L1210 cells and disappeared rapidly following drug removal. Intracellular transport in and out was also rapid. Prolonged exposure of cells to low concentrations of m-AMSA was more cytotoxic than short exposures to high concentrations. These facts would indicate m-AMSA might best be administered to patients by continuous intravenous infusion rather than bolus administration as is commonly done. In a Phase I-II trial of continuous infusion m-AMSA we were able to safely administer the drug with some efficacy seen. However, none of these patients had acute non-lymphocytic leukemia, the disease against which m-AMSA is most active. A new trial treating such patients is about to begin.

Proposed Course:

(1) Basic Approaches - Biochemistry and Cell Biology.

a) Isolated Cell Nuclei

1. Determine the conditions to optimize the production of intercalator-induced DNA strand breaks. Examine pH dependence, effects of polyvalent cations like spermine and spermidine, effect of ATP and possible other co-factors.

2. Possibly utilize nuclei to isolate the protein which binds to DNA following nuclei exposure to intercalating agents.

3. Examine the production of double-strand breaks by these drugs in nuclei; compare with effects in whole cells. Could the activity which produces double-strand breaks be depeleted from nuclei during their isolation and thus account for the absence of breaks in nuclei treated with the double-strand breaking drug, 2-methyl-9-hydroxyellipticinium.

b) Nucleoids

1. Study the rate of reconstitution of normal nucleoid structure following m-AMSA treatment. Is it biphasic as seen following x-ray or does strand ligation and reconstitution of supercoiling occur cotemporally?

2. Do nucleoids from m-AMSA treated cells have DNA-protein crosslinks and, if so, could they serve as a source from which to isolate this protein?

(2) Intercalators as Probes for Normal Cell Function.

a) Hormone-responsive Human Tumor Lines

1. Is the finding of enhanced intercalator scission in hormonally-stimulated breast cancer cells unique for these cells? Glucocorticoid responsive human leukemia cells will be examined.

2. Is the enhanced single-strand breakage accompanied by enhanced DNA-protein crosslinking? If so, is this crosslinked DNA randomly distributed throughout the cell genome or enriched for specific, hormonally - stimulated genes?

b) 5-azacytidine

1. Is the enhanced DNA scission seen here a function of azacytidine-induced hypomethylation or its effects on DNA synthesis? Other DNA synthesis and growth inhibitors as well as inhibitors of methylase will be examined.

2. If hypomethylation is occurring, will the nuclei from azacytidine - treated cells exhibit characteristics of enhanced intercalator break induction? If so, can this be altered by buffer conditions favoring B --Z transitions?

(3) Clinical Studies.

A phase I-II trial of continuous infusion m-AMSA in the treatment of adult, non-lymphocytic leukemia in patients with a past history of other malignancies has been initiated.

In addition to studies of therapeutic efficacy and toxicity, basic work will be performed on the leukemic cells of these patients including transport studies, comparative studies on the DNA effects of various intercalators and probing for the presence of human onc genes.

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13. Zwelling, L.A., Kerrigan, D., Michaels, S., and Kohn, K.W.: Cooperative sequestration of m-AMSA in L1210 cells. Biochem. Pharmacol., in press.

14. Wysor, M.S., Zwelling, L.A., Sanders, J.E., and Grenan, M.M.: Cure of mice infected with Trypanosoma rhodesiense by cis-diamminedichloroplatinum (II) and disulfiram rescue. Science, in press.

15. Zwelling, L.A., Kerrigan, D., Pommier, Y., Michaels, S., Steren, A., and Kohn, K.W.: Formation and resealing of intercalator-induced DNA strand breaks in permeabilized L1210 cells without the stimulated synthesis of poly-(ADP-Ribose). J. Biol. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06151-1 LMPH

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

DNA Damage Produced by Radiation and Antitumor Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Leonard A. Zwelling	Cancer Expert	LMPH NCI
	Michael Mattern	Staff Fellow	LMC NCI
	Donna Kerrigan	Chemist	LMPH NCI
	Yves Pommier	Visiting Fellow	LMPH NCI
	Kurt W. Kohn	Chief	LMPH NCI

COOPERATING UNITS (if any)

Laboratory of Molecular Carcinogenesis, National Cancer Institute

LAB/BRANCH
Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The effects of inhibitors of poly-(ADP-R) synthesis on x-ray break resealing were examined using alkaline elution. These compounds slowed but did not prevent DNA strand break ligation in L1210 cells. Further, this effect was reproduced in human fibroblasts including cells from patients with ataxia telangiectasia (A-T) which are putatively deficient in polymer synthesis following x-ray. Direct examination of poly-(ADP-R) synthesis in normal and A-T fibroblasts revealed no differences in our studies. This slowing of x-ray break resealing was also demonstrated using the nucleoid sedimentation technique. Additionally, this technique allowed the description of a second phase to x-ray DNA damage repair which most probably corresponds to reconstitution of the normal supercoiled structure of chromatin.

Project Description:

(1) To study the kinetics of x-ray-induced DNA break resealing and its alteration by inhibitors of poly-(adenosine diphosphoribose) synthesis in rodent and human cells.

(2) To compare the rate of x-ray-induced DNA break resealing with the rate of reconstitution of normal DNA 3-dimensional structure following x-radiation.

Methods Employed:

(1) Cell culture of various rodent and human cell lines -- treatment with x-radiation and chemical agents in vitro; radioactive labeling of macromolecules.

(2) Nucleoid sedimentation

(3) Cell permeabilization

(4) DNA break assays on membrane filters as developed in this laboratory.

(5) Synthesis of poly-(adenosine-disphosphoribose) in a permeabilized cell system.

Major Findings:

1. Background.

The repair of x-ray-induced, DNA strand breaks necessarily requires the coordination of several connected steps. DNA damage must be recognized, the template must be brought to the biochemical state required to act as a substrate for the repair process, new DNA must be synthesized to fill gaps, the newly synthesized DNA must be ligated to the old, and finally reconstitution of the 3-dimensional supercoiled structure must occur to insure the reproduction of normal structure and, theoretically, function. Several aspects of these various processes have been studied, but the precise means by which these processes occur and are coordinated with one another is incompletely understood. Understanding these processes, their relative rates, degrees of fidelity and their differences among various malignant and non-malignant cells could aid in our understanding of the means by which x-radiation kills cells in general and tumor cells in particular. We have begun to study several aspects of x-ray break repair in rodent and various human cell systems.

2. Effects of Inhibitors of Poly-(Adenosine-diphosphoribose) on DNA Break Repair Rate.

The poly-(ADP-R) inhibitors 3-aminobenzamide and 5-methylnicotinamide slow, but do not block x-ray-induced, DNA break repair in murine L1210 cells. This suggests that x-ray break repair is most rapid when poly-(ADP-R) can be synthesized, but can occur even when polymer synthesis is blocked. This polymer, the synthesis of which is felt to be a requirement for DNA break repair, may not be required for strand ligation. This suggests the presence of 2 systems by which x-ray breaks can be resealed, one dependent on poly-(ADP-R)

synthesis and the other polymer independent. If the second is more error-prone than the first, this could account for the enhanced cytotoxicity of agents which which stimulate polymer synthesis when treated cells are incubated with poly-(ADP-R) synthesis inhibitors following treatment.

3. Poly-(ADP-R) Synthesis in Repair-deficient Human Fibroblasts.

Ataxia-telangiectasia (A-T) is a human disorder characterized by defects in the immune, vascular and central nervous systems of afflicted individuals. Cells from these patients are more sensitive to the cytocidal effects of ionizing radiation than normal cells. A recent report attributed this cell sensitivity in human lmyphoblasts to deficient synthesis of poly-(ADP-R) following x-ray. We have examined 2 normal, 1 xeroderma pigmentosum, 1 Fanconi's anemia and 2 A-T fibroblast lines for their ability to synthesize poly-(ADP-R) following x-radiation. We found no differences among these cell lines. Further, x-ray-induced DNA scission repair was slowed by 3-aminobenzamide in normal and A-T cells just as it had been in L1210 mouse leukemia cells. These data indicate that some A-T cells possess the ability to synthesize poly-(ADP-R) and indicate the earlier finding is not uniformly true for all A-T cells.

4. Comparing the rate of DNA Strand Ligation and Reconstitution of Nucleoid Integrity Following X-ray.

X-ray-induced DNA breaks reseal within 10 min at 37°. Nucleoids from x-irradiated cells do not sediment as controls until 3-5 hr following x-ray. Nucleoid reconstitution is biphasic with an early phase occurring within a time frame comparable to that of strand resealing and a late phase beginning 1-2 after DNA breaks are gone. The early phase, like strand ligation itself, is slowed by 3-aminobenzamide. This inhibitor of poly-(ADP-R) synthesis is without effect on the late phase. The late phase, is however, blocked by novobiocin, an inhibitor of the bacterial topoisomerase, DNA gyrase. Novobiocin is without effect on DNA strand ligation or the early phase of nucleoid reconstitution. It appears that there are at least 2 steps to returning x-irradiated nuclear chromation to its pre-irradiation state.

Proposed Course:

These interlocking studies lead to larger questions. What is the connection between the reconstitution of chromatin structure and its return to normal function such as DNA synthesis? Will intercalator breaks follow similar biphasic reconstitution kinetics or will religation and recoiling occur together? Will inhibitors affect intercalator break reconstitution as they affected x-ray break reconstitution?

Publications:

1. Zwelling, L.A., Kerrigan, D., and Pommier, Y.: Inhibitors of poly-(adenosine disphosphoribose) synthesis slow the resealing rate of x-ray-induced DNA strand breaks. Biochem. Biophys. Res. Commun. 104: 897-902, 1982.

ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1981 - September 30, 1982

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. 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 and normals in different parts of the world are being intensively studied. (2) Studies on the biochemical events preceding mitosis. An understanding of these events appears essential to the control of proliferation, information derived from such studies may lead to more effective inhibitors or neoplastic cell growth. Events leading to mitosis are also of interest since many of the effective antitumor agents are useful only when cells are in DNA replication or in mitosis. Phytohemagglutinin stimulated human lymphocytes and tissue culture cells are the principal tools in these studies. (3) Attempts to develop new approaches to cancer chemotherapy using information gained from basic cellular studies. (4) Studies on the development of biochemical nd immunological markers for malignant cells are carried out. Biochemical and immunological studies are also conducted in individuals with disorders associated with an increased incidence of neoplasia. (5) Controls regulating cellular growth and differentiation, and the process of malignant transformation in hematopoietic cells. (6) Growth factors (and their receptors) that control the growth and differentiation of blood cells have been isolated and are under intensive study, e.g., T-cell growth factor (TCGF), CSF, and related hematopoietic growth effecting molecules.

During the past year a number of findings were reported by investigators from the Laboratory;

Major Findings:

1. T cell growth factor (TCGF) has been purified to homogeneity. Glycosylated, sialylated, and non-modified forms have been identified.

2. The mRNA for TCGF has been purified. The frog oocyte system has been developed for translation of the mRNA.

3. Numerous new neoplastic human T-cell lines have been developed using partially purified TCGF.

4. Ten additional new human T cell leukemia virus (HTLV) isolates have been obtained from eight different areas of the world. (There are now thirteen isolates.) All isolates have been from adult T cell leukemia or lymphoma or in some people in a "pre-leukemic" phase.

5. Antibodies to HTLV p19 and p24 have been found in sera from a large number of T cell leukemia/lymphoma patients and in some healthy normals.

6. Seroepidemiology studies show that HTLV is endemic in Japan, West Indies, South East U.S.A., China, Alaska, various regions of South America, and possibly in Israel.

7. Molecular cloning of HTLV has been achieved. Two subgroups of HTLV have been identified. Most isolates fit subgroup I.

8. Biological activity of HTLV has been demonstrated. HTLV can directly transform human mature cord blood T cells. These cells are OKT-4 positive, lose their requirements for exogenous TCGF for growth, turn on production of endogenous TCGF, develop several new surface markers including modified HLA, gain the ability to grow on agar, grow to high saturation density, and produce tumors in nude mice.

9. The preliminary data on the molecular mechanism of transformation of human T cells by HTLV appears to be very similar to that in avian B lymphomas induced by avian type-C leukemia viruses.

10. In addition to HTLV p24, p19 and reverse transcriptase, p15 has also been purified. Amino acid sequence analysis of HTLV p24 and much of the p15 has been completed.

11. Assays for HTLV antigens in sera of patients with T cell malignancies have been developed. ELISA assays for HTLV antibodies and antigens for clinical laboratory testing have been developed. Some normal blood bank donors (2 of 286) have been found to be HTLV positive.

12. Four human onc genes have been cloned (myb, myc, sis, fes). The study of their expression in human hematopoietic cells of various types has been completed.

13. Complete nucleotide sequence analysis of the sis gene has been achieved.

14. The first example of an amplified onc gene in man has been found, namely the myc gene in HL-60. The amplification is 15 to 32 fold and was found in fresh cells as well as the cell line HL-60.

15. A DNA transfection system has been established and DNA from some fresh uncultured human lymphoid tumors was shown to successfully transform NIH 3T3 cells.

16. Endogenous retrovirus related sequences (not onc genes) have been identified in human DNA and successfully cloned. The sequences include reverse transcriptase gene and other regions.

17. Myeloid cells and monocytes from normal donors have been successfully grown in culture in many instances for close to one year and in one case a permanent normal human monocyte cell line has been established.

18. Retinoic acid and cAMP (e.g., prostaglandin E, cholera toxin, dibutyl cAMP) act synergistically in inducing terminal differentiation of HL60.

19. A differentiation inducing factor (DIF), produced by T cells, acts synergistically with retinoic acid in inducing terminal differentiation of both HL60 and promyelocytes from a patient with acute promyelocytic leukemia.

20. Two human monoblastic-like cell lines (U937 and THP-1) have been induced to differentiate with retinoic acid to monocyte-like cells.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06117-10 LTCB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells and Origin and Pathogenesis of the Leukemias and Lymphomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Robert C. Gallo	Chief, Lab. of Tumor Cell Biology	LTCB NCI
Others:	Prem S. Sarin	Chemist	LTCB NCI
	Theodore Breitman	Chemist	LTCB NCI
	Carl Saxinger	Microbiologist	LTCB NCI
	Flossie Wong-Staal	Microbiologist	LTCB NCI
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COOPERATING UNITS (if any)
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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
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
40	24	16

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

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 to human leukemic cells; (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

Christine Eastment	Postdoctoral Fellow	LTCB NCI
Riccardo Dalla Favera	Visiting Associate	LTCB NCI
Genoveffa Franchini	Visiting Associate	LTCB NCI
Sandra Colombini	Visiting Fellow	LTCB NCI
Hiromichi Hemmi	Visiting Fellow	LTCB NCI
Vittorio Manzari	Visiting Fellow	LTCB NCI
Jorg Schupbach	Visiting Fellow	LTCB NCI
Marco Bregni	Guest Worker	LTCB NCI
Beatrice Hahn	Guest Worker	LTCB NCI
Stephan Lindner	Guest Worker	LTCB NCI
Mika Popovic	Guest Worker	LTCB NCI

COOPERATING UNITS

and Tumor Institute; Bill Hardy, Sloan Kettering, New York; George Vande Woude, National Cancer Institute; Gianmarco Corneo, University of Milan; Yohei Ito, University of Kyoto; Umberto Torelli, University of Modena; Max Essex, Harvard University; Bill Haseltine, Harvard University; Jack Strominger, Harvard University; Henry Kaplan, Stanford University; Luc Montagnier, Pasteur Institute, Paris; Roger Monier, Cancer Institute, Villejuif; Hartmut Schetters and Volker Erfle, Munich; Armand Tavitian, Hospital St. Louis, Paris; Kendall Smith, Dartmouth Medical School; Ron Herberman, National Cancer Institute; Fernando deNoronha, Cornell University; Ivor Royston, University of California at San Diego; Bill Blattner, Epidemiology Branch, National Cancer Institute; Mark Smulson, Georgetown University; Isaac Witz, Tel Aviv University.

Projection Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia. Special focus is on the leukmias and lymphomas.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

2. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization, restriction enzyme analysis and gene cloning.

3. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells were recently set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

4. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

5. Markers: (a) Immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells are being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

6. Cell separation studies are being carried out to enrich subpopulation of leukemic cells which may contain the type-C RNA tumor virus related markers and other biological markers.

7. Techniques are being developed to use monoclonal antibodies, prepared against cell surface antigens for subtyping and separation of peripheral blood and bone marrow cells with the help of a fluorescence activated cell sorter.

8. Antibodies associated with the membranes of human leukemic cells have been isolated. They are under study as to which protein antigens they interact with i.e., are they leukemia cell specific, e.g., viral, etc.

9. Recombinant DNA technique is being utilized to obtain molecular DNA clones of defective and non-defective primate and human viruses. DNA from these clones will be utilized to carry out transfection experiments and for generation of subgenomic fragments for probes and functional analysis.

10. Human T cell growth factor (TCGF) has been purified to homogeneity for further characterization. Studies are in progress to determine receptors on activated T cells for TCGF.

11. The distribution of T cell leukemia virus (HTLV) in human T cell leukemia patients from various parts of the world is being actively pursued.

Major Findings

1. T cell growth factor (TCGF) has been purified to homogeneity. Glycosylated, sialylated, and non-modified forms have been identified.

2. The mRNA for TCGF has been purified. The frog oocyte system has been developed for translation of the mRNA.

3. Numerous new neoplastic human T-cell lines have been developed using partially purified TCGF.

4. Ten additional new human T cell leukemia virus (HTLV) isolates have been obtained from eight different areas of the world. (There are now thirteen isolates.) All isolates have been from adult T cell leukemia or lymphoma or in some people in a "pre-leukemic" phase.

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19. A differentiation inducing factor (DIF), produced by T cells, acts synergistically with retinoic acid in inducing terminal differentiation of both HL60 and promyelocytes from a patient with acute promyelocytic leukemia.

20. Two human monoblastic-like cell lines (U937 and THP-1) have been induced to differentiate with retinoic acid to monocyte-like cells.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanism and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effect on Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72; Proc. Nat. Acad. Sci. 69: 2879-2884, 1972; Proc. Nat. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972).

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol. 12: 431-439, 1973; Biochim. Biophys. Acta 454: 212-221, 1976, 479: 198-206, 1977, 564: 235-245, 1979; Virology 112: 355-360, 1981).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units, focus formation and plaque assays for virus are carried out by conventional techniques.

7. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter techique with RNA covalently attached (Proc. Nat. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S1 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 (SX test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

10. Cell separation studies are carried out using ficoll-hypaque gradients, sucrose density gradients, free flow electrophoresis and centrifugal elutriation. (Lancet 1: 508-509, 1976).

Publications

1. Bodner, A.J., Ting, R.C., and Gallo, R.C.: Induction of Differentiation of Human Promyelocytic Leukemia Cells (HL-60) by Nucleotides and by Methotrexate. J. Natl. Cancer Inst. 67: 1025-1030, 1981.

2. Breitman, T.R., and Gallo, R.C.: New Facts and Speculations on Human Myeloid Leukemias. In Bessis, M. (Ed.): Blood Cells. New York, Springer International, Vol. 7, 1981, pp. 79-89.

3. Dalla Favera, R., Gelmann, E.P., Gallo, R.C., and Wong-Staal, F.: A Human onc Gene Homologous to the Transforming Gene (v-sis) of Simian Sarcoma Virus. Nature 292: 31-35, 1981.

4. Gallo, R.C.: Growth of Human Normal and Leukemic T-Cells: T-Cell Growth Factor (TCGF) and the Isolation of a New Class of RNA Tumor Viruses (HTLV). In Bessis, M. (Ed.): Blood Cells. New York, Springer International, Vol. 7, 1981, pp. 313-329.

5. Gallo, R.C., de-The, G.B., and Ito, Y.: Kyoto Workshop on Some Specific Recent Advances in Human Tumor Virology. Cancer Res. 41: 4738-4739, 1981.

6. Gallo, R.C., Poiesz, B.J., and Ruscetti, F.W.: Regulation of Human T-Cell Proliferation: T-Cell Growth Factor and Isolation of a New Class of Type-C Retroviruses from Human T-Cells. In Neth, R., Gallo, R.C., Graf, T., Manweiler, K., and Winkler, K. (Eds.): Modern Trends in Human Leukemia IV. Germany, Springer-Verlag, 1981, pp. 502-514.

7. Gallo, R.C., and Ruscetti, F.W.: New Human Hematopoietic Cell Systems for the Study of Growth, Differentiation, and Involved Factors: Some Therapeutic Implications. In Sartorelli, A.C., Lazo, J.S., and Bertino, J.R. (Eds.): Molecular Actions and Targets for Cancer Chemotherapeutic Agents. New York, Academic Press, Vol. 2., 1981, pp. 379-394.

8. Gelmann, E.P., Wong-Staal, F., Kramer, R.A., and Gallo, R.C.: Molecular Cloning and Comparative Analyses of the Genomes of Simian Sarcoma Virus (SSV) and its Associated Helper Virus (SSAV). Proc. Nat. Acad. Sci. USA 78: 3373-3377, 1981.

9. Gootenberg, J.E., Ruscetti, F.W., Mier, J.W., Gazdar, A., and Gallo, R.C.: Human Cutaneous T-Cell Lymphoma and Leukemia Cell Lines Produce and Respond to T-Cell Growth Factor. J. Exp. Med. 154: 1403-1418, 1981.

10. Josephs, S., and Wong-Staal, F.: Unintegrated and Integrated Proviruses of Two Strains of Baboon Endogenous Viruses: Comparative Restriction Endonuclease Analysis. Virology 112: 282-288, 1981.

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