Breast Cancer Research: Problems and Progress

During the past 4 months the Research News section has published a series of articles surveying some of the more significant developments in areas of biomedical research related to cancer, from theories of carcinogenesis to trends in therapy. The concluding two articles will examine how this research is being applied to two specific types of cancer.

Breast cancer is the third most prevalent type of cancer in the United States, according to figures compiled by the National Cancer Institute (NCI) in Bethesda, Maryland. An estimated 90,000 new cases and 33,000 deaths as a result of this disease will occur in 1974. The fact that the breast cancer death rate has remained essentially constant for the last 35 years provides a stimulus for ongoing research into the causes, detection, and treatment of the disease. Although fundamental questions remain unanswered, investigators are encouraged by recent developments, especially in the areas of detection and chemotherapy.

Much of the breast cancer research is performed under the egis of the Breast Cancer Task Force (BCTF) of NCI. The BCTF program is the oldest (since 1966) and most extensively funded ($7.1 million in fiscal year 1974) of the "organ site" projects of NCI. These projects are directed at gathering and applying the information necessary for improving the survival of patients with a specific cancer. Virtually all of the research sponsored by the BCTF is targeted—intended to achieve specific research goals—and funded by contracts.

Since more than 99 percent of breast cancer victims are women, it is estimated that one woman out of every 15 will develop the disease sometime in her lifetime. One of the research goals is the identification of those women who are at greatest risk from breast cancer. Epidemiological studies have identified a number of risk indicators. As with other cancers, several biological, environmental, genetic—and, possibly, viral—factors appear to interact in the etiology of breast cancer.

The incidence of breast cancer is five to six times higher in North America and northern Europe than it is in most of Asia and Africa. Among the explanations advanced to account for these geographic variations are differences in genetic and environmental factors, including diet. In high-risk areas the incidence increases with age. Although there is a plateau or even a dip in the incidence curve at about the age of menopause, breast cancer, like most other cancers, is primarily a disease of old age.

Breast cancer appears to run in families. According to figures supplied by the American Cancer Society (ACS), daughters and sisters of breast cancer patients are two to three times more likely to develop the disease than are women not related to breast cancer patients. David Anderson of M. D. Anderson Hospital and Tumor Institute in Houston, Texas, found that the risks were even higher—six to nine times the general risk—if the patient's disease occurred before menopause and involved both breasts. As with most other situations of this type, both heredity and environment could be involved, and sorting out their relative influences is difficult.

Pregnancy and Breast Cancer

A woman's reproductive history is one of the biological factors that determine her chances of developing breast cancer. According to Brian MacMahon of the Harvard School of Public Health, Boston, Massachusetts, the risk increases with the age at which she has her first full-term pregnancy. Pregnancy before age 30 is protective in the sense that these women are less likely to develop breast cancer than are women who have never had a child. Having a first child after 30 increases the risk compared to that of nulliparous women. Although no one knows the mechanism by which reproductive history influences susceptibility to breast cancer, the female sex hormones are probably involved.

Other evidence has also implicated these hormones in mammary carcinogenesis. MacMahon and others have found that both early onset and late cessation of menstruation are associated with an increased risk of breast cancer. Menstruation, of course, depends on the production of the ovarian hormones estrogen and progesterone. Although estrogens especially are known to stimulate the division of sensitive cells—and cancer is characterized by uncontrolled cell division—the exact role of hormones in breast cancer etiology is unclear.

A question frequently raised in conjunction with the relationship between hormones and breast cancer is what effect—if any—contraceptive pills have on the risk of developing the disease. According to Heinz Berendes of the Center for Population Research of the National Institute of Child Health and Human Development, none of the studies conducted thus far show any association between contraceptive pills, most of which contain both estrogens and progestogens, and breast cancer. Most of the studies have been retrospective, but one recent prospective study, conducted by Clifford R. Kay for the Royal College of General Practitioners in Great Britain, also showed no correlation. Nevertheless, Berendes points out that the results of these studies must be interpreted cautiously. The "pill" has been in widespread use for less than 10 years whereas the estimated time period required for the development of most cancers, including breast cancer, is 15 to 20 years.

In addition to the ovarian hormones, those of the adrenal and pituitary glands have been implicated in mammary oncogenesis. Recently, attention has been focused on prolactin, a hormone secreted by the pituitary gland and required for lactation. Evidence acquired by Clifford Welsch of the College of Human Medicine, Michigan State University, East Lansing, indicates that this hormone may be essential for breast cancer development in mice and rats. Welsch also found that addition of prolactin to cultures of malignant human breast tissue stimulated growth of the tissues. A number of investigators are exploring the role of prolactin in the growth and maintenance of human breast cancers.

Viruses play a prominent role in any discussion of the etiology of breast cancer. They have been known as a cause of mammary cancer in mice

since 1936 when the Bittner or mouse mammary tumor virus (MMTV) was discovered. The MMTV is an RNA virus of type B (*Science*, 22 March, p. 1181). Many investigators think that a similar virus is involved in the etiology of human mammary cancer. Although their evidence indicates that such a virus may exist, definitive proof remains elusive—just as it does for all viruses implicated in the etiology of human cancers.

Human milk may be one source of viral particles. For example, Dan Moore and his colleagues at the Institute for Medical Research, Camden, New Jersey, observed particles with the morphological properties of type B viruses in electron micrographs of human milk samples. The presence of the particles did not, however, correlate with the donor's family history of breast cancer.

Substances present in human milk can destroy virus particles. Moore and others have found that human milk causes the breakdown of MMTV. The mechanism of virus damage is not yet known, but it undoubtedly interferes with attempts to detect virus particles by electron microscopy.

Biochemical evidence also indicates the presence of oncogenic RNA viruses (oncornaviruses) in human milk. Such viruses are characterized by their density (1.16 to 1.19 grams per milliter), by the presence of 60*S* to 70*S* RNA (*S* is the symbol for the Svedberg unit, a measure of the rate at which a material sediments in the ultracentrifuge), and by the presence of the enzyme reverse transcriptase. This enzyme synthesizes DNA copies of viral RNA and is necessary for the function of oncogenic RNA viruses. Sol Spiegelman of the Columbia University Medical School, New York, and Jeffrey Schlom, now at NCI, with Moore, found particles with these properties in human milk samples.

In addition to substances that destroy virus particles, human milk contains the enzyme ribonuclease, according to Marvin Rich, Michael Brennan, and their colleagues at the Michigan Cancer Foundation, Detroit. This enzyme interferes with biochemical tests for oncornaviruses which depend on determining the activity of reverse transcriptase because it destroys the RNA template needed for DNA synthesis. Rich said that reverse transcriptase can still be detected if its concentration is high as compared to that of ribonuclease. Nevertheless, the presence in human milk of these interfering substances has complicated attempts to correlate the presence of virus particles with risk of contracting breast cancer.

The presence of particles resembling known oncogenic RNA viruses in human milk does not prove that these particles are involved in the etiology of human cancer. There is no evidence that human mammary cancer is transmitted, as it is in certain strains of mice, by an agent in milk. In fact, although breast-feeding has declined dramatically in the United States in the last 50 to 60 years, the incidence of breast cancer has not decreased and may have increased slightly.

Other evidence implicating viruses in the etiology of breast cancer does not depend on the use of human milk. Spiegelman and Schlom used reverse transcriptase to synthesize DNA complementary to MMTV. They showed that 66 percent of 29 human breast tumors contained RNA that hybridized with this DNA. This indicated that the tumor RNA is itself complementary to the DNA and must therefore have base sequences and information content similar to that of a known oncogenic virus.

Oncornaviruses in Cultured Cells

Rich and his colleagues found a virus with the characteristics of an oncornavirus replicating in a line of cultured cells derived from a patient with carcinoma of the breast. The cultured cells are of human epithelial origin. (Carcinomas are cancers of epithelial cells.) They have certain properties of mammary cells including the presence of receptors that bind estrogen and the ability to synthesize lactalbumin.

Although Rich and his associates have just begun characterization of the virus, preliminary evidence indicates that it may be of human origin. The relationship of this virus to the particles found in human milk and to the etiology of human breast cancer is under investigation. Because of the dismal history of some other "human cancer virus candidates" that have come—and then quickly gone—the investigators are understandably cautious about the meaning of their findings. In any event, a virus caught at the scene of the crime is not necessarily the culprit.

Most investigators think that viruses, if they are involved at all, are necessary but not sufficient causes of mammary carcinogenesis. If they are necessary, then preventive measures based on interference with viral function may be possible. Moore and his colleagues can prevent mammary tumors in mice with a vaccine made from killed MMTV. Such an approach is not feasible for human use because it requires administration of nucleic acid from an oncogenic virus. A vaccine for human use would have to contain only such viral constituents as proteins or glycoproteins. A different preventive strategy, the use of drugs to inhibit viral expression or the activity of reverse transcriptase, is under investigation in a number of laboratories.

Until prevention of cancer becomes a reality, control of breast cancer requires that it be detected and then treated successfully. As with other types of cancer, early detection—before the cancer metastasizes to other parts of the body—is thought to be a prerequisite for successful treatment. Sixty percent of breast cancer patients live for 5 years after their disease is diagnosed. But large differences in survival are apparent between patients with localized disease and those with disseminated disease. Eighty percent of the former live 5 years or longer whereas only 45 percent of the latter do. Thus, many clinicians think that screening (periodic examinations of large numbers of apparently healthy women by trained personnel) might increase the detection of early breast cancers—and improve the survival rate.

One of the largest studies conducted thus far showed that mass screening by clinical examination or manual palpation of the breasts, in conjunction with mammography, can reduce the mortality from breast cancer. Mammography is low-voltage x-ray examination of the breast, with the image recorded on conventional x-ray film. The study, conducted by Philip Strax of the New York Medical College, began in 1963 and includes 62,000 women between the ages of 40 and 64 years. The women, who are members of the Health Insurance Plan of Greater New York, were selected as 31,000 matched pairs, one set of which was the study group and the other the control group. Of the study group, 65 percent agreed to be screened for breast cancer and to have three subsequent annual check-ups.

According to Strax, the death rate of the study group from breast cancer was one-third lower than that of the control group after 5 years. Seventy percent of the patients whose cancers were detected by screening were free of metastases. The comparable figure for the control group was 46 percent. Moreover, the study demonstrated the

utility of mammography in screening: 44 cancers (33 percent of the total) not found by palpation were detected by the x-ray technique. Strax said that only one of the 44 women whose cancers were detected by mammography alone has died of her cancer. On the other hand, 59 cancers not found by mammography were detected by clinical examination. Thus, both techniques contributed to detection of the cancers.

One of the disadvantages of mammography for periodic screening of healthy women is that x-rays are themselves carcinogenic. Use of fast x-ray film has decreased the radiation dose to about 1 to 2 rads (a rad is a measure of the radiation energy absorbed by exposed material) per exposure. Variations of mammography such as xeroradiography, in which a different system is used for recording the x-ray image, also permit a lower radiation dose than older mammographic techniques.

Thermography entails no radiation at all. Instead, an infrared detector is used to produce a photograph of the skin's heat pattern. Cancers are indicated by the presence of "hot spots" due either to the altered metabolism of the tumor or to its increased blood supply. Thermography is not specific for cancer because other, benign, conditions may also produce such hot spots. But it can be used for screening with additional tests used as necessary to confirm the diagnosis.

The ACS sponsored a collaborative pilot study involving several medical centers to evaluate the potential of thermography in cancer detection. The results of the study showed that thermography detects cancer not found by palpation in addition to some not detectable by mammography. Thus, each of the three techniques in greatest use —palpation, mammography, and thermography—detects different groups of cancers. The ACS and NCI now sponsor 27 breast cancer detection centers where women can obtain free examinations. All three techniques are used at the center, and breast self-examination is also taught. At each of these centers, at least 5000 women will be examined every year. There are, however, approximately 40 million women over 40 in the United States. Although both NCI and the ACS stress the importance of early detection, monetary and personnel limitations prohibit extension of screening to all of them.

Once localized breast cancer is detected, the primary treatment is surgery sometimes accompanied by radiation therapy. Cancers too extensive to remove surgically are usually treated with radiation or chemotherapy and often by removal of one or more of the three glands—ovaries, adrenals, or pituitary —known to influence tumor growth. The therapies for advanced disease are palliative, not curative.

For many years, a controversy has raged over how extensive the surgery must be for successful treatment of breast cancer. The Halsted radical mastectomy, the most commonly used operation, entails removal of the entire breast, the underlying chest muscles, and the lymph nodes in the axilla (under the arm). In the simple or total mastectomy, the entire breast is removed. In limited procedures such as the "lumpectomy" or segmental mastectomy, the tumor plus a varying amount of surrounding tissue is removed

Radical Surgical Procedures

Proponents of the more radical procedures think that they are necessary to eliminate all the cancerous tissue and prevent recurrence of the disease. The lymph nodes are removed because cancer is thought to spread first to the nodes and from there to distant sites in the body.

Proponents of the less extensive procedures think that the more radical procedures do not necessarily increase survival. They question the soundness of routine removal of unaffected lymph nodes since the nodes produce lymphocytes needed for cellular immunity which is thought to be one of the body's major defenses against cancer. The radical procedures are also more traumatic both physically and psychologically.

Many investigators and clinicians, such as Bernard Fisher of the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, think that the current data are not adequate to resolve the controversy, which continues unabated. According to Fisher, since the data do not justify the conclusion that any of the procedures is any better or worse than any other, a clinical comparison of them is needed. Such a prospective, randomized clinical trial is now being conducted by the National Surgical Adjuvant Breast Project (NSABP) of which Fisher is chairman.

The study began in August 1971 and will encompass about 1700 patients by August of this year. Those without node involvement, as determined by clinical examination, will be randomly divided among three therapeutic modes: radical mastectomy, total mastectomy, or total mastectomy plus radiation of the nodes. Those who do have node involvement will have either a radical mastectomy or a total mastectomy plus radiation. Fisher estimates that preliminary data on the efficacies of the treatments as measured by the number of recurrent tumors in the different groups will be available within 6 months to a year.

Surgery and radiation have probably reached the limits of their effectiveness. Paul Carbone of NCI points out that approximately 30 percent of breast cancer patients are alive and free of disease 10 years after diagnosis—and this figure has remained constant for 35 years. These treatments can eradicate localized tumors but they cannot eliminate tumor cells or microscopic tumors harbored in other parts of the body. Only systemic treatments can reach these incipient tumors.

The most common systemic treatments now used for breast cancer are hormonal. They are employed only for recurrent or advanced inoperable disease. Some breast tumors depend on hormones such as estrogen for growth and maintenance. Removing the source of the hormone (the ovaries before menopause or the adrenals afterward) or preventing its action by the use of androgens (male sex hormones) can slow tumor growth or even cause it to regress. Since less than half of the tumors respond to such therapy, the problem is determining which are responsive so that the other women can be spared unnecessary surgery.

An in vitro test currently undergoing clinical study may enable physicians to do just that. Elwood Jensen and his colleagues at the University of Chicago Medical School developed the test, which is based on the presence or absence of estrogen receptors in tumor cells. Estrogens, like other steroid hormones, must bind to these receptors before they can exert their effects on the cells. If the tumors lack receptors, they cannot respond to the hormones.

Jensen and his colleagues measured the estrogen-binding capacity of tumor samples taken during surgery or for biopsy. They found that in 26 of 36 patients whose cancer contained receptors, the tumor regressed in response to endocrine therapy. Of 43 patients whose tumors did not bind estrogens, only one responded. Thus, most

patients with receptor-containing cancers benefit from hormone therapy, but there is little chance of benefit if the tumor cells lack receptors.

Chemotherapy is a systemic therapy that, until recently, was used only as a last resort for treating the most advanced cases of breast cancer. The current trend is for chemotherapy to be used as an adjunct to the primary treatment even though the patient may be clinically free of disease. The goal is the elimination of microscopic disseminated tumors that are not yet clinically apparent. In addition, clinicians are turning to drug combinations that are more effective than the agents administered separately.

In a preliminary study, George Cannellos and his colleagues at NCI found that a combination of four drugs—methotrexate, 5-fluorouracil, cyclophosphamide, and prednisone—caused regression of the tumors of 23 out of 33 patients with advanced metastatic breast cancer. Seven patients had complete remissions. The median survival time of those who responded to the drug combination was at least double that of the nonresponders. In a more extensive collaborative study conducted by the Eastern Cooperative Oncology Group, consisting of 39 medical institutions in the eastern United States, the effectiveness of a single agent, phenylalanine mustard, was compared with that of a combination of three drugs. The combination produced both a greater response rate and a longer duration of response than did the single agent.

The NSABP is now coordinating clinical trials of phenylalanine mustard as an adjuvant to surgical treatment of breast cancer. If the single drug improves the prognosis of the patients, Carbone says that combination chemotherapy, which has proved superior for treating advanced cases, can also be tested as a surgical adjuvant.

Adjuvant chemotherapy, which may have hazardous side effects, may not be necessary for all patients. Carbone noted that patients whose breast cancer has not yet spread to the lymph nodes already have a good prognosis. In fact, the extent of nodal involvement is currently the best prognostic indicator, and this must be determined by examination of nodes removed during surgery since some of the involved nodes are not clinically detectable. This is another reason for performing the more extensive surgical procedures which enable evaluation of the condition of the lymph nodes.

Other prognostic indicators, not requiring surgery, may eventually be available, however. Douglas Tormey and his associates at NCI found that more than 96 percent of patients with metastatic breast cancer have one or more of three biochemical markers—carcinoembryonic antigen, methylated guanosine, or human chorionic gonadotropin—in their blood or urine. Markers such as these may permit the identification of patients with a high risk of cancer recurrence without surgical removal of lymph nodes. Ultimately, Carbone said, clinicians hope to use a battery of diagnostic and prognostic tests to design the best treatment for each patient.—JEAN L. MARX

The Long and Short of Lasers (II): The Vacuum Ultraviolet

As in the far infrared region of the spectrum (*Science*, 7 June, p. 1062), the appearance of bright, coherent, and sometimes tunable sources in the vacuum ultraviolet (wavelengths between 100 and 2000 angstroms) is expected to open new avenues of research in spectroscopy and other studies. Photochemical studies of chemical reactions could be made because the energy of many chemical bonds falls in the short wavelength range of the vacuum ultraviolet. Holographic studies of objects that are too small to be resolved by visible wavelength lasers may find application in biological and medical science. High power vacuum ultraviolet lasers potentially have space weapons applications because the short wavelength of their light more easily penetrates (than longer wavelength light) the plasma created around a metal subjected to irradiation by a laser. This property also will be useful for studying the characteristics of the dense plasmas of controlled fusion experiments.

Interest in the vacuum ultraviolet began to accelerate in 1970 when R. W. Waynant, J. D. Shipman, Jr., R. C. Elton, and A. W. Ali at the Naval Research Laboratory, Washington, D.C., and R. T. Hodgson at the IBM Thomas J. Watson Research Center, Yorktown Heights, New York, independently obtained laser action in molecular hydrogen gases. The first laser light from hydrogen was a series of discrete lines near 1600 Å (Lyman band), due to transitions between vibrational levels of an excited electronic state and the ground state. Subsequently, other laser lines at shorter wavelengths (Werner band) were identified in hydrogen, and laser emission was also seen in deuterium and in carbon monoxide.

The Navy scientists applied a method of pumping the laser medium known as traveling wave excitation. In the case of hydrogen, the gas is contained in a long tube, and a high power electrical pulse is applied transversely across one end of the tube to pump the hydrogen into excited states. A sequence of these excitation pulses is applied along the tube in such a way that the excitation proceeds down the tube at the speed of light (traveling wave). Thus, as the light pulse emitted travels from one end of the tube to the other, excited gas molecules are waiting to be stimulated to emit more light by the traveling light pulse (amplification). This method of excitation is necessitated by the very short time before the excited molecules decay, since excited molecules at the far end of the tube could decay before the light pulse from the other end arrived if the entire tube of gas was excited simultaneously. In this arrangement, no mirrors are used, hence there is no optical cavity and no true laser oscillator. This "one pass laser" mode of operation is variously described as superradiance, superfluorescence, and amplified spontaneous emission.

A vacuum ultraviolet laser with a noble gas as the laser medium offers a substantially higher power than can now be obtained from the hydrogen laser (up to 100 megawatts compared to 2 megawatts), and also a limited degree of tunability. A noble gas under high pressure, such as xenon under as much as 20 atmospheres, is excited by bombarding it with a pulse of high energy (relativistic) electrons. Some of the xenon atoms are ionized, but eventually most of this xenon is left in an excited atomic state. Subsequently, an excited xenon atom and one still in the ground state react to form an excited xenon dimer (Xe_2^*), which is only stable in the excited state (excimer). Lasing