THE CANCER LETTER
RESEARCH EDUCATION CONTROL

Vol. 4 No. 18
May 5, 1978

P.O. BOX 2370 RESTON, VIRGINIA TELEPHONE 703-620-4646

Subscription $100 per year

CLEARINGHOUSE AGREES: THREE DYES, HAIR COLOR AGENT ARE CARCINOGENS; RISK TO HUMANS – BROWN

NCI's Bioassay Program has unequivocally identified as carcinogens three of the most widely used commercial dyes in the world—direct blue 6, direct black 38 and direct brown 95, which appear in such consumer goods as shoe polish, clothing and paper products.

The Clearinghouse on Environmental Carcinogens Data Evaluation/-

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In Brief

CANCER PROGRAM BILL RENEWS CONGRESSIONAL COMMITMENT TO PRINCIPLES, REP. CARTER SAYS

LEGISLATION RENEWING the National Cancer Act "represents a renewed commitment to the goals and principles embodied" in the Act, Congressman Tim Lee Carter (R.-Ky.) said of the bill approved by the House Health Subcommittee. "The bill we sent to the full Commerce Committee reflects our subcommittee's view that this country has accomplished a great deal in the cancer field but that many difficult challenges remain. . . . We cannot afford to diminish our efforts. That is why we have provided increased authorizations over the budget request and why we adopted a series of amendments which I believe will strengthen our efforts in meeting the challenges ahead". . . . **HOUSE HEW** Appropriations Subcommittee was scheduled to complete its work on the FY 1979 money bill this week. The figure the subcommittee writes in for NCI will determine to a major extent how much Congress will appropriate for the Cancer Program this year. The Senate figure for NCI is almost always higher of the two, and they usually split the difference. . . . **NEW PUBLICATIONS:** *Clinical Cancer Trials,* edited by Luther Brady, chairman of the Dept. of Radiation Therapy at Hahnemann Medical College, and co-edited by Vincent DeVita, director of NCI's Div. of Cancer Treatment. Quarterly, $45 year ($36 prepublication), published by Masson Publishing Inc., 14 E. 60th St., New York 10022. Also, "Cancer and the Worker," from a 1975 symposium sponsored by the New York Academy of Sciences, Group Health Inc., NCI, NIOSH and Society for Occupational & Environmental Health. $2 per copy, from NYAS, 2 E. 63rd St., New York 10021. . . . **SECOND INTERNATIONAL** conference on adjuvant therapy of cancer will be held in Tucson March 28-31, 1979, sponsored by the Cancer Center Div. of the Univ. of Arizona, Sydney Salmon and Stephen Jones, chairmen. Deadline for submission of abstracts is Dec. 1. Contact Ellen Gerrity, Cancer Center Div., UA, Tucson 85724. . . . **COMBINED MODALITIES:** Chemotherapy/Radiotherapy is topic of conference Nov. 15-18 at Hilton Head Island, S.C. The meeting will focus on the interaction of the two modalities. Deadline for abstracts is Aug. 1. Contact Theodore Phillips, Div. of Radiation Oncology, Univ. of California, San Francisco, 94143.

WIDELY USED 1,2-DICHLOROETHANE FOUND CARCINOGENIC IN BIOASSAY PROGRAM

(Continued from page 1)

Risk Assessment Subgroup concurred with the conclusions in the Program's report on the test. Arnold Brown, Clearinghouse chairman who was the primary reviewer of the report, concluded that the dyes were a potential carcinogenic risk to humans. The Subgroup motion approving the report did not include Brown's conclusion, however, although no other Subgroup members argued against it.

The Subgroup also found that another compound widely used in a consumer product, 2,4-diaminoanisole sulfate, was carcinogenic in the animal tests. This is an important chemical in the cosmetics industry as a key ingredient in hair dyes. Brown, who also was the primary reviewer of this report, again concluded that it was a potential carcinogenic risk to humans. The motion approving the report did not refer to human risk.

The Clearinghouse actions were included in the Subgroup's review of reports on 21 chemicals tested in the Bioassay Program. The increasing number of reviews reflects the Clearinghouse effort to keep up with the flow of reports coming out of the Program's backlog. Members of other Subgroups are assisting with the reviews, which threatened to overwhelm the Data Evaluation/Risk Assessment Subgroup.

The reviewers' primary task is to assess the validity of the reports, based on the tests and the conditions of the tests. They may comment on whether they feel the results lead to the conclusion that the compound is a threat to humans. On occasion, that conclusion has been specifically included in the motion.

The regulatory agencies closely follow Clearinghouse deliberations and actions. So far, they seem to be as interested in the comments of Subgroup members as they are in the formal actions by the Subgroup.

The report on direct blue 6, direct black 38 and direct brown 95 dyes was based on a 13 week subchronic toxicity study. Brown said he agreed with the conclusions in the report that direct blue 6 and direct black 38 were carcinogenic in both sexes of the Fischer 344 rat and direct brown was carcinogenic in the female rat, under the conditions of the test.

Brown noted that the technical grade dyes contained unidentified impurities. Despite the fact that only 10 animals of each sex were used in each treatment group, given the demonstrated carcinogenicity of the materials, it did not compromise the study, Brown said. Determinations of methemoglobin and urinary benzidine levels and the well conducted pathology supported the confidence that could be given to the study, he insisted.

Henry Pitot, the secondary reviewer, was also concerned about the high percentage of impurities in the tested materials, but he agreed that they were strong hepatic carcinogens in the rats.

NCI staff member Kenneth Chu commented that, with respect to the impurities, each batch of the tested material was analyzed for free benzidine and benzidine salt. Since none was found, it was concluded that the urinary benzidine in the treated animals was a metabolic product of the dyes. Subgroup member John Weisburger pointed out that the majority of the impurity probably was sodium chloride, based on analyses in the report. Pitot agreed that the significance of the impurity was diminished if, in fact, most of it was salt.

Program Director Richard Griesemer said that the Program was not planning to conduct a two year chronic study of the dyes, since all of them already have been shown to be carcinogenic. Given the Program's mission and the fact that benzidine was likely the carcinogenic metabolite, it was felt that an additional study was not necessary.

There was more disagreement over the conclusions and significance of the 2,4-diaminoanisole sulfate test, including a challenge from a representative of the cosmetics industry.

Brown agreed with the conclusion in the report that the compound was carcinogenic in both sexes of treated rats and mice, under conditions of the test. Malignant tumors of the skin and its associated glands and malignant thyroid tumors were induced in each sex of the treated mice. Brown made these points:

—Periodic analyses of the stock 2,4-diaminoanisole sulfate should have been made to determine its possible instability.

—Animals were housed in the same room in which other chemicals were under study.

—The high dose level administered to the mice appeared to be below the maximum tolerated dose.

—The high dose rat group was restarted 11 months after the initiation of the study.

Brown added that the oral route of administration for a topically used chemical was questionable. Despite the shortcomings, Brown said they were not significant enough to invalidate the conclusion on the carcinogenicity of the compound. He concluded that it may be a carcinogenic risk to humans.

Pitot, the secondary reviewer, commented that the thyroid neoplasms were of lesser significance than the preputial gland and the Zymbal's gland tumors. He noted that the study gave no information on human risk with respect to the absorption of 2,4-diaminoanisole sulfate through the skin. He questioned rodent skin absorption studies since the structure of rodent skin is quite different than human skin. He suggested that primates would be a more appropriate experimental model to assess human skin absorption.

Subgroup member Lawrence Garfinkel commented that data he had reviewed indicated that some low dose control animals were serially sacrificed with the result that the number of low dose control animals was inadequate. Griesemer argued

that the statistical analysis corrected for the early sacrifices.

John Corbett, of Clairol, Inc., is chairman of the Hair Color Technical Committee of the Cosmetic, Toiletry & Fragrance Assn. He used time allotted for statements by members of the public to dispute the report's findings.

Corbett questioned the value of the study since statistically significant excesses of tumors were found only in the high dose rats and mice when low and high dose controls were combined. He said that the high dose levels established in the 23 day subchronic study exceeded the maximum tolerated doses. In support of this contention, Corbett described results showing that plasma T-3 and T-4 levels of thyroid activity were significantly affected at the high dose level. He also noted a more than 15% weight depression in the high dose animals compared with their associated controls.

With respect to the thyroid tumors, Corbett said that other studies have shown crystals of pigmentary matter in the thyroid after a treatment period as short as 14 days. He pointed out that the bioassay report did not indicate the presence of any particulate matter in the thyroid of the low dose treated animals. He argued that a threshold exists between the low and high doses.

Corbett commented that in studies conducted by industry in which the compound was applied topically, no skin tumors were induced. He said that the NCI bioassay was deficient since the maximum tolerated doses were exceeded and the treated animals were hormonally imbalanced throughout the study.

Brown answered that the relationship of the crystalline matter in the thyroid to the induction of cancer is unknown and that the material had not been characterized. Pitot added that the mechanism for the induction of thyroid tumors also is unknown, although it could be related to a hormonal imbalance. He said that the induction of Zymbal's gland tumors were not related to a hormonal imbalance.

Subgroup member Sheldon Samuels commented that the compound, even at low doses below the level considered to be a threshold by industry, must be regarded as posing a carcinogenic risk to humans because of (1) the multiple exposures to which populations are subjected; (2) the size of the exposed population; (3) the longer exposure period; (4) the variable sensitivities of individuals; and (5) the experimental limitations of the bioassay system to detect carcinogens. He felt that the results were particularly significant since tumor excesses were found in both sexes of mice and rats.

In another action that could have significant regulatory impact on a widely used chemical, the Subgroup agreed with the bioassay report that 1,2-dichloroethane was carcinogenic in the test animals. Subgroup member Joseph Highland, the primary reviewer, also concluded that the chemical is a potential carcinogenic risk to humans.

The compound is a solvent and is a base chemical from which many others are manufactured, including polyvinylchloride.

Highland said that the chemical induced squamous cell carcinomas of the forestomach, hemangiosarcomas, and subcutaneous fibromas in male rats; mammary adenocarcinomas in female rats; mammary adenocarcinomas and endometrial tumors in female mice; and alveolar/bronchiolar adenomas in mice of both sexes.

Although there were shortcomings in the experimental design and conduct of the study, Highland said, they were not significant enough to invalidate the findings.

Weisburger pointed out that 1,2-dichloroethane was one of the few chlorinated hydrocarbons which induced mammary cancer in both mice and rats.

Another intermediate used in manufacture of dyes and in pharmaceutical research is 4-chloro-o-phenylenediamine, which the bioassay report concluded was carcinogenic in animals. Pitot, the primary reviewer, agreed that it was carcinogenic in both sexes of treated rats and mice. Tumors of the urinary bladder and forestomach were induced in the rats and hepatocellular carcinomas in the mice. Pitot said the results were particularly significant since the bladder tumors are similar to ones induced in humans by certain aromatic amine carcinogens.

Brown was critical of the selection of dose levels used in the chronic study. However, he concurred with the conclusion that the compound was carcinogenic in the treated animals, and added that it could pose a carcinogenic risk to humans.

Other chemicals which the Bioassay Program found were carcinogenic in the tests:

–M-cresidine, a dye intermediate. Of little commercial value in the U.S., with only 1,000 pounds produced a year.

–Trimethylphosphate, a chemical intermediate used as a flame retardant in paints and plastics and as a gasoline additive. Induced a statistically significant incidence of adenocarcinomas of the uterus/endometrium in treated female mice. Conclusion that it was carcinogenic in at least the female mice.

–TRIS, the flame retardant. The Clearinghouse made a preliminary report last year, leading to the regulatory action banning its use in children's sleepwear. This was the final report, confirming the previous finding.

–Phenestrin, an anticancer agent. Carcinogenic in both sexes of mice and in female rats. Inadequate study in some respects, difficult to extrapolate to lower doses for purposes of assessing human risk. Human risk a secondary consideration if phenestrin is an effective anticancer drug.

–ICRF–159, an experimental anticancer drug.

Induced uterine adenocarcinomas in female rats and lymphomas in female mice.

—Acronycine, another chemotherapeutic agent. Dose related incidence of osteosarcomas occurred in the high dose male rats; other tumors were found in the peritoneal cavity of both sexes of treated rats.

—1,4-dioxane, an industrial solvent. Induced squamous cell carcinomas of the nasal turbinates in treated rats and hepatocellular carcinomas in treated mice.

—Daminozide, a plant growth regulator. At most a borderline carcinogen, primary reviewer David Clayson said. Subgroup member Sidney Wolfe disagreed, arguing that the report did find it was carcinogenic in female rats, was associated with induction of hepatocellular carcinomas in male mice, and that there were other evidence of increased carcinogenicity to conclude that daminozide was a risk to humans.

The Subgroup approved two motions—one to accept the report, another stating that the data was insufficient to assess carcinogenic risk to humans.

The Subgroup reviews of other reports and conclusions:

—N-phenyl-p-phenylenediamine, an industrial intermediate in making photographic chemicals, pharmacueticals and rubber products. Not carcinogenic in the test. Samuels argued that the higher incidence of total tumors in treated mice compared with matching controls and a shortened treatment period should qualify the compound for a retest. The motion to approve the report carried, but with Samuels, Highland and Wolfe opposed.

—Trichlorofluoromethane, an ingredient in the refrigerant, freon. Not carcinogenic in either sex of treated mice; the rat portion of the study was inadequate for evaluation.

—Tolazamide, an oral hypoglycemic agent. Not carcinogenic. Primary reviewer Michael Shimkin noted a pure grade compound was tested and the study was well conducted. But Garfinkel felt that the number of surviving animals at the end of the study was too little to draw any conclusion and voted against the motion accepting the report.

—Acetohexamide, another hypoglycemic agent. Results of the study inadequate to reach a conclusion regarding its carcinogenicity.

—2-amino-5-nitrothiazole, a veterinary antiprotozoa agent. May be associated with induction of hematopoietic tumors in male rats. The motion included the notation that the result were unusual with respect to induction of granulocytic leukemias in only one sex of treated rats.

—L-tryptophan, an essential amino acid. Not carcinogenic in rats or mice.

—Phenoxybenzamine hydrochloride, a drug used for treatment of hypertensive crises. Produced sarcomas in rats and mice upon interperitoneal injection but that should not lead to discontinuation of its use, especially since it is administered intravenously in humans, Shimkin said.

—Mixture of aspirin, phenacetin and caffeine (APC), commonly used for pain control. Higher incidence of endocrine tumors in treated male rats, but not statistically significant. Evidence inconclusive on carcinogenicity; motion asked for a retest.

—3-sulfolene, an intermediate in petroleums, plastics and textiles, and in the synthesis of some fungacides. Poor survival among high dose male rats prevented evaluation in that group; no carcinogenic effect in low dose males or either treatment group of female rats. An increased incidence of hepatocellular carcinomas in male mice, but not statistically significant. Study inadequate to assess carcinogenicity.

ANNA BARKER SUCCEEDS BOREK AS HEAD OF CENTER CORE GRANT REVIEW COMMITTEE

The new chairman of the Cancer Center Support Grant Review Committee has some advice for applicants renewing their core grants or seeking new ones.

Anna Barker, who will succeed Ernest Borek July 1 as chairman of the review committee that must pass on more than $60 million in the all important center core grants, told ***The Cancer Letter*** there are two major improvements she thinks applicants could make in their presentations:

- Applications should be concise, with emphasis on the science they are going to pursue.
- Oral presentations should address the budget requests.

"You have to get across to the site visit teams your rationale for the budget requests," Barker said. "There may be 35-50 budgets in one grant application. You can't ask the visitors to understand them if they are not explained."

Applicants should make every effort to finalize their budgets when the application is submitted, Barker said. "It doesn't look good if the budget is changed six times after it goes in. It's disillusioning (for reviewers) to review a lengthy, complicated application, then on the night before the site visit to have to re-review it because of revisions. I realize there is a long lag time between submission and review. But center changes are part of the planning process. The problem rests with the center directors."

Barker is senior research immunologist and manager of biomedical sciences research at Battelle Memorial Institute in Columbus, Ohio. She received her PhD at Ohio State Univ., considers herself an immunologist and basic scientist. Barker has been a member of the committee for two years. "Cancer centers are working," she said. "They are effective in bringing really good multidisciplinary research to bear on cancer problems. Basic scientists can talk to clinicians, work on their problems. We can make centers what Congress intended them to be when the Cancer Act was passed. They are facilitators, places where there really is a feeling, a commitment,

to the Cancer Program.

"It is very exciting seeing the interactions at centers, especially where basic scientists are seeing their findings translated to the treatment of cancer patients."

Barker agreed with Richard Steckel, director of the UCLA Jonsson Comprehensive Cancer Center, who contends that NCI and its advisors have not adequately or consistently enforced core grant guidelines (*The Cancer Letter,* Feb. 17). "The reason they're not being enforced is because the guidelines themselves are not very clear," she said.

Despite the problems, "the bottom line in the centers program is that peer review works," Barker said. "We try to select against poor quality research. I'm not sure how much we can improve on the process by changing the guidelines."

NCI STILL TRYING TO WORK OUT GROUP B DRUG DISTRIBUTION TO INVESTIGATORS

NCI's Div. of Cancer Treatment is still wrestling with the problem of distributing "Group B" drugs to clinical investigators who are unable to develop working relationships with their nearest cancer centers.

Group B drugs are those which have been studied in some phase II trials and have been shown to be tolerated by patients at specific doses and schedules. There usually is some evidence of therapeutic benefit.

In the past, NCI distributed free to physicians on request for humanitarian purpose some phase II and phase III drugs which were shown to be effective in certain protocols. They were drugs not available from any other source.

FDA cracked down on this somewhat liberal distribution, so DCT devised a system with investigational drugs in three categories—Group A, available only to phase I contractors; Group B, described above; and Group C, drugs proven sufficiently in trials to be cleared by FDA for marketing as prescription drugs but for one reason or another have not cleared the lengthy NDA process.

DCT will send Group C drugs directly to qualified physicians who file the proper forms with NCI and agree to use the drugs in approved protocols.

NCI is distributing Group B drugs through those cancer centers which have agreed to participate in the program. The problem remains of fulfilling requests from physicians in areas where centers are not participating, or where no center is conveniently available. DCT has worked out a plan for handling those situations, and will discuss it this week with FDA.

ABSTRACTS OF SELECTED PAPERS PRESENTED AT ANNUAL AACR MEETING

Following are the remaining abstracts of selected papers presented at the 69th annual meeting of the American Assn. for Cancer Research. These papers were selected by the program committee as worthy of special attention. The others chosen by the committee appeared last week in *The Cancer Letter.*

INHIBITION OF THE PHOSPHORYLATION OF NONHISTONE CHROMOSOMAL PROTEINS OF RAT LIVER BY CORDYCEPIN AND CORDYCEPIN TRIPHOSPHATE – Michel Legraverend, Robert Glazer and David Johns, NIH

Cordycepin (3'deoxyadenosine), an inhibitor of nuclear RNA synthesis, has previously been found to act as a competitive inhibitor of cyclic nucleotide-independent forms of protein kinase from nuclei of rat liver. The present study was initiated to assess the effect of cordycepin, and its metabolite, cordycepin triphosphate on the phosphorylation of isolated hepatic nuclei in vitro, in order to determine the relationship of this process to inhibition of transcription. Cordycepin as well as cordycepin triphosphate competitively inhibited the phosphorylation of nonhistone urea-soluble chromosomal proteins in isolated hepatic nuclei in vitro with a K_1 of 1.2×10^{-3}M and 8×10^{-5}M, respectively. Isoelectric focusing of urea-soluble proteins indicated that inhibition occurred predominantly in nuclear proteins with isoelectric points of pH4-7. QAE Sephadex chromatography of extracts of nuclei incubated with the inhibitors also showed inhibition of phosphorylation of nonhistone chromosomal proteins with similar isoelectric points. RNA polymerase I and II were not affected by cordycepin and cordycepin triphosphate after QAE Sephadex chromatography of nuclear extracts incubated with either agent. However, RNA polymerase I and II in isolated nuclei were competitively inhibited by cordycepin triphosphate but not by cordycepin.

These results suggest that cordycepin triphosphate, and perhaps cordycepin too, may affect transcription via interference with the phosphorylation of nonhistone chromosomal proteins.

MALIGNANCY OF HUMAN LUNG CANCER CELLS IS SUPPRESSED IN HYBRIDS FORMED WITH MOUSE FIBROBLAST CELLS – Desmond CArney, Cora Edgell, Adi Gazdar, John Minna, NCI/VA Medical Oncology Branch

These experiments were performed to determine whether malignancy or non-malignancy was expressed dominantly in interspecies hybrid cells. A thioguanine resistant human lung adenocarcinoma cell, A549/8, was fused with a bromide-oxyuridine resistant mouse fibroblast cell, 3T3-4E, using polyethylene glycol. Hybrid clones were selected in medium supplemented with hypoxanthine, aminopterin and thymidine. Isozyme analysis was used to determine the chromosomal constitution of the hybrids, and with the exception of chromosomes 3, 8, 22, and Y, all human chromosomes were analyzed. All hybrids selectively lost human chromosomes while retaining the complete mouse genome. All of the human chromosomes analyzed were present in at least 5 of the 14 hybrids studied. Tumorigenicity of the parental and hybrid cells was tested in athymic nude mice. A549/8 cells (5×10^6) produced tumors in 100% of the mice within 8 days. All hybrid and 3T3-4E cells (1×10^7) failed to induce tumors over a mean observation period of 84 days (44-130). Lack of substrate adherence, another widely used in vitro test for malignancy was also determined. A549/8 cells formed progressively growing colonies in soft agarose (efficiency less than 10^{-5}). These experiments indicate that, in the human x mouse hybrid system employed, the non-malignant mouse genome is dominant over the malignant human genome.

NITROSOUREAS: INTERACTION WITH CHROMATIN AND EFFECT ON POLY (ADP-RIBOSE) POLYMERASE ACTIVITY AT THE NUCLEOSOME LEVEL – Swaroop Sudhakar and Mark Smulson, Lombardi Cancer Research Center

Treatment of HeLa cells with N-methyl-N-nitrosourea (MNU) results in (a) depression of intracellular NAD levels; (b) activation at the nuclear level of the chromatin associated enzyme, poly (ADP-ribose) polymerase. This enzyme catalyses the successive transfer of ADP-ribose units from NAD to acceptor chromosomal proteins. A similar activation of the enzyme has been observed at the subunit structure of chromatin, in the nucelosome dimers, and its higher oligomers. Incubation of cells with MNU results in an increased accessibility of nucleosome monomer histones and its amino acid variants for modi-

fication by poly ADP-ribosylation. In contrast to the above results, no effect on enzyme activity is observed by the interaction of chloroethyl nitrosoureas (CENU) with chromatin. CENU do not affect cellular NAD levels. A non-random in vivo alkylation of chromatin DNA by equimolar concentrations of MNU and cyclohexyl-CENU (CCNU) is revealed by digestion of nuclei from drug treated cells with micrococcal nuclease versus pancreatic DNase I. The former enzyme cleaves internucleosomal DNA. Accordingly, (methyl-^{14}C)-MNU interacts preferentially with the more accessible regions of chromatin, the internucleosome linkers, whereas the (chloroethyl-^{14}C)-CCNU alkylates more to the nucleosome DNA. This difference in the reactivity of methyl and chloroethyl nitrosoureas with chromatin might correspond to their differential effect on poly (ADP-ribose) polymerase activity.

PROTECTION OF CHEMOTHERAPY TOXICITIES BY INTRAVENOUS HYPERALIMENTATION (IVH) – Brian Issell, Manuel Valdivieso, Howard Zaren, Edward Copeland and Gerald Body, M.D. Anderson Hospital

Weight loss prior to therapy is a major adverse prognostic factor for lung cancer patients. In order to test the hypothesis that nutritional replenishment may increase response and tolerance to therapy, a prospective randomized trial was conducted comparing the addition of IVH to chemoimmunotherapy with C. parvum, ifosphamide and adriamycin (CIA) in 26 patients (pts) with extensive squamous lung cancer. 13 pts were entered on each arm of the study and weightloss prior to therapy, performance status and schedule of C. parvum administration were evenly distributed between each arm. IVH was administered before and over the first course of CIA for a total of 31 days. Tumor regressions greater than 50% were seen in 4 pts on IVH and 1 pt on CIA alone (31% vs 8%). The major dose limiting toxicity was leukopenia. A significant difference was found in the lowest recorded leukocyte (WBC) and neutrophil (PMN) counts between the 2 groups (p=0.03 and 0.01, respectively) for the 1st course of therapy. The medium lowest recorded WBC, PMN and platelet counts x $10^3/mm^3$ respectively were 2.5, 1.6, 259 for the IVH pts and 1.5, 0.4 and 150 for the CIA alone pts. Also a significant decrease (p=0.006) in nausea and vomiting associated with chemotherapy administration was found for the IVH group. 10 of 13 pts in the CIA alone group had persistent nausea and vomiting lasting 12 hours following chemotherapy compared to 2 of 13 pts in the IVH group. The differences in toxicities between each group were not maintained over subsequent courses of therapy when both groups received CIA alone. The protection of chemotherapy toxicities by IVH suggests a means of giving higher chemotherapy dosage with the intent of increasing tumor response and pt survival.

OPEN COMMUNICATION IN THE DIAGNOSIS OF PEDIATRIC CANCER: LISTENING TO LONG-TERM SURVIVORS – J.E. O'Malley, G.P. Koocher, D.J. Foster, J. Gogan, N. Jaffe and W.E. Fine, Sidney Farber Cancer Institute and Children's Hospital Medical Center

As part of a multifaceted investigation of the psychosocial sequelae of childhood cancer, 78 families of children who have survived at least 60 months in remission post-treatment were asked whether the diagnosis should be shared with the patient (pt). Most parents had been told not to reveal the diagnosis in order to "protect" the child. The impact of this advise on subsequent adjustment, coping mechanisms and family interactional patterns was assessed. It was found that those pts who were judged to have good or excellent adjustment (78%) stated that children have the right to know the diagnosis, to be able to share openly with their family concerns and questions and to facilitate treatment. Those pts who were judged to have poor adjustment to cancer (22%) by clinical psychiatric interview, psychological testing and indices of current functioning felt angry, still, that they were not told the diagnosis and had much more difficulty cooperating with treatment. Families that were open about problem areas with their children prior to the diagnosis of cancer either could not follow the advice not to share the diagnosis (12%) or shared the diagnosis 2-5 years after it was made (48%). The results of this current study and a review of the literature suggest that a shift from a "protective stance" to an open, direct, simple explanation of the cancer to children, based on a child's current cognitive development, should be investigated.

PREDICTING RESPONSE OF AML PATIENTS TO REMISSION INDUCTION THERAPY BY PREMATURE CHROMOSOME CONDENSATION – Walter Hittelman, M.D. Anderson Hospital

The purpose of this study was to determine if the technique of premature chromosome condensation (PCC) is useful in predicting response to remission induction chemotherapy of patients with leukemia. Sequential bone marrow aspirations were obtained from 21 patients with the diagnosis of AML or AMML during the course of remission induction therapy. Mononuclear cells from bone marrow aspirations were concentrated by Ficoll-Hypaque separation, washed with Hanks balanced salt solution, and fused with mitotic Chinest hamster ovary cells (using Sendai virus) to induce PCC in the bone marrow cells. Slides containing PCC were scored for the fraction of cells in G1, S and G2, the fraction of G1 PCC with highly extended morphology (i.e. potential proliferative index, PPI), and the degree of chromosome damage evident in the PCC due to therapy. Initially, patients had an average PPI of 34.0%, 6.7% S-PCC, and little or no chromosome damage in the PCC of the bone marrow cells. During therapy, responding patients generally exhibited an initial drop in the PPI (if their initial PPI was high) with evidence of moderate to extensive chromosome damage, followed by a rise in the PPI and the fraction of S-PCC. In these patients the PPI dropped once again as normal peripheral blood counts were achieved. Two patients not responding to therapy showed little change in PPI and little or no chromosome damage in the PCC. These results suggest that the PCC technique might be useful in the prediction of response early in induction therapy.

CHANGES IN LIPID COMPOSITION OF FRIEND ERYTHROLEUKEMIA CELL MEMBRANES UPON INDUCTION OF DIFFERENTIATION WITH DIMETHYLSULFOXIDE – Lana Rittmann, Carole Jelsema and Alan Sartorelli, Yale Univ. School of Medicine

This study was designed to examine the composition of membrane lipids during dimethylsulfoxide (DMSO) induced differentiation of Friend erythroleukemia cells. Friend erythroleukemia cells, clone 745, were grown in the presence of 1.5% DMSO and their degree of differentiation was followed by the production of heme. The phospholipid composition of the cells was examined immediately after addition of DMSO and on day six when maximal induction occurred. Lipid extracts of the membranes of control and DMSO-treated cells contained the same complement of phospholipids. Phosphatidylcholine represented over 50% of the total phospholipids in both control and DMSO-treated cells. GLC analysis of the fatty acid components of the lipid extracts, however, showed significant differences between control cells and differentiated cells. The fatty acid composition of the total chloroform-soluble lipids was the same in the control and induced cells, whereas examination of the individual lipid classes, including neutral lipids, neutral glycolipids and phospholipids, showed significant differences in chain length and degree of saturation upon differentiation. The ganglioside fraction of the untreated cells contained long chain fatty acids (greater than 20 carbons) which accounted for approximately 25% of the total fatty acids. These acids were not detectable in the gangliosides of differentiated cells. These data indicate that differences occur in the composition of membrane lipids which are related to DMSO-induced Friend erythroleukemia cell differentiation in vitro.

ARYL HYDROCARBON HYDROXYLASE INDUCIBILITY IN CULTURED LYMPHOCYTES FROM LUNG CANCER PATIENTS – Marilyn Rasco, Toshio Yamauchi, Dennis Johnston and Charles Shaw, M.D. Anderson Hospital

Aryl hydrocarbon hydroxylase (AHH) inducibility was measured in culture lymphocytes from approximately 1000 healthy donors and cancer patients. The inducibility in the normal population showed a unimodal distribution, arguing against a single-locus control of this enzyme. The observed inducibility decreased with increasing age of the donor but was unaffected by the sex or smoking history of the donor. Cancer patients undergoing chemo- or radiotherapy gave poor lymphocyte growth and AHH activity, and were not used for studies reported below. The bronchogenic carcinoma (n=79) and oropharyngeal squamous cell carcinoma (n=25) patients had significantly higher AHH inducibility than the normal population over 40 years of age (n=273). Patients with other types of cancer (n=219) had AHH indicubility in-

distinguishable from the normal population. To circumvent problems of variability in the AHH activity observed in cultured lymphocytes, a case-control study was undertaken. Blood was collected from 32 lung cancer patients and their spouses over a 5-month period. Lymphocytes from patient and spouse were processed simultaneously. Lung cancer patients had significantly higher AHH inducibility than their spouses. Taken together, these studies indicate a strong correlation between AHH inducibility and the occurrence of two types of cancer: bronchogenic carcinoma and oropharyngeal squamous cell carcinoma.

DEMONSTRATION, IN LEUKEMIA L-1210 CELLS, OF A PHOSPHODIESTERASE (PDE) ACTING ON 3',5'-CYCLIC CMP BUT NOT ON CYCLIC AMP OR CYCLIC GMP – Yung-Chi Cheng and Alexander Bloch, Roswell Park

Since demonstrating the presence of cyclic CMP in nature, we have been involved in identifying the enzymes responsible for the metabolism of this cyclic nucleotide. We have now observed cyclic CMP specific PDE activity in leukemia L-1210 cell extracts prepared by disruption of the cells, centrifugation at 100,000 x g, streptomycin sulfate precipitation, ammonium sulfate fractionation (0-30, 30-60, 60-80 and 80-100% saturation) and dialysis for 24 hours against 0.1 M Tris-HC1 buffer, pH 7.5, containing 1 mM dithiothreitol. The cyclic CMP specific activity was present in the 80-100% fraction, as assayed in the presence of Tris-HC1 (0.1 M), pH 7.5, dithiothreitol (2 mM), ^{3}H-cyclic CMP (1 mM), and $MgSO_4$ (10 mM). The reaction was linear with respect to enzyme concentration and time (up to 1 hour), and half maximum velocity was reached at 200 µM cyclic CMP. The cation requirement was met by Mg^{++} (1 mM for half maximum velocity) greater than Mn^{++} greater than Fe^{++} greater than Ca^{++}=Zn^{++}. Under the same assay conditions as employed for cyclic CMP, no degradation of cyclic AMP or cyclic GMP was observed. This is the first demonstration of a cyclic CMP specific PDE in mammalian cells, a finding that may aid in assessing the metabolic role of this cyclic pyrimidine nucleotide.

ELEVATED SISTER CHROMATID EXCHANGE RATE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA – Marlene Otter, Catherine Palmer and Robert Baehner, Indiana Univ. School of Medicine

The sister chormatid exchange (SCE) technique is a recent cytogenetic development which provides a sensitive assessment of subchromosomal changes related to DNA damage, repair and replication processes in normal and disease states. Studies in cell cultures and experimental animals have already revealed an increase in SCE levels after exposure to known mutagens and/or carcinogens at doses far below those which cause overt chromosomal breakage. To investigate DNA damage as evidenced by SCE events in childhood acute lymphoblastic leukemia (ALL), peripheral blood samples were obtained at time of diagnosis prior to treatment from 5 children ages 2 to 4 years with null cell ALL. Peripheral blood blasts in these patients ranged from 80% to 100%. Leukocyte samples from patients and from 5 age-matched controls were incubated for 96 hours in the dark in the presence of phytohemagglutinin and 10^{-4} M 5-bromodeoxyuridine, a thymidine analogue which is preferentially incorporated into replicating DNA and allows differential staining of sister chromatids in subsequent metaphases. SCEs are demarcated by sharp reciprocal changes in Giemsa stain intensity along the paired chromatids in cells which have replicated twice before harvest. Twenty to 35 well-spread metaphases from each subject were scored for SCE frequency determinations. The ALL group SCE rate was 11.59 $\pm$ .13 SCE/mitosis ($\bar{x} \pm$ SE) while the control group SCE rate was 6.96 $\pm$ .12 (p less than .01). These results provide the first documentation of an increased sister chromatid exchange rate in the circulating lymphoblasts from children with newly diagnosed ALL.

PROGESTERONE RECEPTOR (PR) AS A MARKER OF HORMONE RESPONSIVE HUMAN BREAST TUMOR – Peter Young, Lawrence Einhorn, Clarence Ehrlich, Robert Cleary and Robert Rohn, Indiana Univ. School of Medicine

This study was designed to test the hypothesis that PR, alone or together with estrogen receptor (ER), may serve as a better marker of hormonal dependence. Medroxyprogesterone acetate (MPA) has been found to interact with PR of human breast cancer specifically and with high affinity. The receptor-MPA complexes had sedimentation pattern in sucrose gradients almost identical to those of the receptor-R5020 and receptor-progesterone complexes. Data on the binding kinetics of MPA and its receptors revealed that there were at least two components which bound MPA with different affinities. Using (^{3}H)MPA and (^{3}H)estradiol as the tracers, and unlabeled progesterone and Nafoxidene as the competitors, respectively, the activity of PR and ER were determined in 138 breast tumors by a dextran-coated charcoal assay. The results on the distribution of ER and PR in these tumors are: ER+, PR+, 53 (38%); ER+, PR-, 20 (15%); ER-, PR+, 17 (12%); ER-, PR-, 48 (35%).

Twenty-nine of these cases have been evaluated for their responses to a trial of endocrine therapy. 13 out of 18 (72%) breast tumors that contained both ER and PR responded favorably to hormonal therapy, whereas only 1 out of 7 (14%) tumors that contained ER alone responded. Of the 3 tumors which lacked both ER and PR, 1 was found to be hormonally responsive. One tumor which contained PR but lacked ER responded to endocrine therapy. Our results indicate that PR may be a better marker of hormonally responsive breast tumors than ER.

REVERSAL OF METHOTREXATE (MTX) HIGH AFFINITY BINDING IN L1210 CELLS – M. Cohen, R. Bender, C. Myers and B. Chabner, NCI

MTX is known to bind tightly to intracellular sites (thought to be dihydrofolate reductase (DHFR), but the affinity and reversibility of this binding has only been estimated by extrapolation from experiments with cell-free enzyme preparations. We have used competitive binding principles to characterize this binding in L1210 leukemia cells in vitro. In the presence of 2 µM extracellular ^{3}H-MTX, a peak intracellular ^{3}H-MTX level of 10.2 nmoles/gm dry weight was reached in 20 min. Resuspension of loaded cells in drug-free medium led to a fall in intracellular ^{3}H-MTX to a plateau level of 6.3 nmoles/gm dry weight, a level which held constant for up to 120 min. Cell viability was unaffected by experimental conditions, as indicated by trypan blue exclusion, cloning, and in vivo tumorigenesis. Chromatography and other biochemical evidence confirmed that plateau ^{3}H-MTX was bound to DHFR. Reversibility of the ^{3}H-MTX:DHFR complex was demonstrated by resuspending cells in unlabelled MTX, at concentrations of 1 to 100 µM or greater, with loss of 90% of bound ^{3}H-MTX within 60 min. The rate of loss of bound intracellular ^{3}H-MTX was proportional to the concentration of unlabelled drug. Other anti-folates (aminopterin, pyrimethamine, Baker's antifol) but not leucovorin or dihydrofolate successfully competed off the bound ^{3}H-MTX. At 100 µM extracellular MTX - an "off" rate constant of -3.8 x 10^{-2} per min. was calculated, a value somewhat faster than the off rate of MTX from highly purified DHFR from L1210 cells ("k off"=1.0 x 10^{-3} per min.). These differences may result from differences in co-factor concentration or species, ionic constituents, or other aspects of the enzyme's intracellular environment. In conclusion, these experiments demonstrate the reversibility of "high affinity" MTX-DHFR binding in cells, and constitute an approach to the quantitation of drug-enzyme binding in intact cells.

OFFSPRING OF CHILDHOOD-CANCER SURVIVORS – Frederick Li, Frederick Holmes, Grace Holmes and Norman Jaffe, NCI, Sidney Farber and Univ. of Kansas

Genetic effects of cancer in childhood were examined among offspring of patients enrolled in the tumor registries of the Sidney Farber Cancer Institute and Univ. of Kansas Medical Center. Fifty-five women and 44 men who survived diverse childhood cancers reported the outcome of 219 subsequent pregnancies: 25 (11%) spontaneous abortions, 11 (5%) induced abortions, 1 (less than 1%) stillbirth, and 183 (84%) live births. Five liveborn infants died within the first day of life, and 2 died thereafter. The remaining 176 are alive at ages 0-33 years (median 7 years). Immunoglobulin levels and chromosome analysis of 9 of them revealed no evidence of genetic damage as a consequence of parental radiotherapy and chemotherapy. The offspring had no clear excess of congenital malformations or acquired diseases, but 2 have developed

cancer. One girl died at 2 years of age with bilateral retinoblastoma, which also affected her father. A second girl, whose mother had received radiotherapy for a brain tumor, developed acute myelocytic leukemia at age 15 years. Large collaborative studies are needed to determine the risk of neoplasia among these offspring, as compared with the 1 in 500 risk of cancer during the first 15 years of life among the general U.S. population.

ANDROGEN RECEPTORS IN CARCINOGEN-INDUCED AND TRANSPLANTABLE RAT MAMMARY TUMORS – M.M. ip, R.J. Milholland, U. Kim and F. Rosen, Roswell Park

Androgen receptors (AR) have been characterized in the DMBA-induced rat mammary tumor (MT) and in a series of transplantable MTs in W/Fu rats. Binding of dihydrotestosterone (DHT) to DMBA tumor cytosol was demonstrated to be specific, with a K_d of 1.3 nM and a total binding capacity of 31 fmoles/mg protein. The ^{3}H-DHT receptor complex sedimented at 7S on sucrose gradients in low salt buffers, at 4S in high salt, and was completely displaced by cold DHT. ^{3}H-testosterone also bound, but with lower affinity. Estradiol (E_2) competed as well as cold DHT for ^{3}H-DHT binding, but diethylstilbesterol did not compete. The assay did not detect any specific binding of ^{3}H-DHT to rat serum indicating that SBG was not being measured. In vivo injection of 15 mg DHT caused 76% loss of cytoplasmic ^{3}H-DHT binding in 8/9 tumors and 50% depletion of ^{3}H-E_2 binding in 5/9 of the same tumors. However, low doses of testosterone propionate (5 mg/kg; 3 x wk) could neither replace E_2 in maintaining the growth of the DMBA tumor in ovariectomized rats nor suppress E_2 stimulation when injected simultaneously. Pharmacologic doses of androgen are thought to act through the estrogen receptor (ER), however the presence of AR in a variety of both E_2-dependent and -independent transplantable MTs, with binding parameters similar to those of the DMBA tumor suggests a physiological role for AR and the potential for this receptor in the treatment of MT with androgens and/or antiandrogens. Phosphorylation of nuclear proteins is currently being examined as an indication of the functional significance of the AR under conditions in which ER is not translocated.

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 SHP-78-133

Title: *Macroeconomic model services*
Deadline: *May 15*

We desire access to a macroeconomic model which should have the following characteristics:

a) Provide forecasts through the year 2000.
b) Be available via a time-sharing system
c) Contain a demographic sector as well as an extensive industrial sector
d) Have well supported data bases
e) Be easily accessible to run alternative solutions
f) Have a policy orientation
g) Be well documented and have assistance provided in initial running of the model and interpretation of output.

Enviro Control Inc.
Subcontract Administration
George Hall
11300 Rockville Pike
Rockville, Md. 20852

CONTRACT AWARDS

Title: Development and implementation of at-home rehabilitation programs, renewal
Contractor: The Cancer Center Inc., Cleveland, $373,171.

Title: Transplacental carcinogenesis in erythrocebus patas, supplemental
Contractor: Meloy Laboratories, $131,425.

Title: Demonstration of cancer rehabilitation facilities and/or departments, renewal
Contractor: Institute for Cancer Research/Fox Chase Cancer Center, $322,119.

Title: Breast Cancer Detection Demonstration Project, continuation
Contractor: Univ. City Science Center, $322,638.

Title: Biomedical computing software services in support of clinical and diagnostic trials program, continuation
Contractor: Information Management Services, $252,445.

Title: Maintenance of immunodeficiency cancer registry, continuation
Contractor: Univ. of Minnesota, $51,526.

Title: Studies of the Marek's disease herpesvirus, continuation
Contractor: Life Sciences Inc., $454,369.

Title: Role of stroma in the growth of neoplastic and preneoplastic lesions of the mammary gland, continuation
Contractor: Stanford Univ., $95,000.

Title: Demonstration of cancer rehabilitation facilities and/or departments, renewal
Contractor: Medical College of Virginia, $323,013.

Title: NCI histocompatability testing center
Contractor: Duke Univ., $244,943.

The Cancer Letter –*Editor* JERRY D. BOYD
Published fifty times a year by The Cancer Letter, Inc., P.O. Box 2370, Reston, Virginia 22090.