Symposium on Mammary Neoplasia

William F. Feller, M.D., Ph.D.

Introduction

An international conference on mammary neoplasia, sponsored by the Institute for Medical Research and the National Institutes of Health was held in Cherry Hill, New Jersey on November 11-13, 1971. Although the focus of the program was on experimental studies of mouse mammary tumors, a large segment was devoted to breast cancer in humans.

One of the key questions reviewed: Is human breast cancer caused by or related to an oncogenic virus? It is now well established that a specific RNA oncogenic virus plays a major role in the development of mouse mammary cancer. Recent experimental work indicates that mammary cancer in rats and monkeys may also be related to an oncogenic RNA virus. The evidence that a virus may be involved in human breast cancer rests primarily on the presence of particles in human milk which very closely resemble animal RNA tumor viruses. These electron microscopy findings have been reported by three different groups of investigators in studies of 300 milk samples. Further evidence that a specific RNA virus may be involved in human breast cancer is based on the observation that human milk samples known to contain virus-like particles also contain two biochemical markers (reverse transcriptase and 70 S RNA) which are uniquely characteristic of RNA tumor viruses.

In addition to these investigations, a number of reports and discussions also tried to assess the role of genetic and hormonal factors in the etiology of human cancer. The discovery of the enzyme, reverse transcriptase, and the understanding of its role in tumor viruses as well as normal cells may provide the link to unify the genetic and viral hypotheses of cancer.

Genetics, Epidemiology and Pathology

Two reports focused on the genetic aspects of breast cancer. From clinical and pathological data, Haagensen has been able to define six groups of women in whom the risk of breast cancer is significantly increased. These six groups include: (1) women whose breast physiology has been atypical; (2) those who have a family history of breast carcinoma; (3) women who have previously had carcinoma in one breast; (4) those who have had gross cystic disease; (5) women who have had lobular carcinoma in situ; and (6) women who have multiple intraductal papilloma. Haagensen noted that women with gross cystic disease had four times the expected incidence of breast cancer. He also reported that in a series of 53 women with lobular carcinoma in situ who were followed for 10 years, 20 percent subsequently developed breast cancer. He also noted that 14 percent of patients with lobular carcinoma in situ had mothers with breast cancer.

Dr. Feller is Associate Professor of Surgery, Georgetown University School of Medicine, Washington, D.C.

The entire proceedings of the Symposium on Mammary Neoplasia are published in the Journal of the National Cancer Institute 48: 1013-1244, April, 1972.
Although most genetic studies have reported a two- to three-fold increase in the incidence of breast cancer in first degree relatives of patients with breast cancer, Anderson's genetic study revealed that the increased risk for relatives was confined entirely to those patients with premenopausal breast cancer. Furthermore, when the malignant disease was bilateral, the risk of breast cancer for these patients' relatives was five-fold. If the patient had both bilateral breast cancer and was premenopausal, the risk for first degree relatives was nine-fold that of age-matched controls.

For the past 15 years, Bulbrook has studied the urinary steroid excretion patterns in women. He has now formulated that a long standing metabolic abnormality resulting from the hyposecretion of androgens is related to the development of breast cancer. In a prospective study of urine samples of 5,000 British women, he found low urinary androgen metabolites in those women who subsequently developed breast cancer. This low androgen secretion level was noted at least nine years before the diagnosis of breast cancer was made.

Because some studies have suggested that ductal hyperplasia may predispose to cancer, Brennan and Wolfe studied the usefulness of xeroradiography in detecting ductal hyperplasia. Comparing the results of breast biopsies with the findings of breast xeroradiography they found that they could readily detect significant degrees of ductal hyperplasia with a breast xerogram.

Paymaster reviewed the epidemiology of breast cancer in India. In Hindu women, breast cancer represented 13 percent of all cancers in women while in Parsi women breast cancer accounted for a striking 49 percent. He also noted twice the incidence of breast cancer in women who had never been married or pregnant. Recently, Moore reported finding particles resembling animal RNA tumor viruses in the milk of 40 percent of the Parsi women studied.

Ozzello, using thin section electron microscopy, showed the prominence of granular endoplasmic reticulum, ribosomes, Golgi complexes and secretory material, suggesting that human breast cancer cells perform a variety of synthetic and secretory activities. Electron microscopic studies of human breast cancer cells in culture showed that these cells retain their basic ultrastructural features even after several years.

**Virology**

Sarkar reviewed the extensive electron microscopic studies of human milk done with Moore over the past five years. They concluded that B-type particles, because of their unique surface structure, can be identified by negative staining techniques. They defined three varieties of virus-like particles: (1) One type resembles, in all details, the B-particle or mouse mammary tumor virus. This particle, which appears as an almost exact replica of the mouse virus, is found only in about five percent of all women. (2) A second type closely resembles the mouse C-type particle, or perhaps the monkey mammary tumor virus, and is seen in about one third of all American women. (3) Another type resembles the other two particles in size and morphology but has spikes on its surface different from the spikes of the mouse B-particle. Moore's group showed that human milk can damage the spikes of the mouse B-particle virus. The results of these investigations of human milk along with the work of others strongly suggest an association between human breast cancer and RNA viruses.

Dalton presented the ultrastructural detail of mouse, feline and allegedly human RNA tumor viruses. Using specific and controlled fixing and staining techniques, he showed that it was possible to distinguish C-type particles from different species by ultrastructure alone. Dalton maintained that the recently dis-
covered ESP virus is possibly of human origin and is definitely not a mouse contaminant as suggested by other investigators. In the last two years, accumulated evidence suggests that breast cancer in two other species—the rat and the monkey—may also be related to a specific oncogenic virus.

In 1970, Chopra and Mason discovered a budding RNA virus in the spontaneous breast cancer of a Rhesus monkey. This virus has been grown in quantity in both monkey and human cells and has been isolated and highly purified. Biochemically, it resembles other RNA tumor viruses in that it possesses the enzyme reverse transcriptase and a 70 S RNA. While the evidence suggests a relationship between this virus and monkey breast cancer, with no evidence of tumor formation in monkeys or other animals, and no "in vitro" transformation, the link has not been proven. Chopra also noted budding C-type particles in the R-35 rat mammary adenocarcinoma, which developed in a subline of the Huggins spontaneous fibroadenoma A during serial transplantation. This rat virus propagates in tumor cell cultures. Inoculation of the virus into newborn rats produced some mammary adenocarcinoma but in only a very few animals. The virus shares biochemical properties with other oncogenic RNA viruses including: (1) a density of 1.16; (2) the presence of 60-70 S RNA; and (3) the presence of RNA dependent DNA polymerase (reverse transcriptase). Ahmed reported seeing morphological transformation of rat mammary cells in culture after infection with R-35 virus. Interestingly, transformation in other types of cells such as rat embryo, muscle, spleen or kidney failed to occur.

It has been known for some time that the blood and spleen of mice often show viral activity one month following oral or parenteral infection with mouse mammary tumor virus long before any other tissues are involved.

Nandi discussed his recent studies which attempt to locate the mammary tumor virus activity in blood. His work suggests that the MTV activity in blood is associated with nucleated cells, most likely the hematopoietic colony forming cells found in the spleen. This conclusion was based on studies which showed that irradiation completely destroys the infectivity of blood and spleen cells.

One question long puzzling to biologists is the occurrence of mammary
tumors containing virus in supposedly "virus free" mice. Bentvelzen's experimental data strongly suggested that all mice carry genetic information necessary for the formation of the mammary tumor virus. In some situations, complete virus particles are released spontaneously; often, however, the genetic information is expressed in an incomplete form, i.e., virus antigens only or, as in blood, infectivity not associated with a mature virion. Irradiation and chemical carcinogens can induce the appearance of virus antigens in mammary epithelial cells or nucleated blood cells in so-called virus free strains. Even natural aging may allow the pertinent genetic information, necessary for virus production, to be switched on. In this latter regard, Hagemen found B-particles in the mammary glands of old "virus free" breeding BALB/C females. Cell free homogenates from these glands when inoculated into newborn BALB/C mice caused a high percentage of mammary tumors.

**Tissue and Organ Culture**

There is reason to believe that a long-term culture of human breast cancer cells might, under proper circumstances, yield an oncogenic virus. The crucial problem, unresolved at this time, is that of establishing such long-term human breast cancer cultures. Presently there are only two, or possibly three, long-term cultures of human breast cancer. However, Lasfargues outlined a new method of growing human breast cancer cells in suspension cultures by introducing three innovations: (1) a serum-free media in which all the ingredients could be autoclaved; (2) 3.5 percent polyvinylpyrolidone in the culture media, which, because of its charge properties, keeps the epithelial cells in a suspended state; and (3) a cocultivation technique utilizing a human lymphoblast cell line, NC37, which permits much longer periods of maintenance and growth of human cancer cells.

With more traditional methods, Feller and his colleagues reviewed their experience with the cell cultures of 130 breast cancer specimens. These investigators maintained 41 viable epithelial cell cultures for 30 days; 12 of these survived for 120 days. Both Lasfargues and Feller reported briefly on electron microscopic studies of their cultured human breast cancer cells, and both noted seeing a few oncornavirus-like particles in the cell material. These observations did not show budding and were not totally convincing.

Sherwin presented some unusual observations of the interactions of lymphocytes with human breast cancer cells using short term tissue culture preparations and time-lapse photomicrography. Apparently, lymphocyte interaction between normal and cancer cells is a more complex process than previously thought.

**Immunology and Interferon**

Immunological studies of the mouse mammary tumor virus system have been performed for several years. Blair attempted to elucidate the observation that immunodepression depressed MTV-induced mammary tumor development. She reported that treatment of MTV infected mice with both antilymphocyte and antithymocyte serum reduced, rather than increased, the subsequent development of mammary tumors, suggesting that components of the immune system, or their interactions, may actually facilitate MTV-induced onogenesis. Mice neonatally infected with MTV apparently do not produce cell-associated immune reactivity against MTV-associated antigens on mammary tumor cells, although they may produce humoral antibodies against MTV antigens, when immunized as adults. They can, however, respond to antigens of mammary tumor cells which are tumor-specific and apparently not virus-associated. This suggests that immune surveillance, if present in the mammary
tumor system, probably operates against tumor-specific but not virus-associated antigens.

Charney noted that formalin inactivated MTV given to four- to six-week-old noninfected mice completely protected the mice against the subsequent challenge of infective virus given one month later. As proof of protection, this study demonstrated that immunized mice did not show MTV antigen in milk from the third lactation. Similar immunization of RIII mice, which spontaneously carry the mammary tumor virus, did not significantly reduce infection but did delay tumor development by approximately one month. Charney also showed that the administration of massive doses of infective MTV resulted in immunization and reduced infection of virus-free mice.

Nowinski described the results of a series of studies, designed to elucidate the antigenic components of mouse MTV. Utilizing Tween-80 ether treatment to disrupt the virion, he identified five distinct antigens. He has shown that MTV S1 and MTV S2 are group specific and are antigens of the viral nucleoid. MTV S3 has been shown to be a structural protein of the viral membrane. Two other antigens, MTV S4 and S5, are type specific, being found in MTV of only certain strains. Nowinski also performed polyacrylamide gel electrophoresis on mouse MTV and demonstrated five major proteins of the virus. The major protein, p3, is found in the nucleoid of the virion and carries the S1 antigen.

Sibal compared the antigens of the mouse mammary tumor virus with similar antigenic components found in murine leukemia virus. He pointed out that while both MTV and mouse leukemia virus (MLV) contain reverse transcriptase activity, they appear to be biologically and antigenically distinct. The sera of C3H tumor bearing mice, have been shown to contain antibodies which precipitate the MTV virion. Several investigators have now shown that mice also respond to an internal antigen of the virion.

Although an immunological relationship between the mouse mammary tumor virus and a possible human breast cancer virus does not appear likely, sporadic observations have kept alive the hope that such may be the case. Priori discussed her experiments which seemed to support such a conclusion. She showed that sera from 58 percent of patients with breast cancer reacted positively to mouse mammary tumor cells in culture known to contain B particles (MTV). Edynak reported on immunofluorescent studies with sera of breast cancer patients on human breast cancer cells in culture. Using human sera, he demonstrated a new antigen in two primary breast cancer cultures. Cell lines derived from normal cells and from tumors other than breast were negative when tested with positive human sera. Antibody titers of 1:640 were found in the serum of 91 percent of patients with breast cancer within one week of mastectomy.

Studies have shown striking regression of spontaneous breast cancers in animals following the administration of interferon inducers, i.e., poly I:C. Cane noted that interferon treatment significantly delayed the onset of tumors in RIII mice, a strain of mice known to carry the mammary tumor virus. There was no effect on tumor development in C57 B1 mice, a virus-free strain, which had been inoculated with MTV virus shortly after birth.

**Biochemistry**

Although the role of specific RNA viruses in the oncogenesis of many animal tumors is widely accepted, the exact genetic mechanisms by which the viral RNA is expressed and causes malignant transformation in the intact animal are not clear. The recent discovery of the enzyme, reverse transcriptase, and the subsequent demonstration that it is an in-
tegral part of all RNA tumor virions has opened a new field of investigation. If human breast cancer is caused by a specific virus, Spiegelman believes that genetic information in the tumor cell that is being actively transcribed should correspond to genetic information within the virus particle. Spiegelman developed a probing technique which can demonstrate nucleotide sequences related to viral RNA in tumor cells. This technique utilizes a specific product DNA which reflects the genetic sequence of mouse mammary tumor virus RNA. Using this viral derived 'mouse DNA probe,' he can detect evidence of some genetic relatedness when he hybridizes the mouse probe DNA with both polosomal and nuclear RNA from human breast cancer cells. This indicates that genetic information that is being transcribed in the cancer cell is complementary to at least part of the genetic information contained in the RNA of the mouse mammary tumor virus. Spiegelman believes that scientists now have a technique for looking at a human virus in human tissue. If enough human virus particles could be obtained from human milk to make a 'human DNA probe,' this technique should demonstrate the role of viral genetic information in human breast cancer.

Schlom discussed a new oncogenic RNA virus assay method which simultaneously detects reverse transcriptase and 60-70 S RNA, and thus increases the accuracy of interpreting positive results.

The test itself relies on oncogenic RNA viruses exhibiting two unique biochemical properties: (1) a high molecular weight 70 S RNA; and (2) reverse transcriptase, an enzyme capable of employing the viral RNA as a template to generate a DNA complementary copy. The assay was used to detect several known RNA oncogenic viruses including mouse leukemia virus in plasma, and cat leukemia virus in tissue culture fluid. This assay method could readily detect mouse MTV in milk and also in blood.

Gallo presented data suggesting that reverse transcriptase activity is required for the initiation of malignant transformation by RNA tumor viruses. He found a direct correlation between the inhibition of focus formation by murine sarcoma virus and the inhibition of reverse transcriptase by different classes of rifampicin derivatives. These studies suggest that reverse transcriptase must play some critical role in the malignant transformation process.

Das reported on some biochemical studies of milk from Indian women of the Parsi community. Essentially he found the milk of four out of 12 women studied had RNA dependent DNA polymerase activity. He attempted to characterize the nature of the DNA product formed by the milk RNA/reverse transcriptase and he found that the DNA product was attached to RNA sedimenting in either the 70 S or 30-45 S region.

Temin, one of the discoverers of the RNA-dependent DNA polymerase added a note of caution about over-interpreting experimental data concerning reverse transcriptase. He made two points: (1) not all RNA viruses that contain the enzyme reverse transcriptase are transforming (cancer) viruses; and (2) the presence of the enzyme in a particle does not necessarily identify it as a virus. His first point is generally accepted since at least three or possibly four nontransforming viruses are known to contain reverse transcriptase. His second point, also generally accepted, would not appear to invalidate the work of Schlom and Spiegelman on the human milk particles; their modified assay of "milk particles" uses two markers—a 70 S RNA and the reverse transcriptase. Currently, the presence of a high molecular weight 70 S RNA is generally accepted as a unique property of certain kinds of RNA viruses.
Endocrinology and Therapy

It has long been known that certain hormones have a profound effect on the growth and development of breast cancer in nearly all mammals studied. Even in mice where a virus is known to be a critical etiological factor, hormones still appear to play a decisive role. The two hormones most strongly implicated in the development of breast cancer are prolactin and estrogen. Muhlbock’s studies support the view that prolactin is the dominant hormone in murine mammary tumor development. He and his colleagues have shown that estrogenic hormones act mainly as an inciter for prolactin production and that progesterone is active only in the presence of prolactin, both hormones acting synergistically. Whether hormonal stimulation alone can produce mammary tumors in mice has not been resolved, although many so-called MTV free strains of mice will develop mammary tumors after hormonal stimulation. If the virus is present as a provirus in all mice, as Bentvelzen maintains, one cannot rule out some role for the MTV genome. Observations from Muhlbock’s work on mammary gland transplantsations have shown that the genetic susceptibility is localized in the mammary gland itself. Genetic factors may alter the susceptibility of the mammary gland to hormonal action, or to the virus, or may allow an easy transformation from a normal to a malignant cell.

Meites reviewed his work and that of others concerning the relationship of prolactin and estrogen to mammary tumor growth in rats. Estrogen is known to stimulate prolactin secretion and acts with prolactin directly on the mammary tissues to promote tumorigenesis. Large doses of estrogen can inhibit mammary tumor growth, not by suppressing prolactin secretion but by interfering with the peripheral action of prolactin on mammary tissue. Estrogen can neither induce nor maintain growth of mammary tumors in the absence of the pituitary gland, but prolactin may have a limited capacity to induce and maintain mammary tumor growth in the absence of the ovaries. Thus, it seems that in rats, as in mice, prolactin plays a very important, if not the key, role in the development of mammary tumors.

If the genetic loci being transcribed in neoplastic mammary cells is different from those transcribed in normal mammary cells, the messenger RNA in carcinoma cells would be different and would presumably consist of different nucleotide sequences. Turkington described his experimental studies which tested this hypothesis. Using RNA-DNA hybridization and hybridization competition, he compared the rapidly labeled RNA population of normal and neoplastic cells. He showed an incomplete competition of the normal cell RNA tested against a radioactive labeled mammary cancer RNA—homologous DNA hybridization as compared to the nuclear RNA from the cancer itself. He inferred from these studies that normal and neoplastic mammary cells differ with respect to the specific genes which are actually being actively transcribed.

Pearson speculated on the role of prolactin and estrogen in the development of human mammary cancer. He pointed out that ablation of the ovaries, adrenal glands and pituitary gland can induce significant regression of breast cancer in some women. Pilot studies have shown that following an oophorectomy-induced remission, estrogen administration could reactiviate tumor growth. However, after hypophysectomy-induced regression, estrogens failed to reactivate tumor growth, suggesting that a pituitary factor was involved in breast cancer growth.
Symposium on Mammary Neoplasia

List of Participants

M. Ahmed, Ph.D.
The John L. Smith Memorial for Cancer Research
Pfizer, Inc.
Maywood, New Jersey

David E. Anderson, Ph.D.
Department of Radiology
The University of Texas M. D. Anderson Hospital and Tumor Institute
Houston, Texas

P. Bentvelzen, D.Sc.
Radiobiological Institute TNO
Rijswijk (Z. N.)
The Netherlands

Victor V. Berga, Ph.D.
Department of Microbiology
University of Miami School of Medicine
Miami, Florida

Phyllis B. Blair, Ph.D.
Department of Bacteriology and Immunology
and the Cancer Research Genetics Laboratory
University of California Berkeley, California

Michael J. Brennan, M.D.
Michigan Cancer Foundation
Detroit, Michigan

R. D. Bulbrook, M.Sc., Ph.D.
Imperial Cancer Research Fund
London, England

Paul E. Card, Ph.D.
Scherer-Pugh Corporation
Bloomfield, New Jersey

Jesse Charnay, M.S.
Institute for Medical Research
Camden, New Jersey

Harish C. Chopra, Ph.D.
National Cancer Institute
Bethesda, Maryland

Albert J. Delton, Ph.D.
National Cancer Institute
Bethesda, Maryland

M. R. Das, Ph.D.
Tata Institute of Fundamental Research
Bombay, India

Eugene M. Edyvan, M.D.
Department of Surgery
Hospital of the University of Pennsylvania
Philadelphia, Pennsylvania

William F. Eiler, M.D., Ph.D.
Georgetown University School of Medicine
Washington, D.C.

Jaco R. Furt, M.C., D.Sc.
Institute of Cancer Research and Department of Pathology
Columbia University New York, New York

Robert C. Gallow, M.D.
Laboratory of Tumor Cell Biology
National Cancer Institute Bethesda, Maryland

C. D. Haagenen, M.D.
Columbia-Presbyterian Medical Center
New York, New York

Philoema Hageman, Ph.D.
Mammary Tumor Virus Research Unit
Anton van Leeuwenhoekhuis
The Netherlands Cancer Institute
Amsterdam, The Netherlands

G. H. Hegner, Ph.D.
Division of Bio-Medical Science
Brown University and Department of Medicine
Roger Williams General Hospital Providence, Rhode Island

Jo Higjera, D.Sc.
Department of Biology
The Netherlands Cancer Institute Amsterdam, The Netherlands

K. H. Hoffmann, Ph.D.
Laboratoire de cytopathologie Hospital Broussais
College de France Paris, France

Robert L. Kassel, Ph.D.
Division of Bio-Medical Science
Brown University and Department of Medicine
Roger Williams General Hospital Providence, Rhode Island

Elianne Lastargues, D.V.M.
Department of Cytological Biophysics
Institute for Medical Research
Camden, New Jersey

Richard P. Mason, M.D.
American Cancer Society, Inc
New York, New York

Joseph Meltzer, Ph.D.
Department of Physiology
Michigan State University East Lansing, Michigan

Dan H. Moore, Ph.D.
Institute for Medical Research
Camden, New Jersey

Otto Mulliken, M.D.
The Netherlands Cancer Institute Amsterdam, The Netherlands

S. Hand, Ph.D.
Department of Zoology
and its Cancer Laboratory
University of California Berkeley, California

Robert C. Nowinski, Ph.D.
McArdle Laboratory for Cancer Research
University of Wisconsin Madison, Wisconsin

Teakam Okh, Ph.D.
National Institute of Arthritis and Metabolic Diseases
Bethesda, Maryland

Luciano Ozzello, M.D.
Section of Surgical Pathology
Michael Reese Hospital and Medical Center Chicago, Illinois

and Department of Pathology
University of Chicago Chicago, Illinois

J. C. Peaymaster, M.D.
Tata Memorial Centre
Bombay, India

Olaf Pearson, M.D.
Cleveland, Ohio

Elizabeth S. Priori, Ph.D.
Departments of Virology and Biology
The University of Texas M. D. Anderson Hospital and Tumor Institute
Houston, Texas

Nural K. Sarkar, Ph.D.
Institute for Medical Research
Camden, New Jersey

George Shchidlovsky, B.S.
The John L. Smith Memorial for Cancer Research
Pfizer, Inc.
Maywood, New Jersey

Jeffrey Schloim, Ph.D.
Institute of Cancer Research
College of Physicians and Surgeons
Columbia University New York, New York

Kerri V. Shah, M.D., Ph.D.
Department of
The Johns Hopkins University School of Hygiene and Public Health
Baltimore, Maryland

Russell P. Sherrin, M.D.
Department of Pathology
University of Southern California School of Medicine
Los Angeles, California

Louis R. Sibal, Ph.D.
Viral Oncology Area, Etiology
National Cancer Institute
Bethesda, Maryland

Dharm Vir Singh, Ph.D.
Michigan Cancer Foundation
Detroit, Michigan

Joseph G. Sinkovics, M.D.
Section of Clinical Tumor Virology and Immunology
and Medical Breast Service
Department of Medicine
The University of Texas M. D. Anderson Hospital and Tumor Institute
Houston, Texas

Soti Spiegelman, Ph.D.
Institute of Cancer Research
College of Physicians and Surgeons
Columbia University New York, New York

Howard Ternin, Ph.D.
McArdle Laboratory for Cancer Research
University of Wisconsin Madison, Wisconsin

Roger W. Turckington, M.D.
Department of Medicine
University of Wisconsin Medical Center
Madison, Wisconsin

John Wolfe, M.D.
Michigan Cancer Foundation
Detroit, Michigan