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## NCI IMPLEMENTS CHANGES TO SPEED UP NEW DRUG DEVELOPMENT; COMPOUNDS IN PIPELINE LISTED

NCI's Drug Research & Development Program is still in the process of carrying out the reorganization and streamlining of its Drug Development Branch as recommended by the Div. of Cancer Treatment Board of Scientific Counselors (*The Cancer Letter*, March 19). The changes are aimed at speeding up the flow of new compounds through the "pipeline" and to provide more emphasis on finding and testing natural products with antitumor potential.

The Drug R & D Program is directed by Saul Schepartz. The Drug Development Branch, headed by Harry Wood, has been split into three branches—Drug Synthesis & Chemistry Branch, which Wood will head; Natural Products Branch, with John Duoros as chief; and Pharmaceutical Resources Branch, headed by Paul Davignon.

The three new branches will get additional professional staff, another recommendation of the Board. Still another recommendation, per-

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*In Brief*

## LET SALARIES RISE TO LEGAL LEVELS, BIOMEDICAL PANEL SAYS; PROSTATIC CANCER BLOOD TEST FOUND

**PRESIDENT'S BIOMEDICAL** Research Panel made one finding that NIH executives and scientists in the upper grade levels will not dispute. The Panel noted that top scientists and administrators are leaving NIH because of the salary problem. The panel recommended that salaries for grades GS-15 through GS 18 be allowed to rise to their current legislated levels, past the current limit of $37,800. The legislated level for GS-18 is $48,654; for GS-17, $42,066 through $47,674 in five steps; GS-16, $36,338 through $46,026 in nine steps; and GS-15, $31,309 through $40,705 in 10 steps. . . . **ERNEST PARK**, new president of the National Assn. of Life Science Industries, let it be known that NALSI will fight for a bigger share of federal health research dollars. "We will oppose discriminatory practices that impede scientific progress in finding cures for diseases and which inevitably add to the heavy economic burden already being carried by the taxpayer," Park said. The "discriminatory practices" include HEW's rule that private industry may not receive research grants and the fact that, in competing for contracts, non-profit institutions frequently have the advantage of indirect costs subsidized by state and local government. NALSI would like to have an equalization factor built into contract negotiations. . . . **ROSWELL PARK** announced it has developed a blood test for possible use as a mass screening tool in detecting prostatic cancer among men at high risk. Gerald Murphy, director of the institute and chairman of the National Prostatic Cancer Project, and T.M. Chu, chief cancer research scientist, said that the test detects specific enzymes secreted into the blood by an abnormal prostate gland.

## NCI SPEEDS UP DRUG DEVELOPMENT; COMPOUNDS BEING TESTED LISTED

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mitting natural products principal investigators to be involved in the decision making processes as their drugs move through the pipeline, has been implemented, Schepartz said.

The major cause of delays between identification of a potential anticancer agent and clinical trials has been getting the drug into production. This can be particularly difficult with natural products, especially when rare or exotic plants are involved. Preparative laboratory contractors have been responsible for both synthetic and natural product scale up and production; the Board suggested that an RFP be issued to obtain a contractor responsible only for natural products. That RFP was issued and generated a number of proposals which are now being reviewed.

There are three major decision points once a drug has been found with antitumor activity, either in NCI's experimental tumor screens or in foreign clinical trials:

1. Based on the experimental or foreign data, the decision is made that it is a compound of interest and development will proceed toward clinical trials. Steps are taken for bulk procurement and development of a suitable formulation.

2. When suitable quantities and formulations are obtained and optimal dose schedules for experimental animals are worked out, tests are undertaken with large animals—dogs and monkeys. These tests determine the type of toxicity—whether it is reproducible, predictable and reversible—and the starting dose in humans.

3. When that data has been collected and analyzed, it forms the basis for an investigational new drug application (IND) to the Food & Drug Administration which if approved permits the start of phase I clinical trials.

That is the point when the drug passes into the hands of the clinicians, but it may be returned to Schepartz' program for reformulation or other changes found necessary through clinical use. The program is responsible for continued production of new drugs through all clinical trials, until FDA approves a new drug application and permits it to go into commercial distribution.

Following are the list of drugs in development. Included is the NSC number, type of compound (synthetic, natural, fermentation), program status and, when available, a description of the drugs's activity.

**Amino thiadiazole**, 4728, synthetic. An older drug now being reconsidered because clinical trials in the 1950s are considered inadequate. Decision to go to phase I trials was made nearly three years ago, but toxicity problems caused delay. Now in phase I study at Sloan-Kettering.

**Dihydrate chartreusin**, 5159, fermentation. Large animal trials are waiting bulk procurement. An older drug that was re-evaluated after activity was picked up in newer, more sensitive screens. A protein synthesis inhibitor, it binds to ribosomes and prevents acceptance of the next amino acid.

**Gallium nitrate**, 15200, synthetic. In phase I trial.

**Pyrazoloimidazole derivative**, 52243, synthetic. In animal toxicity testing. Inhibits DNA synthesis, possibly by ribonucleotide-reductase inhibition; production of dCDP most affected.

**Hematoporphyrin**, 59265, plant. A light-sensitizing agent in clinical use at Mayo Clinic and requested by Roswell Park. NCI will supply resources necessary for RP to file the IND, but problems with purification are holding up production.

**Thalicarpine**, 68075, plant. Awaiting phase II clinical trial. There has not been a great amount of interest shown yet, but the Southwest Oncology Group is planning a phase II protocol.

**Metronidazole**, 69587, synthetic. Awaiting production. One of several radiation sensitizers; should be worked up with NSC-261036 and NSC-261037, the Roche compounds.

**Ellipticine**, 71795, plant. A prime example of difficulties caused by production problems and its toxicity. It passed the first decision point in August 1967, and the second five years later. It is now awaiting large animal toxicology. NCI has looked at analogs, without satisfactory results, and is considering oral formulations. The drug intercalates into DNA, also interferes with processing of ribosomal precursors.

**Alphadeoxythioguanosine**, 71851, synthetic. In phase I clinical trial, in Canada. Normal tissues do not activate the alpha form (to triphosphate) while some tumor tissues in man do.

**Fluorodopan**, 73754, a Russian synthetic. One of several drugs obtained in the exchange with USSR. The IND, based on Russian clinical data, has been approved, and phase I trials are imminent at Roswell Park.

**D-tetrandrine**, 77037, plant. Phase I clinical trials have been completed, and efforts are being made to interest clinical groups in doing phase II trials.

**S-trityl-L-cysteine**, 83265, synthetic. Dropped from phase I clinical trial in June, 1975, because of phlebitis from high pH of Na salt. May inhibit electron flow. NCI is trying to develop an oral formulation.

**PCNU** (one of the nitrosureas), 95466, synthetic. Awaiting formulation development and procurement. Proposed by Levin (Univ. of California) for use against brain tumors, based on screening data and decreased lipophobicity.

**Tetrahydroouridine**, 112907, synthetic. In phase I trial at Sloan-Kettering. No antitumor activity alone, but prevents deamination of AraC, thus in-

creasing its activity.

**Dichloroallyl Lawsone (DCL)**, 126771, plant originally, now produced synthetically. In phase I clinical trial.

**Deazauridine**, 126849, synthetic. Completed animal tests a year ago, now awaiting preparation of an IND.

**N-oxide indicine**, 132319, plant. Animal trials were completed in February, 1975, but NCI has been unable to obtain quantities large enough for phase I trial.

**Piperazinedione derivative**, 135758, fermentation. In both phase I and phase II trials now. Inhibits DNA synthesis.

**Selenoguanosine**, 137679, synthetic. Awaiting accumulation of enough material for formulation development.

**5-methyl-tetrahydrohomofolic acid**, 139490, an analog of methotrexate, synthetic. Selected for its activity against a methotrexate-resistant experimental animal tumor. Large animal toxicology starting. Doses are high, and NCI has had a big production problem in getting it in sufficient quantities.

**Anguidine**, 141537, fermentation. In phase I clinical trials.

**Homoharringtonine**, 141633, plant. Approved for animal toxicology in February, 1972. Inhibits initiation of protein synthesis. Delayed by difficulty of production, and since there was only marginal interest, no big effort was made.

**Hycanthone**, synthetic. Used for years as an anti-infectious disease drug, primarily in Africa. FDA held up the IND when it couldn't decide whether review should be by its Oncology or Anti-infectious Drug Div.—the decision finally went to the latter, but the IND still has not been approved.

**Nitidine, chloride, dihydrate**, 146397, plant. Problems with formulation, concerns over possible cardiovascular toxicity, and difficulties in securing the plant material have delayed progress since 1971.

**Ftorofur**, 148958, synthetic. Another drug obtained from the Russians. In phase II trials at M.D. Anderson and the Southwest Oncology Group, substituting it for 5-FU.

**L-alanosine**, 153353, fermentation. In large animal toxicology now.

**Maytansine**, 153858, plant. In phase I trials at the NIH Clinical Center, M.D. Anderson and Sidney Farber Cancer Center. This is the drug, identified by Kupchan at the Univ. of Virginia (one of four Drug R & D Program natural products contractors) that has aroused intense interest around the country.

**Pentaazacentopthylene**, synthetic. Awaiting bulk production and formulation. Lack of solubility has made it difficult to develop a formulation; NCI is trying to find a soluble derivative.

**Sulfoacetate coralyne**, 154890, synthetic. Large animal studies have been completed, and serious toxicity problems are delaying filing of an IND. It will be presented to clinicians for possible consideration.

**Crystalline neocarzinostatin**, 157365, fermentation. A Japanese drug, this has been around for years. FDA has given preliminary approval of an IND subject to additional Japanese clinical data.

**Tripdiolide**, 163063, plant. Another Kupchan product, it is now in large scale development and production.

**Isoxazoleacetic acid derivative**, 163501, synthetic. A glutamine antagonist. Very expensive to produce, and NCI is trying to find a more econimically feasible way to make it.

**Bruceantin**, 165563, plant. Now in toxicology, will be up soon for a decision on an IND.

**Asaley**, 167780, synthetic. Another Russian drug. Phase I trials have been completed, and phase II studies are imminent.

**Soluble ICRF**, 169780, synthetic. A chelating agent, possible ribonucleotide reductase inhibitor, reverses the toxicity of daunomycin. Has been in clinical trials in England. NCI is waiting to find out if clinicians are interested before going into production.

**Macromomycin**, 170105, fermentation. Another Japanese product. A glycoprotein, it has its effect on the cell membrane, blocks cells in G2, increases immunogenicity. NCI has had difficulty in getting pure, stable, active material.

**Spirohydantoin mustard**, 172112, synthetic. Developed in NCI's own labs. It's a nitrogen mustard derivative of hydantoin, designed to pass the blood-brain barrier. In production now, will soon go into animal toxicology.

**Butocin**, 172755. Obtained from Czechoslovakia. An IND will be filed soon based on Czech clinical data.

**Quinolinium derivative**, 176319, synthetic. Developed by New Zealand chemist Bruce Cain. A methionine synthetase inhibitor. Large animal studies have shown serious toxicity problems; will be presented to clinical groups for consideration before filing an IND. Very active but very toxic.

**Chlorozotocin**, 178248, a nitrosurea. It has more activity than some other nitrosureas without the toxicity. Designed by Phil Schein, Georgetown Univ., to have no marrow and delayed liver toxicities in man. Is water soluble. An IND has been filed.

**Spirogermanium derivative**, 192965, synthetic. NCI decided to go into production with it, but has not proceeded with toxicology. Has been in clinical trials at Georgetown Univ., showing responses in breast cancer and malignant melanoma (An IND has not yet been filed; FDA regulations apply only to material that crosses state lines. In this case, the drug was developed by Schein in the District of Columbia and used there. Many states have laws requiring adherence to FDA regulations; D.C. does not).

**Sterigmatocystin**, 204985, fermentation. Proceeding into production. A highly carcinogenic agent.

**Polyene**, 208642, fermentation. An antibiotic, now in the procurement phase.

**Deoxycoformycin**, 218321, fermentation. NCI is proceeding with procurement, will go into large animal studies when sufficient supplies are obtained. This is an adenosine deaminase inhibitor, without antitumor activity alone. Very active in the NCI screening systems when combined with AraA.

**PALA**, 224131, synthetic. In the procurement stage. This compound is a rationally synthesized antimetabolite which is a transition-state inhibitor of aspartyl transcarbamylase.

**Bouvardia ternifolia** (the name of the plant from which this drug is derived—a name has not yet been selected for the compound), 227262. Actually consists of two compounds, which NCI did not realize until after the decision was made to go into procurement.

**Thiosemicarbazone derivative**, 246112, synthetic. In the procurement stage. More potent than agents now in the clinic in inhibiting ribonucleotide reductase because it has the potential of being less susceptible to inactivation in man, and is water soluble.

**Adriamycin derivative**, 246131. May be more active than adriamycin in experimental tumors. Problems with production and formulation are delaying tests in large animals.

**Ledacrine**, 247561, synthetic. A Polish drug, it has shown clinical responses in ovarian, gastric and colon cancers as well as control of pleural effusions with intracavital use. Supplies are being sought in Poland, and some large animal studies will be done before an IND is filed.

**Acridine derivative**, 249992, synthetic. Large animal studies will be completed this fall.

**Propanediol**, 261036, synthetic. A radiosensitizer developed by Roche. Will go into phase I clinical trial as soon as an IND is filed and approved.

**Imidazole-1-ethanol**, 261037, synthetic. Another Roche compound, same status as above. Other radiation modifiers are being developed which NCI feels may be better, but have not yet entered the system.

**Cesaline**, 264879, plant. Going into production and formulation, will proceed into toxicology when enough material is available. Problems in getting enough pure material have delayed large animal studies since 1971.

## DCCR SURVEY DETERMINES MANPOWER NEEDS FOR QUALITY CANCER CARE

A survey sponsored by NCI's Div. of Cancer Control & Rehabilitation has determined that a total of 296,000 "full time equivalent" health and allied health personnel would be required to provide quality care for every cancer patient in the U.S.

That figure represents gross need, not the shortage, "and is based on a lot of assumptions," according to the survey contractor, Messer Associates of Silver Spring, Md.

J. Daniel Recer, chief of DCCR's Resources Branch, told the DCCR Advisory Committee that the study "assessed cancer incidence at 38 body sites. It then tried to determine the approximate amount of time spent by various professionals and allied health professionals in treating a specific site cancer. . . The contractor then assessed the available manpower in each of 104 manpower categories, and from incidence and time data estimated the manpower needs in each of the categories by intervention and state.

"Naturally, some degree of error exists in the time determinations, but in our judgment this is the most accurate information available," Recer said.

The RFP did not permit Messer to use primary data sources and thus prohibited the in depth type of study that would be needed to determine the actual shortages that may exist in the various manpower categories. The firm did identify 20 categories for which shortages were presumed, based on its estimates of need and on existing supply.

The 20 categories, the full time equivalent (FTE) need, and the national supply presented available (FTE is defined as the number required if each worked entirely with cancer patients):

Family physician—16,248.66 FTE, 50,363 available; cancer control thus requires (according to the estimates for quality care for all patients, which of course is not now the case) 32% of the national supply.

"We know that family physicians do not spend 32% of their time with cancer patients," a Messer spokesman told ***The Cancer Letter***. "We don't know what the true percentage is—the RFP did not permit us to attempt to answer that question." Whatever the true percentage is, the difference between that figure and 32% represents the shortage.

Gynecologist—8,318.73 FTE, 18,807 available; cancer requires 44% of the supply.

Colon-rectal surgeon—924.25 FTE, 632 available; cancer needs exceed the supply by 46%.

Dermatologist—954.8 FTE, 3,826 available; cancer requires 25% of the supply.

Anaesthesiologist—2.225.75 FTE, 11,020 available; cancer requires 20% of the supply.

Pathologist—4,865.73 FTE, 8,215 available, cancer requires 50% of the supply.

Diagnostic radiologist—14,410.75 FTE, 9,708 available; cancer needs exceed the supply by 48%.

Physical medicine and rehab physician—271.05 FTE, 1,171 available; cancer requires 23% of the supply.

Nuclear medicine physician—1,052.54 FTE, 1,354 available; cancer requires 78% of the supply.

Endoscopist—309.92 FTE, 4 available. (Messer said that figures for this category were difficult to obtain since accreditation is usually not required; there certainly are more than 4, but a national shortage is presumed).

Health social workers—3,305.59 FTE, 10,300

available; cancer requires 30% of the supply.

Respiratory therapist—1,792.21 FTE, 3,461 available; cancer requires 52% of the supply.

Enterostomal therapist—317.19 FTE, 156 available; cancer needs exceed the national supply by 103%.

Thermography technician—24,299.14 FTE; no data available on the national supply.

Cytotechnologist—24,797.37 FTE, 4,341 available; cancer needs exceed the national supply by 471%.

Nuclear medicine technician—2,504.39 FTE, 2,793 available; cancer requires 90% of the supply.

Blood bank technician—602.27 FTE, 273 available; cancer needs exceed the supply by 120%.

Radiotherapy technician—1,407.68 FTE, 887 available; cancer needs exceed the supply by 59%.

Lab technician—9,301.92 FTE, 18,875 available; cancer requires 49% of the supply.

Histologic technician—25,186.8 FTE, 6,720 available; cancer needs exceed the supply by 275%.

While it may be difficult to determine precise needs in some categories without a further and more detailed study, those with major shortage are obvious. Recer said DCCR would undertake an assessment of the figures and planned to distribute the survey results to various groups and agencies for their evaluation. To help make proper use of the information, DCCR awarded another contract for "Education for Cancer Control." The objective is to develop suitable modes, settings and personnel qualifications needed to assist in the education of health professionals and others who are most specifically concerned with conducting, administering and participating to a major degree in cancer control activities, Recer said. The contract will be completed later this year.

"Preliminary information from this study indicates that a plethora of occupational titles are often currently engaged in similar tasks," Recer said. "For instance, interviews at several sites showed that lay people often perform duties traditionally reserved to professional and technical people. Also, many instances of technical-level people performing professional work were noted.

"Consequently, the contractor decided upon a task-oriented, as opposed to an occupational-title oriented, approach to learning, and then, identified a learning module for each of the identified tasks. Conceptually, these task modules will cover a single task, will be fully transportable and will function independently or in concert with other modules. Examples of modules for the treatment of breast cancer for instance are interviewing (one entire module), physical examination and staff operations.

"Hopefully, the experience gained in producing a complete set of descriptor modules on one body site will enable us to expand our capacity, both in range to other sites and in depth to a greater degree on each site."

Recer described DCCR's effort to develop a history of cancer control.

"The exact purpose [of the contract] is to determine the extent and limitations of progress in cancer control from 1946-1971; to elucidate the factors that favored or inhibited the application of the available technology; to describe and evaluate the successes and failures of various national and state voluntary programs and to suggest directions for future development.

"Recognizing that most of the people who significantly contributed to this history are yet alive and that a substantial portion of the history is undocumented, project staff set about to interview many principals.

"Bibliographic material has been identified, screened, used, and in many cases, abstracted and entered into a specially designed computer program where it can be readily retrieved. Future division plans include adopting means of making the knowledge gained by this project as widely known as possible."

## BREAST CANCER TASK FORCE TO ISSUE 16 NEW RFPs FROM JULY TO SEPTEMBER

The Breast Cancer Task Force has started issuing new RFPs this month, with a minimum of 16 to go out before the end of September. Most of them will have a deadline for proposals in November, permitting review and award of contracts by next March.

That's the schedule set up by BCTF Chairman Pietro Gullino, who took over that job last year and immediately developed a reputation as perhaps the most thorough, detailed and demanding organizer on NCI's staff.

Gullino produced a syllabus which contained a list of all BCTF contracts grouped by program area; narrative descriptions of each of the programs including suggested areas needing more research and new initiatives and the supporting literature references; and a tough new schedule for the year which, so far, the task force has been able to maintain.

The meeting schedule seemed backbreaking, with the entire group meeting in plenary session one day

**"Breast Cancer: A Report to the Profession, 1976" is the theme of the conference sponsored by the White House and NCI, scheduled for Nov. 22-23 at the Washington Hilton Hotel. The conference is supported by funds from the sale of President Ford's inaugural medals. The program will include discussion of major problems concerning biology, epidemiology, diagnosis and treatment of breast cancer. It is open to the public. Advance registration is requested, no registration fee. Write to Sally Simpson, Breast Cancer Conference, 1501 Wilson Blvd. 6th Floor, Arlington, Va. 22209.**

every two months to hear reports from about a dozen contractors, followed by another full day of meetings by the committees. The reports are in the form of papers describing the contractor's work and progress to date.

Gullino said the meeting schedule is necessary "to keep everybody informed of the whole program. In the past we had a general meeting each year to review the whole program. Today the program is too vast for one meeting a year."

That schedule will be maintained at least for the rest of the year, "unless they just refuse to keep it up," Gullino said. In January, "we'll take a look and see where we're going" to determine if the same schedule will be followed next year.

The first of the RFPs, probably four, will come from the Diagnosis Committee. They will be available in a matter of days. The next group probably will be those developed by the Experimental Biology Committee, four and perhaps five more. The Epidemiology Committee will have four ready by the end of July, and the Treatment Committee will have four sometime in August, Gullino said.

The Task Force experimented with Cancer Research Emphasis Grants and found that the quality of proposals was on par with contract proposals. Since CREGs are reviewed by study sections in the NIH Div. of Research Grants, the BCTF committees did not know what would be coming out of the study sections and thus was hampered in determining research areas that would have to be covered with contracts.

Gullino said that although he agrees that CREG is an important mechanism, its use in the structured and well-defined BCTF program would result in overlapping in some cases, gaps in others.

The Task Force issued both a CREG announcement and an RFP relating to the role of glycoproteins of the cell surface in breast cancer. Thirteen CREG proposals and 11 contract proposals were received. The study section approved eight CREGs, four of which scored high enough to be funded. Three contract proposals were approved and two were funded.

Gullino said he felt the measurement of quality was the relationship between the number approved and number funded.

Following are abstracts from contractor reports at recent BCTF bimestrial meetings. Additional abstracts will appear in next week's ***Cancer Letter.***

### THE ISOLATION AND CULTIVATION OF CELLS FROM HUMAN MILK – Edwin Gaffney, Frank Polanowski, Susan Blackburn and Robert Burke

The establishment of pure cultures of indisputably normal human mammary epithelium has been partially hampered by the lack of an appropriate source of cells. An apparent solution to this problem was suggested through studies which demonstrated the growth of cells isolated from samples of breast secretions. This possesses the inherent difficulty that mammary gland secretions contain a variety of cell types. The question of the origin and function of these cells becomes important when using in vitro cultivation as a diagnostic tool or when determining cellular hormone responsiveness.

This project concerns the identification and cultivation of cells from milk. Cells were isolated from milk samples collected during the following stages: colosltrum (1 to 2 days postpartum), early lactation (3 to 10 days), mid-lactation (1 to 2 months) and late lactation (1 to 10 days postweaning). The two major cell types observed were vacuolated foam cells and epithelial cells in clusters. Relatively large, pure populations of single foam cells were observed in early lactation samples, while populations enriched in epithelial cell clusters were derived from samples of post-weaning fluids. Foam cells were identified as differentiated mammary gland epithelium by comparing the structural characteristics of those cells partially exfoliated in lactating breast tissue, those isolated from milk samples, and those maintained for several weeks in vitro. Light and electron microscopic studies revealed similarities in the types and distribution of cytoplasmic organelles and appearance of the nuclei.

Foam cells and colony forming epithelial cells exhibited differences in response to lactogenic hormones. Insulin stimulated DNA synthesis in cultures of proliferating colony forming cells but had no significant effect on non-dividing foam cells. Prolactin initiated mitogenic responses in cultures of either cell type. DNA synthesis in milk cell cultures was inhibited by serum regardless of hormone treatment. Hydrocortisone alone or in combination with other hormones inhibited human embryonic lung fibroblasts and amnion epithelial cells.

The study of human milk cells in culture may serve as a diagnostic tool for the early detection of breast disease. A major finding which defends this theory was the potential of cells to replicate sufficiently to form epithelial cell lines (HBL-6 and HBL-100). In general, the HBL-100 cells are not tumorgenic in nude mice, but do form colonies in soft agar supplemented with 10% serum. Cells demonstrate a capacity to synthesize lactose, but lack alphalactalbumin at a level detectable by radioimmunoassay. This cell line has been useful in establishing a method for the measurement of casein synthesis in mammary epithelial cell cultures. Both prolactin and estradiol enhanced the synthesis of casein and/or the phosphorylation of pre-existing milk proteins. Thus, HBL-100 appears to be a non-malignant but transformed cell type which maintains certain functions characteristic of the tissue of origin.

### APPLICATIONS OF HISTOCULTURE – Russell Sherwin

With solid cancers in general, and human breast cancer in particular, it has been demonstrated that histocultures of the cancer tissue can be obtained in the majority of primary explantation attempts. Histocultures are architecturally intact, mixed cell populations. They can usually be maintained in culture for direct phase contrast observations for an average of two weeks, and in a limited study have remained intact for as long as 11 months. The intact architecture not only permits a conclusive identification of cancer in culture but allows an investigation of cancer cells in their tissue form.

From experience with over 300 human histocultures, the following potentials are indicated: 1) the nutrient medium can be varied while the cells are under direct observation in order to a) evaluate nutrient requirement for long-term maintenance of both the histoculture form of the cancer and the corresponding documented cancer monolayer, b) evaluate nutrient factors which influence the conversion of histoculture to monolayer. 2) the testing of chemotherapeutic and other agents is possible beginning immediately after biopsy. Metaphase plate time and rate of necrobiosis are two discriminants suggested from earlier investigations. 3) studies of lymphocyte and macrophage interactions with cancer tissue as well as cells can provide data not available by other methodology.

For example, an initial study of 50 cancers yielded 45 cancer histocultures. Of these, 16 showed a newly described lymphocyte activity, juxtanuclear orientation, and all lymphocytes were counted, from 12 to 539 per cancer. In six of the 16, the ratio of juxtanuclear orientation to random lymphocyte activity varied from 1.3 to 5.2, the other nine having ratios less than unity. Of the six, five were two year or more survivors.

### THE DISSOCIATION OF MAMMARY EPITHELIAL CELLS FROM SOLID TISSUE – Robert Zucker

Different rat and mouse transplanted mammary tumors have been studied to acquire information on the procedures necessary to obtain single cell suspensions from human and animal solid tumors. The tumors were morphologically characterized by light and electron microscopy to study the intercellular bonds and cell types. The dissociation began by gently slicing the tumor into small sections which increases the surface areas for enzyme exposure. The tumor slices were then

placed in a $Ca^{++}$ free 199 media containing collengase, trypsin, DNA'se, BSA and gentamycin.

The bovine serum albumin was added for cell protection and the DNA'se was added to help reduce cell clumping. This dissocation lasted for approximately 1 hour at 37°C during which time constant mechanical agitation was applied. The tumor cell viability measured by flourescin diacetate and trypan blue exclusion was consistently over 80% with this methodology.

### CELL SURFACE ALTERATIONS ASSOCIATED WITH HUMAN BREAST CANCER – Elinor Spring-Mills and Joel Elias

The scanning electron microscope (SEM) reveals previously undetected differences between the surface morphologies of duct cells from cancerous and noncancerous human breasts. The alterations appear to extend throughout the affected breast. By processing all tissue samples examined in the SEM for histology, it appears that when a primary carcinoma is present there are substantial changes in the morphology of the ducts within histologically "normal" areas away from invasive nodules as well as hyperplastic regions. The observations call attention to real and potential problems incurred by using so-called "normal" areas from diseased breasts for establishing or extrapolating either the structural or functional characteristics of normal breast tissue.

Infiltrating duct carcinoma cells have fewer surface microvilli than duct cells and can be readily identified in the breast with the SEM on the basis of their shape and surface topology. It appears that the plasma membrane undergoes profound changes at some point when these cells leave the ducts and enter the stroma of the breast. The loss of microvilli may be an important step in the invasive process or a consequence of the loss of surface polarity.

Whether the observed alterations turn out to be either an accurate indicator of malignant change or an essential part of the neoplastic mechanisms remains to be established. Nevertheless, it is possible that such morphological differences may facilitate: (1) the differentiation of certain confusing benign conditions from infiltrating duct carcinoma and (2) the diagnosis of noninvasive duct carcinoma.

### THE IN VITRO CELL AND MAMMARY CANCER – William McLimans

The contract investigations encompass the following, as based on the rationale that in many, if not most instances, cancer in man is caused by environmental factors that may be operative over long periods of time before the symptoms of the overt disease occurs. If this is valid, it follows that long term cultures of human mammary cells—representative of those found in the tissue of origin and as cultured under controlled environmental conditions—may be prerequisite to the identification of these subtle cancer inciting agents.

Employing our controlled environmental culture system we initiated cultures of normal lactating mammary gland (rat) on March 15, 1974. Environmental conditions maintained included 37.0°C, 2% $CO_2$, 21% $O_2$, thin film culture, pH 7,36, frequent automatic feed of media; – after 29 weeks – cultures were fed every hour with insulin, hydrocortisone, prolactin in McCoys 5A supplemented with 10% serum.

Cultures of the above type were maintained in the same flask for nearly two years i.e. 15,416 hours elapsed time, 15,028 feed cycles. At that time heavy cell growth and/or tissue was removed and sectioned. Subcultures from four areas of this thick growth resulted in the rapid establishment of several "cell lines". These "lines" are currently in their 6 and 7th passages.

### CHARACTERIZATION OF MAMMARY CELLS BY ISOENZYME PATTERNS – Russell Hilf, Regina Ickowicz, S. Abraham, J.C. Bartley

Goal and Approach: Examine multiple molecular forms of glucose 6-phosphate dehydrogenase (G6PD) and lactate dehydrogenase (LDH) to characterize normal, preneoplastic (HAN) and neoplastic mouse mammary tissue by studying mammary tissue during pregnancy and lactation as well as HAN and tumors exposed to the same hormonal milieu in vivo.

Results: Preparations from mammary tissue of lactating BALB/C mice showed on polyacrylamide electrophoresis considerable amounts (up to 50%) of a slower-migrating species (G6PD-III), which was essentially absent from glands of pregnant mice, HAN and carcinomas All tissues possess a faster migrating species, G6PD-II, which accounted ca. 85% of total G6PD activity in glands of pregnant mice. A third species, G6PD-I, migrating most rapidly was found in HAN and tumors and represented ca. 35% of total activity; G6PD-I was low (< 15%) in glands during pregnancy and essentially absent (ca. 5%) during lactation. Dithiothreitol (DTT) did not influence total G6PD activity but increased relative activity of G6PD-II and G6PD-I. Mild oxidation increased the relative amount of G6PD-III; oxidation with DTT largely prevented appearance of G6PD-III. Addition of NADP did not alter G6PD patterns but stabilized G6PD against heat inactivation; G6PD-III appeared to be the most stable species. Full recovery of G6PD activity was achieved after overnight oxidation by addition of NADP and heating at 47°, suggesting recombination of inactive subunits. Thus, a role for –SH groups in G6PD activity and multiple molecular forms is indicated.

### CASEIN mRNA AS A PROBE FOR CHARACTERIZING MAMMARY CELLS – Susan Socher and Jeffrey Rosen

Casein is widely used as a marker of differentiated function in both normal and neoplastic mammary cells. Recent evidence has suggested that the presence of casein may be useful in the early detection of breast cancer. Thus, we have developed procedures to measure both the levels of casein mRNA and of casein synthesis in mammary tissues. Casein mRNA can be detected either by direct translation of total cell RNA extracts in the wheat germ cell free system or by hybridization with a sensitive complementary DNA probe synthesized from purified casein mRNA. Casein synthesis can be measured by the incorporation of $^3$H-proline into specific immunoprecipitable material. Finally, intracellular levels of casein can be quantitated by the modified radioimmunoassay. The specific antibody used in these procedures was prepared against purified casein isolated from skim milk by isoelectric precipitation followed by DEAE cellulose chromatography in the presence of urea.

Casein mRNA can be readily quantitated using a wheat germ translation assay and can be detected if it comprises as little as 5% of the total mRNA activity. Since casein mRNA constitutes 50% of the total mRNA activity in extracts from the normal lactating gland, it can be purified by a combination of specific sizing techniques and affinity chromatography. The purified mRNA is used as a template for viral RNA-directed DNA polymerase for the synthesis of a high specific activity complementary DNA probe. Using this probe, casein mRNA sequences can be detected in RNA extracts from hormone-dependent mammary tumors at levels ranging from 0.003 to 10% of the level in the normal lactating rat. In order to examine the role of hormones in casein production, levels of polysomal casein synthesis represents 15% of the in vitro protein synthesized at midpregnancy and greater than 40% of the protein made during lactation. Within 48 hours of ovariectomy of a midpregnant animal, casein production rises to 30% of the in vitro synthesis; this increase can be blocked by treatment with progesterone at the time of ovariectomy. These changes in casein synthesis show a good correlation with the activity of isolated casein mRNA assayed in vitro.

### TRANSPLANTATION OF HUMAN BREAST TUMORS IN NUDE MICE – Beppino Giovanella

One hundred and eight primary and metastatic human breast carcinomas and 20 cell lines from human breast cancers have been transplanted in 1,356 nude, thymus deficient mice. Of 57 breast cancers transplanted directly from the patient into untreated nudes and observed for 180 days or more, 28 grew slowly. Many regressed, some reappearing after months of latency. Four tumors showed indefinite growth between six months and a year. A fifth primary duct cell carcinoma grew rapidly and has been transplanted serially: it is now in its 8th passage. Twelve human breast cancer lines grew vigorously in the nudes; seven of them have been transplanted serially for 2 to 5 passages. Of 52 tumors transplanted into anti-lymphocyte-B serum treated mice, 32 grew, 13 of them indefinitely. Five of these tumors have been transplanted serially for 2 to 4 passages. Four of the serially transplanted human breast carcinomas have been screened for their sensitivity to different doses of anticancer drugs.

### MORPHOLOGICAL INSTABILITY AND DEDIFFERENTIATION OF NORMAL HUMAN MAMMARY LOBULES AND ATYPICAL LOBULES TRANSPLANTED INTO NUDE ATHYMIC MICE – S.R. Wellings and H.M. Jensen

There is circumstantial evidence that normal appearing lobules which persist after the menopause, and certain atypical lobules, may be precancerous. In our study we are transplanting human lobules (L) and atypical lobules (AL) into host gland free fat pads of nude athymic mice in order to determine their growth potential. Our results so far show that L and AL do not progress to cancer in six months. We are, therefore, following changes in histology and cytology which could reflect differences in precancerous potential of the transplanted tissues. Fresh biopsy tissues and mastectomy specimens provided L and AL which were individually dissected and isolated.

One half of each L or AL (control) was fixed and histology slides prepared and stained with hematoxylin and eosin. The other half (experimental) was transplanted into the host gland free fat pad of a female "nude" athymic mouse. After 2-25 weeks in the host, the transplants were harvested, fixed, and histology slides prepared for comparison with the control slides. Survival with intact epithelium was observed in 120 of 135 (89%) of transplanted L recovered, and 25 of 30 AL (83%). There was no significant difference in transplant survival between L or AL from women younger than age 50 (pre-50) and from women older than age 50 (post-50). However, 14 of the surviving 89 pre-50-L (16%) showed morphological epithelial change interpreted as dedifferentiation; whereas, 17 of 31 post-50-L (55%) showed the same change. In the instance of AL, 1 of 11 pre-50-AL (9%) changed, while 4 of 14 post-50-AL (29%) changed.

This increased phenotypic instability of L and AL after age 50 may reflect a greater precancerous potential.

### THE IN VITRO PROPERTIES OF TWO NEW HUMAN MAMMARY CELL LINES – A.J. Hackett

Two cell lines have been derived from a 74 year old woman with a carcinosarcoma, one from a sample of the tumor and the other from peripheral breast tissue.

The tumor derived line is epithelial, shows polygonal shaped cells, desmosomes, tonofibrils, a well developed golgi, and duct-like cytoplasmic vacuoles. At confluence the cells form small secretory domes demonstrable by both scanning and transmission electron microscopy. The domes contain cells that are secreting a product with the ultrastructural features of casein (Dr. L. Springer). The cell line derived from the normal breast tissue is fibroblastic by both light and electron microscopy.

Both cell lines grow well in culture. The tumor line has a doubling time of approximately 2-3 days, a saturation density of $22 \times 10^4/cm^2$ and has undergone 30 subcultures to date with no evidence of senescence. The fibroblast line has a saturation density of $7.4 \times 10^4/cm^2$ and has been subcultured 30 times. The growth rate at the later passages is slowing, suggesting that the cells are approaching senescence. Both cell lines are negative for mycoplasma by bioassay (L. Hayflick, Stanford Univ.) as well as by electron microscopy and uridine incorporation. The tumor line has a unique karyotype with a modal chromosome number of 57, while the fibroblasts are normal diploid (W.A. Nelson-Rees), and both lines have glucose-6-phosphate-dehydrogenase isoenzyme type B. (W. Peterson, Wayne Univ.). The tumor line shows no evidence of Mason-Pfizer Mammary Tumor Virus p25 antigen by radioimmune precipitation (H. Charman, Flow Laboratories) or by immunofluorescence (J. Riggs, California Dept. of Health). The tumor line also lacks estrogen binding protein.

The lines have been characterized for a number of properties thought to correlate with malignancy. The tumor cells show a high nuclear-cytoplasmic ratio, irregular nuclear margin with condensed chromatin, and cytoplasmic basophilia. The tumor cells grow well on both epithelial and fibroblastic contract-inhibited monolayers, but do not grow in methocel, produce no tumors in immuno-suppressed mice, and are negative for plasminogen activator. The fibroblast culture is consistently negative in all of the tests (Dr. H. Smith).

The tumor line thus shows some but not all of the properties usually associated with malignancy. Dr. Smith has found that tumor cell lines derived from other types of carcinomas and sarcomas also possess various combinations of these malignant growth properties. To determine whether the observed characteristics are those of the original tumor rather than random changes induced by cultivation, two cell lines derived from metastatic tumors of a single individual were characterized. The cultures were found to be identical. These studies suggest that human tumors vary in growth properties and that the differences are retained in culture.

### BIOLOGICAL CHARACTERISTICS OF MCF-7 – Marvin Rich, Charles McGrath, Jose Russo, Herbert Soule and Bruce Voyles

It seemed likely that a favorable source of "endogenous" human breast cancer viruses would be human breast cancer cells, and that the most favorable source of these would be a line of homogeneous breast cancer epithelium in continuous passage. Availability of such a cell line would allow both the standardization of techniques for virus recovery and yield the necessary amplification of gene expression. The use of cell lines however demands the exercise of extreme care in establishing that such cultured cells are indeed human mammary epithelial cells of neoplastic origin and that they are free of adventitious viruses.

The MCF-7 cell line was established from a pleural effusion of a patient with a hormone responsive, disseminated malignant adenocarcinoma of the breast (Soule et al, JNCI 51:1409, 1973). It has maintained its original epithelial growth characteristics through more than 160 serial passages. Ultrastructurally, the MCF-7 cell is epithelial as evidenced by several markers including both a well-developed junctional complex system between cells and defined cell polarity with microvilli.

MCF-7 cells have a human subtetraploid karyotype, contain human plasma membrane antigens, and synthesize the human type B glucose-6-phosphate dehydrogenase isozyme. The molecular weight of the 28 ribosomal RNA of MCF-7 cells is identical to human ribosomal RNA and is clearly distinguishable from that of mouse and other animal species.

It has been demonstrated that the synthesis of an endogenous oncorna-like virus can be stimulated in these cells (McGrath et al, Nature 74:247, 1974). The characterization of this agent and its role in human mammary neoplasia is being intensively studied in our own and other laboratories.

MCF-7 cells possess specific 17 $\beta$-estradiol and progesterone receptors. The presence of these receptors is stornglY suggestive of both a parenchymal endocrine origin, and of the maintenance of a differentiative state through-out long-term cultivation. We have also demonstrated $\alpha$-lactalbumin synthes is in MCF-7 cells using a specific and sensitive radioimmunoassay for the human milk protein.

## CONTRACT AWARDS

**Title:** Prototype network demonstration project in breast cancer
**Contractor:** Univ. of Vermont, $338,823.

**Title:** Comprehensive cancer center communications network
**Contractor:** Illinois Cancer Council, $118,943.

**Title:** Studies on nucleic metabolizing enzymes associated with RNA tumor viruses, and synthesis of synthetic templates
**Contractor:** Univ. of Wisconsin, $55,144.

**Title:** Solid tumor pharmacology in laboratory animals
**Contractor:** George Washington Univ., $33,268.

**Title:** Standard protocol for evaluation of imaging techniques in cancer diagnosis
**Contractor:** Bolt Beranek & Newman Inc., Cambridge, Mass., $467,805.

**Title:** Continuation of investigations of possible correlations between morphological and epidemiological characteristics of breast cancer
**Contractor:** M.D. Anderson, $44,900.

**Title:** Studies and investigations on therapy of patients with stage II and stage III carcinoma of the breast
**Contractor:** Case Western Reserve Univ., $200,000.

**Title:** Biochemical analysis of human breast cyst fluid
**Contractor:** Sloan-Kettering, $35,191.

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