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THREE FOURTHS OF PEDIATRIC PATIENTS ARE GETTING BEST TREATMENT; NCI GOAL: REACH ALL THE OTHERS

Some of the most notable successes in cancer treatment during the past 15-20 years have occurred in the treatment of childhood cancer. Although there will be only 6,400 new pediatric cancer patients in 1977 in the U.S. compared with 690,000 adults, the problem of childhood cancer "transcends the figures," in the words of Donald Buell, program director for medical oncology in NCI's Div. of Cancer Control & Rehabilitation.

"The effort that childhood cancer brings forth perhaps accounts for

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In Brief

HOME HEALTH CARE COST EFFECTIVENESS DATA SOUGHT THROUGH COMMUNITY BASED PROGRAMS

COST EFFECTIVENESS assessment of home health care programs should be added as a task for Community Based Cancer Control Program contractors, the Cancer Control & Rehabilitation Advisory Committee recommended. Ruby Isom, NCI project officer for the program, said the contractors already were planning for data collection on costs and cost reimbursement and felt most could get home care costs without too much trouble. . . . **TOBACCO INDUSTRY** objected to the saccharin-cigarette analogy drawn by Congressman Henry Waxman and reported in ***The Cancer Letter*** April 15. Frederick Panzer, vice president of the Tobacco Institute, contends that the Canadian tests showing increased incidence of bladder cancer in saccharin exposed animals are more valid than any animal tests with tobacco. Panzer also said Waxman's "analogy falls down" when he complained that there is no government regulation of cigarettes, citing advertising and marketing controls and government-financed antismoking education programs. . . . **RAY CRAMPTON**, listed as the principal investigator for the Long Island Community Based Cancer Control Program (***The Cancer Letter***, April 29), is the president of the Long Island Cancer Council, the CBCCP contractor, and is also president-elect of the Nassau County Medical Society. . . . **NEW PUBLICATIONS**: "You Can Fight Cancer and Win," by New York Times medical science writer Jane Brody and ACS vice president for medical affairs Arthur Holleb (Quadrangle/New York Times Book Co., $12.50). Written for the general public, it's a positive guide on how cancer can be prevented, detected and treated. It includes useful information on where to find the best treatment, with names and addresses of comprehensive cancer centers and hospitals with approved cancer programs. *Journal of Environmental Pathology & Toxicology* will make its debut in July. Edited by Myron Mehlman, NIH interagency liaison officer, it will sell for $33 for a one year subscription (six issues), $58 for two years. Manuscripts are being solicited—write to Mehlman, P.O. Box 34790, Bethesda, Md. 20034.

Betty Mascivia reported that 100 paperbacks copies have been ordered for distribution. We will be receiving them.

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TWO NEW GRANT PROGRAMS IN PEDIATRIC CANCER ARE BEING CONSIDERED BY DCCR

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the improvement," Buell told the Cancer Control & Rehabilitation Advisory Committee. That improvement is reflected in the death rate, which has decreased from 8.4 per 100,000 in 1950 to 5.3 per 100,000 in 1974. The incidence of childhood cancer has been stable over the last 30 years at about 12 cases per 100,000.

There will be an estimated 2,800 deaths attributed to pediatric cancer this year, and five-year survival is now recognized at about 33%, the same as adults, "but I suspect it will be better than that for children this year," Buell said.

One of the more emotional arguments put forth in favor of the Cancer Control Program when the National Cancer Act was being debated in 1971 was that, while treatment for childhood cancer had improved, optimal treatment was not available to most of the patients. The situation has improved considerably, and Buell presented figures which show that approximately 4,800 of the 6,400 patients will be treated either under the auspices of three cooperative groups, four major centers or other centers, all with strong pediatric cancer programs.

The cooperative grups are Children's Cancer Study Group, 1,200 patients; Southwest Oncology Group, 1,200 patients; and Cancer & Leukemia Group B, 1,000.

The major centers are Roswell Park, Memorial Sloan-Kettering, St. Jude's, and Sidney Farber, with a total of 700 (not including those on cooperative group protocols).

Other pediatric centers account for an estimated 700. These are children's hospitals offering a good level of care, such as Denver, Cincinnati, Stanford, and other large pediatric hospitals, Buell said.

One of the goals of the DCCR pediatric cancer control effort is to reach the remaining one fourth, or 1,600 children.

The Children's Cancer Study Group headed by Denman Hammond, with a $648,000 contract from DCCR, hopes to reach an additional 400-500 patients in satellite hospitals. The Southwest Oncology Group headed by Barth Hoodstraten, with a $228,000 contract from DCCR, estimates it will have access to 300-400 more patients in 10 participating institutions.

Another goal is to upgrade the knowledge and treatment skills of community physicians, to minimize the chances of incorrect initial management decisions and to permit treatment whenever possible to be administered in the child's own community.

"Can children be treated appropriately and optimally within the community?" Buell asked, and answered it with, "Yes, for some diseases."

Buell commented that in the past it was generally believed early treatment offered no significant benefit for leukemia patients. "The word is out now that for leukemia, get treatment fast," he said. Most physicians can administer vincristine and prednisone, the most common of antileukemia drugs.

DCCR supports a leukemia-lymphoma network in a prototype clinical chemotherapy program in which seven centers receive a total of $726,000 a year. The program at Children's Hospital in Los Angeles, headed by Gussie Higgins, "is the star of the show," Buell said. Others are Children's Hospital, Denver, David Tubergen; Dartmouth Medical School, O. Ross McIntypre; Univ. of Alabama, John Durant; New York Hospital, Cornell, Richard Silver; and Mount Sinai School of Medicine, Louis Wasserman.

Other DCCR pediatric cancer treatment programs are:

—Delaware Valley Pediatric Oncology Program and Central Tumor Registry, $159,000. Audrey Evans, at Children's Hospital of Philadelphia is principlal investigator.

—Community Based Therapy for Children with Cancer, at the Univ. of Iowa, $84,500 a year. C. Thomas Kisker is PI. Treatment is performed by private physicians in the state.

—Regional Ambulatory Cancer Chemotherapy Program, Ohio State Univ., $9,700, with James Battles as PI.

Max Myers, head of the End Results Section in NCI's Biometry Branch, reported on a study of 101 children with acute lymphocytic leukemia. All were diagnosed in 1972. Five died in the first month after diagnosis, and all the rest achieved remission, treated as follows:

—Eighty six received vincristine and prednisone. Of those, 15 were treated with 6-mercaptopurine in combination with vincristine and prednisone; seven were treated with L-aspariginase in combination with vincristine and prednisone; and four others received other drugs in combination with vincristine and prednisone.

—Ten were treated with 6-mercaptopurine and prednisone.

—One received vincristine and dexamethasone.

—One died before treatment was started, and the treatment for three others was unknown.

The median time from first visit to a physician to the diagnosis of leukemia was nine days. Some were longer, and for at least one it was six months.

All remission maintenance regimens used either 6-mercaptopurine, cyclophosphamide or methotrexate singly or in combination. Eleven received the single drug, eight two drugs, 20 three drugs and 48 four or more drugs for remission maintenance.

Two year survival was 66%, and that includes the five who died in the first month.

Buell commented that Cincinnati Children's Hospital has achieved a two year survival rate of 67% for ALL patients, with a remission rate of 97%. He also

mentioned the program at Denver, which through its outreach efforts reaches 80% of pediatric cancer patients in Colorado and Wyoming. Comparing those treated at Denver with those at outlying hospitals, six of 14 ALL patients in Denver remain in remission and 10 of 13 in outlying areas are in remission.

"We had an interesting problem at Alabama. The physicians at the university did not want patients treated in outlying hospitals," Buell said. "They wanted to stay with the established patterns."

DCCR supports seven psychosocial programs in childhood cancer. "We need to take a hard look at them when evaluation time comes," said DCCR Director Diane Fink. They are:

—Psychological adaptations to childhood leukemia, St. Jude Children's Hospital, George Marten, PI, $66,800.

—Coping in families with a leukemia child, Children's Memorial Hospital, Chicago, Jerome Schulman, $107,100.

—Studies of long term survivors of childhood cancer, Sidney Farber Cancer Center, Norman Jaffe, $165,900.

—Home care for children with cancer, Univ. of Minnesota, Ida Martinson, $95,000.

—Childhood cancer: psychosocial rehabilitation, Univ. of Kansas, Shirley Lansky, $117,500.

—School problems of children with malignant neoplasms, Children's Hospital, Denver, William Zwartjes, $95,000.

—Pain control through hypnosis in childhood cancer, Stanford Univ., Ernest Hilgard, $34,900.

Other programs in pediatric cancer control supported by DCCR include:

—Study of oncogenesis and other late effects of cancer therapy at Children's Hospital of Philadelphia under Giulio D'Angio. The study includes 10 hospitals with many childhood cancer patients. It originally was based on radiation but now includes all late effects.

—Study of the incidence and natural history of genital tract anomolies and cancer in offspring exposed in utero to synthetic estrogen (DES studies). There are four contracts totaling $981,000.

DCCR is considering establishing grant programs in adolescent oncology and family competence in coping with cancer.

ASCO URGED TO BE MORE AGGRESSIVE ON NATIONAL ISSUES

A member of the American Society of Clinical Oncology this week urged the organization to become a more aggressive advocate of positions adopted by the membership, including questions on national issues which affect clinical oncologists. David Fisher, Yale Univ., said, "The surgeons have such a group that speaks loudly. Radiologists have one. But we oncologists come to this meeting once a year and discuss drug methodology, and the organization doesn't do anything about these problems. Do we have to form a new organization? I hope not. This organization ought to serve that kind of representation."

Fisher made his comments in a session on "Perspectives in Cancer Care Planning" at the annual ASCO meeting in Denver, responding from the floor after presentations by panel members. The major problem he cited that needs attention from ASCO involves reimbursement for anticancer drugs prescribed for outpatients. "Medicare will pay 80% of the redbook price, and few physicians can get drugs for the redbook price," Fisher said. "The Blues won't pay for any drugs in the physician's office. So what do we do? Bill the patient for the 20% Medicare doesn't pay, or for the entire amount if he's not covered by Medicare, and hope to collect it? Or tell him his disease is treatable but he can't have the drugs?"

"That is not what usually happens," Fisher said. "We admit the patient into the hospital for 24 or 48 hours, so he can get $200 worth of drugs, and the hospital charge is $600. Then the President screams about hospital costs."

David Johnson, administrator of Deaconess Hospital, Evansville, Ind., commented that Blue Cross-Blue Shield will authorize payment for outpatient drugs provided it is built into the premiums. Johnson was a member of the panel, speaking on "Problems of Cancer Care Implementation as Viewed from the Community Hospital."

Diane Fink, director of NCI's Div. of Cancer Control & Rehabilitation, was scheduled as a panel member on the topic "Cancer Control in Community Oncology." She did not appear, however, sending Donald Buell, program director for medical oncology, who said Fink could not get away because of the problems related to the "mammography issue."

Buell described community programs supported by DCCR and commented that one of the division's major problems is determining when new methods or technology is ready. "When information transfer is implemented by DCCR, it tends to become standard therapy, so we have to be cautious."

Johnson said the major problem he has encountered is the shortage of trained personnel, especially medical oncologists. Oncology nurses are badly needed, with each community cancer center needing one. Johnson said he could find only 12 institutions that award advance degrees in oncology nursing.

In Congress

HEW MONEY BILLS MARK UP MAY 26

The HEW appropriations bill which will include NCI's funds for the fiscal year starting next Oct. 1 is nearing the final stages of committee work. Both House and Senate HEW Subcommittees are scheduled to mark up their bills May 26. Full committee action could be taken in the following two weeks, and both could reach floor votes by the end of June.

FORMER NCI DIRECTOR STILL IN THERE PITCHING FOR THE CANCER PROGRAM

Frank Rauscher left his job as NCI director more than six months ago, but he hasn't given up the job of selling the National Cancer Program with the upbeat approach that made him such an effective spokesman before scientific and lay audiences, and Congress.

Rauscher has carried on the task as chief defender of NCI and the Cancer Program, partly because the appointment of a permanent director has been delayed so long (Acting Director Guy Newell, who has been an effective spokesman when required, has been reluctant to thrust himself into that role), and partly because that was one of the jobs, if not the primary one, for which the American Cancer Society hired him.

Rauscher was scheduled to deliver the David A. Karnofsky Memorial Lecture at the American Society of Clinical Oncology meeting this week. His topic: "The National Cancer Program—Reflections and Current Issues." It's a topic he could talk about in his sleep; in fact, he hasn't talked about much else since 1972 when Richard Nixon made him the first director of NCI under the vastly expanded Cancer Program.

It was more of the same last month at the ACS Science Writers Seminar. As a member of a panel on "The Fragility of Basic Research Support," Rauscher came out swinging:

—"There isn't going to be any decrease in support for basic research by NCI. It's a myth that Congress won't support basic research. I think we can use the fact that this year we will pay only 30% of competing approved grants to present a case to Congress for more money.

—"Since 1938, 55% of all money spent by NCI has been for basic research. Over the past four years, contracts have declined by 8% adn grants (including construction but most of it for basic research) have increased by 8%. Total dollars for basic research is still going up as a proportion of the NCI budget.

—"There's no question that cancer dollars have funded a lot of basic research applicable to other diseases."

Now that he's out of government, Rauscher feels he can talk about the maneuvering that went on to get around some of the crippling restraints the Nixon Administration put on biomedical research, including cancer.

"I felt we had to get along with NIH," Rauscher told ***The Cancer Letter.*** "If NIH is not healthy, the Cancer Program won't be healthy. I did everything I could to get along with NIH, but never hesitated to exploit the direct access we had to OMB and the White House."

The National Cancer Act of 1971 permitted NCI to submit its budget requests directly to the Office of Management & Budget. NIH and HEW could comment on NCI requests and make their suggestions on what NCI should have but couldn't change NCI's own figures. The Act also created the President's Cancer Panel with authority to take complaints and suggestions directly to the White House, which the Panel frequently has done.

"When the Act is renewed, it's vital that we retain that independence," Rauscher said. "But it's a tricky thing to handle. People resent it. I told Bob Marston (former NIH director) that we would work with him, to try to exploit our special authority for the benefit of NIH."

NCI quietly channeled $3 million a year to other institutes to enable them to continue funding their best grantees, always with the stipulation that their work had to have some relevance to cancer, however distant it might be.

After the Administration killed the NIH training grant program, NCI used its separate training authorization to help fund trainees at other institutes, Rauscher said.

Now that he's no longer involved in the day to day job of running NCI, Rauscher looks at its organization with a more critical eye.

"It's good as it is, but I think it is unhealthy to have what is essentially two institutes, one for grants and one for contracts. The grants division on one side and everything else on the other."

Rauscher feels that NCI divisions each should be permitted to fund a program "across the board, irregardless of funding mechanism. One division would have responsibility, for epidemiology, nutrition, virology, whatever. In virology, for instance, duplication is rampant (between contract supported research in the Div. of Cancer Cause & Prevention and grant supported research in the Div. of Cancer Research Resources & Centers).

"One of the dangers would be that this would require more manpower."

Rauscher suggested that an Office of Grants Management located either in the director's office or in DCRRC could handle the processing of grants.

Rauscher feels his plan would not inhibit investigator initiated research.

The suggestion by National Cancer Advisory Board Chairman Jonathan Rhoads, that NCI support (with major funding increase from Congress) development of a network of 200 clinical centers around the country has Rauscher's enthusiastic backing. Before he left NCI he had suggested that many regions may not be capable of or may not want full fledged comprehensive cancer centers with the basic research requirement but could and should have the best facilities for clinical treatment and research.

"Henry Kaplan's center at Stanford and Isaac Djerassi's in Pennsylvania (Mercy Catholic Medical Center in Darby) are examples of superb clinical centers," Rauscher said.

Rauscher said the scientific community should get itself "revved up, get out and talk to people and to Congress about Cancer Program needs. We can justify it, $815 million is a drop in the bucket when you consider that cancer costs us $25 billion a year. We've got to have substantial increases in funding. It's nonsense to say we can pay for new initiatives by taking money away from viral oncology, or something else."

RFPs AVAILABLE

Requests for proposal described here pertain to contracts planned for award by the National Cancer Institute, unless otherwise noted. Write to the Contracting Officer or Contract Specialist for copies of the RFP, citing the RFP number. Some listings will show the phone number of the Contract Specialist, who will respond to questions. Listings identify the respective sections of the Research Contracts Branch which are issuing the RFPs. Their addresses, all followed by NIH, Bethesda, Md. 20014, are:
Biology & Diagnosis Section — Landow Building
Viral Oncology & Field Studies Section — Landow Building
Control & Rehabilitation Section — Blair Building
Carcinogenesis Section — Blair Building
Treatment Section — Blair Building
Office of the Director Section — Blair Building
Deadline date shown for each listing is the final day for receipt of the completed proposal unless otherwise indicated.

RFP NCI-CM-87157

Title: *Operation of an animal disease diagnostic laboratory*
Deadline: *Approximately July 15*

NCI is seeking proposals for the operation of an animal disease diagnostic laboratory. The successful offeror shall supply NCI with viruses, bacterial, and parasitic profiles of rodents from the animal suppliers serving the Drug Screening Program.

The successful offeror will supply services, qualified personnel, material, equipment and facilities not otherwise provided by the government under the terms of the contract to perform the following procedures: (1) Gross physical observations including activity, alertness, condition of hair, etc.; (2) Necropsy with observations for gross lesions (followup histopathological observations when indicated); (3) Culture of respiratory tract (nasal passages through lungs), ear canal, and intestinal tract for pathogenic microorganisms; (4) Examination for ectoparasites and endoparasites; and (5) virus antibody determination.

It is recognized that offerors with the ability to perform this workscope will have varying areas of expertise. However, the successful proposal should reflect staff capability in overall rodent health diagnosis including viruses, bacteria and parasites. The principal investigator should have achieved professional recognition in one or more of these areas and, of equal importance, he should have acquired sufficient practical experience to be able to evaluate the significance of diagnostic findings in concert with the project officer. The principal investigator should be a veterinary pathologist or PhD in microbiology and devote 15% of his time to the project.

It is anticipated that award will be for three years, incrementally funded, periods of performance.

Contracting Officer: Daniel Abbott
Cancer Treatment
301-427-7463

BALTIMORE, COLLEAGUES REPORT ON NEW METHOD TO AID LEUKEMIA THERAPY DESIGN

Reports on research involving biological markers and other methods of assisting in detection and diagnosis of cancer were presented at the American Cancer Society's annual Science Writers Seminar. One of the reports, by Nobel Prize winner David Baltimore of MIT, explains how his research is leading to development of methods to design better treatment for leukemia patients. That report and others follow:

TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE AS A MARKER FOR LEUKEMIAS AND LYMPHOMAS — David Baltimore, Patrick Kung, John Long and Ronald McCaffrey

Terminal deoxynucleotidyl transferase (TdT) is a unique form of DNA polymerase found only in the thymus and the bone marrow of both human beings and other vertebrates. In 1973, when we were attempting to search for DNA polymerases of RNA tumor viruses in human leukemic cells, we accidentally observed that the cells of a child with acute lymphoblastic leukemia (ALL) contained TdT. The enzyme we found in the cells of this patient was indistinguishable from the enzyme found in normal human thymus.

The initial observations on TdT in human ALL cells have led us to an extensive characterization of the types of cells that contain TdT in both human beings and in mice. The enzyme was first described by Bollum and Krakow and has been carefully characterized as to its biochemical properties. It is differentiated from all other known DNA polymerases by its inability to copy a template. Because of this inability, TdT could not be an enzyme involved in standard, replicative DNA symthesis.

The function of TdT in immature lymphocytes has not been ascertained but because the enzyme acts to synthesize DNA it seems likely that its role has something to do with DNA synthesis or organization. We suggested a number of years ago that TdT might be involved in the generation of the diversity of specificities found in lymphocytes. Such an hypothesis would make TdT the central enzyme in the generation of the multiple reactivities possible in the immune system. Whether this is the correct function for TdT remains to be shown.

We and others have assayed for TdT in a wide range of human leukemias. We have found that human leukemias can be classified according to their content of TdT and this classification appears to provide a more rational base for design of therapy than classifications based solely on morphology. We discuss below three situations in which TdT appears to be valuable as a marker.

Acute lymphoblastic leukemia (ALL)

We have now assayed 66 cases of acute lymphoblastic leukemia, both in children and in adults. Of these cases, 63 contained a large fraction of TdT-positive cells. The occurrence of TdT-bearing cells in 95% of ALL cases makes it a very precise marker for the identification of the disease.

In 42 cases of acute myeloblastic leukemia (AML) only two have been found to be positive and of these one was very low. Thus TdT provides an almost perfect correlative to the morphologic distinction between ALL cells and AML cells. This distinction enables us to use TdT in less certain situations as a marker for whether the leukemia has

arisen in lymphoid cells or in myeloid cells.

Using TdT assays in cells from acute undifferentiated leukemia, a disease of uncertain status, we have found that six of 10 cases are TdT-positive. This enables us to place those six cases in the lymphoid category of diseases and suggests that patients having TdT-positive acute undifferentiated leukemia should be treated as if they were ALL patients.

TdT has not been found in any chronic lymphatic leukemia (CLL) cells. This disease involves much more mature cells than ALL, suggesting that TdT is a marker for undifferentiated lymphoid cells. Such a conclusion is consistent with the data in experimental animals. Similarly, TdT has not been found in the cells of patients with Sezary's syndrome and other tumors of relatively mature T-lymphocytes.

Chronic myelocytic leukemia

Chronic myelocytic leukemia (CML) is a disease that usually occurs in two phases. During the "stable phase" of the disease the patient has an excess of immature white cells in both the bone marrow and in the blood stream. These cells are clearly of the myeloid cell series and do not contain TdT (in 13 cases). The disease in its stable phase inevitably progresses to a "blastic phase." In the blastic phase the bone marrow is almost completely replaced by extremely primitive cells which also enter the blood stream. Because in the stable phase the immature cells are clearly myeloid, the blast phase cells have been usually thought to be myeloblasts in spite of their relatively uninformative morphology.

Contrary to the belief that blast phase CML involves myeloid cells, we have found that in 19 of 53 cases, the blast cells contain TdT. Other laboratories have made similar observations. Therefore, blast phase CML may be more like ALL than has previously been recognized and may involve lymphoid cells. Surface marker studies have strengthened this conclusion.

At our suggestion, a number of hospitals have begun to treat blastic CML patients who are TdT-positive as if they were ALL patients. Remarkably, such patients respond to an ALL chemotherapy protocol using vincristine and prednisone. Blast crisis CML patients whose cells are TdT-negative have not responded to this therapy. These are preliminary but very encouraging observations and a more extensive series is in progress.

Lymphomas

Lymph node biopsy and splenectomy specimens of adults and children with malignant lymphoma have been examined for TdT at the Massachusetts General Hospital. To date, the enzyme has been demonstrated only in cases of lymphoblastic lymphoma. The enzyme was detected by immunofluorescence within the nuclei of neoplastic cells in diagnostic lymph node biopsy specimens, and in the blood and bone marrow cells of patients who have subsequently developed disseminated disease. Microscopically, lymphoblastic lymphoma involves the thymus-related portion of the lymphoreticular system. The presence of an anterior mediastinal mass in some patients has suggested a thymic origin of the tumor. Involvement of the marrow, blood, and central nervous system may occur, and, when disseminated, the disorder has features similar to ALL.

Lymphocyte surface studies have indicated the cells of lymphoblastic lymphomas are related to T-lymphocytes. The neoplastic cells usually form E-rosettes, and lack surface immunoglobulin. As in acute lymphocytic leukemia, however, there is a heterogeneity of surface markers; some of the TdT-positive cases did not form E-rosettes, a criterion thought to be diagnostic of T-lymphocytes.

TdT has not, to date, been detected in cases of lymphocytic lymphoma (nodular or diffuse), chronic lymphocytic leukemia, or Hodgkin's disease.

We conclude from these observations that TdT is both a fasctinating enzyme from the point of view of basic science and an important tool for the classification of human leukemias and lymphomas. We believe that it is possible to group these diseases according to their content of TdT and thus provide a more rational method for classification than has previously been possible. This classification has therapeutic implications because ALL is a disease that has been brought under control by chemotherapy. We believe that TdT determinations along with assays of surface markers can provide therapists with a precise method of classifying leukemias and lymphomas so that a given disease may be treated with the optimum therapy.

DETECTION OF HUMAN TUMOR STEM CELLS – Sydney Salmon, Anne W. Hamburger, Univ. of Arizona Cancer Center

Scientists at the Univ. of Arizona Cancer Center have developed a new technique to make the key cells of the cancer (tumor stem cells) which are present in biopsy samples, grow and form colonies in culture plates. The tumor stem cells are the cells in a cancer which are responsible for uncontrolled growth and spread of cancer. The test employs special nutrients and a gelatin-like culture medium and identifies tumor stem cells by their ability to grow into multicell tumor colonies in the gel medium. The method permits direct testing of tumor stem cells from patient biopsy samples for their sensitivity to anticancer drugs and radiation. This new approach should permit individualized cancer treatment based on the results of the culture and sensitivity tests.

To date, over 100 patients with widespread cancer have been studied with the biopsy samples obtained with a needle and syringe from the bone marrow or from a body fluid which contains cancer cells. The most extensive work has been done with two types of cancer involving the bone marrow (multiple myeloma and malignant lymphoma) as well as abdominal fluid from women with cancer which has arisen in ovary. Occasional patients with other forms of cancer have also been studied.

Thus far, we have been able to establish that the tumor stem cell colonies from different types of cancer have different appearances and growth rates in the agar medium. Tumor colonies can be grown regularly from patients with myeloma or ovarian cancer who are studied prior to treatment or at a late stage of illness when the cancer recurs. Tumor colony formation has also been observed in samples from approximately 50% of patients with lymph gland cancer (lymphoma) as well as in neuroblastoma. We are now beginning studies on other forms of cancer as well, including breast cancer.

THE USE OF STABLE ISOTOPES IN CANCER RESEARCH – J. Cymerman Craig, Univ. of California (San Francisco)

Some chemicals, themselves innocuous to cells, have been shown to be transformed by enzymes present in living organisms into highly toxic molecules which can attack the genetic control center of the cell.

In the development of new diagnostic tools for cancer, the use of stable isotopes offers a highly sensitive and accurate means by which to search for and identify such toxic molecules, and by which to follow the metabolic paths taken by drugs and chemicals in the body.

It is a strange fact that many known environmental carcinogens (such as benzopyrene, which induces skin cancers in mice) are harmless in themselves, but become transformed in living organisms, through the action of enzymes, into highly toxic molecules. This makes it necessary to test not only new chemicals, drugs, or food additives for potential carcinogenic or mutagenic properties, but also to examine their metabolic transformation products. New and ingenious tests, using tissue culture, have recently been developed and are now coming into routine use for this purpose (Ames).

It appears that certain chemicals tend to produce cancer in specific organs of the body, and it is believed that this is the result of a particular metabolic activity occurring more readily in some tissues than in others. Previous work with highly toxic substances has indicated that such materials, being highly reactive, are invariably found in very low concentrations and are correspondingly difficult to identify.

The breast cancer problem presents such a question. Do "foreign" compounds such as environmental chemicals, drugs, food additives, or even normal food constituents get into breast tissue or into mothers milk? The method recently devised by Sartorius makes it possible (using a simple plastic suction pump applied to the nipple) to obtain a drop of breast fluid from non-lactating women. By examining samples of breast fluid from normal women and from women from high-risk and low-risk families, as well as from actual breast cancer patients, for the presence of viruses, normal biochemical constituents such as fatty acids, and abnormal constituents derived from "foreign" chemicals or drugs, the group headed by Petrakis has been obtaining the data needed to establish a future epidemiological correlation with breast cancer frequency in these patients.

In animal studies, the use of radioactive tracers is an extremely sensitive method for following the metabolic fate of a drug. However, the risk of radiobiological damage forbids the extensive use of radio-

active tracers in most clinical studies, particularly in pregnant women and children.

It is now possible to use stable (non-radioactive) isotopes to follow drug disposition in man, and molecules labelled with stable isotopes such as deuterium (heavy hydrogen, ^{2}H), or the ^{13}C isotope of carbon, the ^{15}N isotope of nitrogen, or the ^{18}O isotope of oxygen, have been used to study the human metabolism of drugs. A mass spectrometer is used for the certain identification of the molecules in question, and the presence of the particular isotope verifies the origin of the metabolite formed as arising from a labelled precursor.

Recently, a second application of stable isotope methodology has been devised. This consists of addition to the biological fluid, as an internal standard, of a known quantity of a stable isotope-labelled variant of the compound (e.g. a toxic metabolite) to be looked for, usually present only in very small amount. This serves two purposes: the stable isotope-labelled variant acts as a "carrier", helping to isolate minute amounts of the suspected toxic metabolite by "carrying" them with it in the extraction process, and the ratio of the labelled to unlabelled material found gives the actual quantity present of the searched-for metabolite. This is achieved by using the mass spectrometer to count, in very rapid alternation, the ions of the two different masses corresponding to the labelled and unlabelled molecules, respectively. This technique is known as "Specific Ion Recording", and gives a very accurate ratio from which amounts as low as 10^{-12} of a gram (i.e. a picogram) of a substance may be identified with certainty.

In this way we have recently shown (in association with Petrakis) that it is possible to detect, identify and measure, e.g., the amount of nicotine and its metabolite found in breast fluid of women within minutes after a single cigarette is smoked. In the same way, commonly used drugs such as barbiturates can be shown to be present in breast fluid within a short time after an oral dose is taken.

A suspected source of a toxic metabolite is a substance such as cholesterol, which is present in large amounts in fatty tissue such as breast tissue and breast fluid. Cholesterol may form, under the influence of enzymes, a metabolite (cholesterol epoxide) which has been found in the blood of patients with high cholesterol levels, and which has carcinogenic properties. It is thus the kind of potentially toxic metabolite mentioned above, which may, although present in very small amount, exert harmful effects.

We are therefore (in association with Petrakis) engaged in an active search for the presence of this type of toxic metabolite, using material labelled with stable isotopes as a "carrier" to assist in the identification and isolation of this type of substance in breast fluid.

VESICLES AND LIPOSOMES IN BIOLOGY AND MEDICINE — I.R. McDougall and J.P. Kriss, Stanford Univ. Medical Center

In spite of major developments in tumor seeking radiopharmaceuticals and detecting devices in nuclear medicine, there has been a lack of progress in the early diagnosis of primary malignant disease. One approach to tackle this problem, by encapsulation of packets of diagnostic radiopharmaceuticals inside tiny spheres of lipid, has been undertaken in this laboratory. These lipid spherules may have therapeutic as well as diagnostic values.

The drug (radiopharmaceutical) to be entrapped within the central cavity of these spherules must be water soluble. In the simplest form, the spherules have a single central cavity surrounded by a bilayer of phospholipids; these are called vesicles. More complex spherules which have multiple concentric lipid bilayers separating multiple aqueous compartments are called liposomes.

Liposomes are made by adding aqueous buffer to phospholipids which have been thoroughly dried. The lipids used most frequently are lecithin (phosphatidylcholine), other amphipathic lipids, such as gangliosides and cholesterol. Agitation of the lipid-buffer mixture produces a milky solution which contains an inhomogeneous population of liposomes, differing in both overall size and number of concentric lipid bilayers. A proportion of the buffer is enclosed within and between the lipid bilayers, and if the radioactive marker had been dissolved in the buffer, a proportion of the marker would also be encapsulated.

Ultrasonic irradiation of the milky liposomal solution, using a probe or bath sonicator, causes the liposomes to fragment and reform as smaller spheres, resulting eventually in the formation of vesicles with a single cavity separated from the aqueous environment by a single lipid bilayer. Vesicles and liposomes loaded with drugs can be separated from the nonentrapped drug by Sephadex or Sepharose gel filtration.

Vesicles, whose limiting membrane consists of lecithin, are relatively permeable to molecules of small molecular weight. The rate of egress of entrapped materials can be slowed by the addition of gangliosides or cholesterol, or both, to the lecithin. A similar effect can be produced by subjecting the material to longer periods of ultrasonic irradiation.

Recently, Dunnick and Kriss have made vesicles by subjecting whole plasma of experimental animals and humans to ultrasonic irradiation in vitro. These vesicles contain lecithin, cholesterol and cholesterol esters in the same proportions as plasma; they also contain protein fractions (probably lipoproteins). Entrapment of radiopharmaceuticals in plasma vesicles is possible, and therefore, they could be prepared from native plasma constituents of the patient.

The lipid bilayer of vesicles is similar to the lipid bilayer structure of biological membranes, albeit the composition of the artificial vesicles is much simpler. These artificial membranes provide an excellent, simple, in vitro model to test permeability characteristics and the effects of drugs, such as anesthetics, on lipid bilayers.

The distribution of a radioactive marker encapsulated in vesicles and injected intravenously in mice or rats is very different from the distribution of the free radioactive marker. In most circumstances the liver and spleen extract the greatest quantity of vesicles from the circulation. The proportion removed by these organs can be increased or decreased by appropriate alterations of the lipid composition of the limiting membrane. We have been able to demonstrate intact vesicles in the circulation of mice for as long as 3 hours after I.V. injection. Studies have been conducted on the distribution of vesicles injected subcutaneously and intraperitoneally in experimental animals, and recently Dapergolas et al have shown that insulin entrapped in vesicles produces its hypoglycemic effect after oral administration. If this is confirmed, it would have very important therapeutic implications.

The rate of growth of tumor cells in vitro can be slowed by incubating the cells with vesicles of specific compositions. This is due to an interchange of phospholipids of the vesicles and tumor cells; vesicles with stearylamine in the membrane have been most effective. In vivo testing of this finding has still to be undertaken.

If vesicles or liposomes are to gain a place in diagnosis, or therapy, the major problem to be answered is the one of direction to selected target sites. Uptake in the liver and spleen can be achieved with certainty. However, diseases confined to these organs are rare. In certain situations, the dose of a drug, which if given systemically would produce side effects, could be reduced and the drug effect restricted to the liver and spleen.

Although the distribution of vesicles in vivo is altered by techniques discussed above, specific localization in other organs is less successful. We have addressed ourselves to this problem by attaching proteins, e.g. antibodies, onto the outside of preformed loaded spherules. In vitro, this approach has been modestly successful in attaching vesicle-antibody complexes to the appropriate antigen.

Vesicles and liposomes, once the research tools of the membranologist, are now the tools of the cell biologist and even the clinician. These spherules might provide a method of delivering packages of diagnostic or therapeutic agents to target sites and the possibility of using the patient's plasma to provide the membrane constituents is of great interest. If the results from animal experiments can be extended to humans, drugs which cannot be given by mouth, because of intestinal degradation, could possibly be administered by mouth inside vesicles. The possibility of altering tumor cell growth by incorporating lipids from vesicle membranes also deserves further evaluation.

ANALYTICAL CYTOLOGY AND CANCER DETECTION — Mortimer Mendelsohn, Lawrence Livermore Laboratory

A major focus of the Biomedical Program at the Lawrence Livermore Laboratory is the development and application of analytical cytology to environmental and health problems including cancer detection and prevention. Analytical cytology concerns the measurement of chemical and physical properties of single cells. In the context of cancer research, our studies in analytical cytology are relevant to:

—Cancer diagnosis of cervix, bladder, lung and other organs which shed (exfoliate) cells.

—Screening of worker populations for premalignant changes indicative of carcinogen exposure.

—Short-term laboratory tests for carcinogenic substances.

Conventional cytological cancer diagnosis, as in the Papanicolaou Test, is based on the visual recognition of precancerous and cancerous cells. Changes in shape, size, texture and staining characteristics of the cell and its parts are the usual criteria. By providing the cytochemical definition and quantitation of such changes, analytical cytology contributes to our understanding of the mechanism of change and the optimization of criteria. It also leads to automation of the cytological method.

Two classes of machines have been used for analytical cancer cytology.

1. Image analyzers operate much like the human vision. They capture an image of the cell with an electronic camera, dissect, measure and interpret the image by digital computer, and generally operate from conventional preparations of cells on slides. An excellent example is the TICAS system of George Wied and colleagues at the Univ. of Chicago. Such machines collect large amounts of information per cell, tend to be too slow and expensive to be used to find rare abnormal cells in routine screening, and are ideal for characterizing preselected cells.

2. Flow cytometers operate on cells in suspension as they flow past stationary physical and optical probes. They collect one or a few measurements per cell and hence are information poor; they sense little or no internal morphology of the cell and can resort to few classical cytological criteria. However, they can process 1000 cells per second, and can measure cell size and stain content with great precision and facility. They can also sort those cells with particular characteristics, thus making them available for a cytologist, a biochemist or a mechanical image analyzer. The application of flow cytometers to clinical cytology is recent, but the preliminary results are exciting and suggest that such instruments can serve as prescreeners and perhaps as definitive diagnostic machines. The key to their success is the development of cytochemical methods to mark the dysplastic and cancer cells and to allow them to be distinguished from the variety of normal and otherwise abnormal cells.

This laboratory has contributed to the development of scanning and flow cytometers, preparative methods for Pap specimens, and cytochemical methods for enzymes and DNA. Two recent examples of cytological application perhaps will show why we are excited about the potential.

A team, headed by Ron Jensen, has been studying human Pap material with a combination of the above methods. By simultaneously measuring cell size and cell DNA content in a flow cytometer, they can structure the usual popuation of cervical-vaginal cells into several subgroups. Three subgroups each contain one common, non-cancer-related cell type, while the fourth contains the cancerous and precancerous cells mixed with several types of noncancerous cells. Sorting the fourth region concentrates abnormal cells perhaps 50-fold and is already a valuable prescreening process for the human cytologist. New cytochemical probes are now being designed to further discriminate within this region.

A second team, under Frank Dolbeare, is using similar methods to discover differences between normal and transformed cells in culture. The transformation of cultured cells under the influence of carcinogens is rapidly gaining credence as a model of human carcinogenesis and as a short-term laboratory approach to evaluating substances for carcinogenic potential. Dolbeare has surveyed many cytochemical markers for their discriminatory potential by comparing them directly on normal and transformed cells. He has concentrated primarily on enzyme markers and has already found several which allow complete discrimination of normal and transformed cells in a flow cytometer.

CONTRACT AWARDS

Title: Collect sera from high cancer risk populations
Contractor: Univ. of Minnesota, $80,096.

Title: Circulating antigen-antibody complexes in cancer
Contractor: Univ. of Alabama, $92,741.

Title: Continue research on isolation of type C viruses from cultured leukemic cells
Contractor: Sidney Farber Cancer Institute, $142,900.

Title: Continue studies of leukemia virus DNA polymerase
Contractor: Massachusetts Institute of Technology, $177,250.

Title: Continue houseing and maintenance of a chimpanzee breeding colony
Contractor: Southwest Foundation for Research & Education, $69,152.

Title: Continue maintenance of an irradiated monkey colony
Contractor: Emory Univ., $26,890.

Title: Continue studies of epidemiology and geographic pathology of cancer
Contractor: Louisiana State Univ., $206,199.

Title: Conduct studies of the molecular basis of viral carcinogenesis
Contractor: Johns Hopkins Univ., $428,510.

Title: Procurement of melanoma cell vaccine and in vitro assays for humoral and cellular cytotoxicity
Contractor: Litton Bionetics, $335,444.

Title: Resources modelling and analysis
Contractor: JRB Associates, $295,990.

SOLE SOURCE NEGOTIATIONS

Proposals are listed here for information purposes only. RFPs are not available.

Title: Systems planning support services for the National Cancer Institute, Office of the Director, National Cancer Plan
Contractor: JRB Associates.

Title: Programming services in support of the contract management system
Contractor: Sigma Data Computing Corp.

Title: In vitro malignant transformation of human and subhuman primate cells by interaction between viruses and chemicals
Contractor: Microbiological Associates.

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