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SUMMARY AND REPORT

Report of the Scientific Committee

Part I of this report states the findings, sets forth recommendations, and delineates the "means and measures necessary to facilitate success in the treatment, cure and elimination of cancer—at the earliest possible date" as required by S. Resolution 376. A principal finding by the Panel of Consultants is that "advances in the fundamental understanding of cancer in the past decade have opened up far more promising areas for major advances in cancer prevention and treatment than have ever before existed."

Part II outlines the status of current work in the fields of cancer prevention, detection, treatment, and research and marks out areas which have special promise for intensive exploration and exploitation. A Summary of Part II precedes the more detailed report.

In addition, there is in preparation further material developed by the Panel, which includes much valuable background material including a historical review of cancer research, work being done by Government agencies and private organizations, the growth and funding of categorical institutions, and state laws and regulations pertaining to cancer research.

SUMMARY OF REPORT

In 1969, 323,000 persons died from cancer in the United States. Deaths from cancer were greater than all U.S. battle deaths in the four years of World War II and four times the sum total of battle deaths in the three years of the Korean War and six years of the war in Vietnam. Of the 200 million people now living in the United States, approximately 50 million will develop some form of cancer and, unless better methods of treatment and cure are developed, 34 million of those now alive will die of cancer. The rate of increase in deaths from cancer is more rapid than the rate of increase in the population. Most of this increased mortality is a result of the rise in deaths from lung cancer among males in the past 30 years. Although one usually thinks of cancer as a disease of maturity and old age, it is also the largest cause of death—except for accidents—among persons between the ages of 1 and 35.

There are no complete figures on the economic impact of cancer in this country. It is estimated, however, that direct costs for hospitalization and medical care, coupled with losses of productivity and earning power, are probably in the range of $10 to $15 billion per year. In spite of these appalling facts, the 1969 allocations in our national budget, per citizen, were $410 for national defense and only 89¢ for cancer research.

Present Status of Cancer Treatment

It has been estimated that there are now 1,500,000 individuals who are alive and well more than five years after treatment from cancer and that another 700,000 who have been treated within the past five years are alive and well. Whereas, in 1930, only one in five individuals with cancer survived five years after treatment, the figure has since risen to one in three, and under optimum circumstances, one in two may be cured. This improvement in cure rate is tangible evidence of the beneficial impact of the many advances in diagnosis and treatment which have accrued from the nation's investment in cancer research in the past 25 years.

THE AVENUES OF ADVANCE

There are four chief avenues of advance toward the conquest of cancer:

1) *Cancer prevention*—by the elimination from the external and internal environment of chenical and other agents that cause or promote cancer.

2) *Earlier cancer detection*—through improved methods, whose application will allow earlier curatuve therapy.

3) *More effective application of present treatment methods*—by making more widely available the best present techniques of surgery, radiation, and chemotherapy.

4) *Development of new methods of cancer treatment and prevention*—through research. It is the judgement of the Panel that major gains in the effort to eradicate cancer will come from new developments achieved through research.

Cancer prevention

Cancer prevention offers greater possibilities for the control of cancer and the saving of human lives than any other measure now at hand. Many, perhaps most, human cancers can now be prevented. The most important environmental causal agent in the production of internal cancer today is the prolonged inhalation of cigarette smoke. Cigarette smoking is now recognized as the major cause of lung cancer and a significant factor in the causation of cancer of the oral cavity, pharynx, and larnynx. Other cancers that can now be prevented include: cancers of the skin from prolonged overexposure to sunlight, to ultraviolet lamps, to arsenic, to coal tar, and to certain oils and chemicals; cancers of the bladder in workers exposed to betanaphythylamine; cancers of the lung in miners in uranium, chromate and asbestos mines; cancers of the nasal sinuses in nickel miners; and leukemias and thyroid cancer from excessive radiation. Studies of the differences in the geographic distribution of various cancers throughout the world should lead to the identification of other specific and presumably avoidable carcinogens in the environment.

Cancer detection

If cancers are discovered when they are still localized, the majority of them can be cured by surgery and radiotherapy. The past decade has witnessed striking innovations in the development of methods and instruments for cancer detection, as well as the perfection of established procedures. Exfoliative cytology is extremely valuable in detecting early cancer of the cervix and also in studying high-risk populations for cancer of the lung, bladder, and stomach. It may soon be applicable to cancers of the colon, kidney, and oral cavity.

Diagnostic radiology also contributes to cancer detection by means of chest x-rays, G.I. series, and barium enemas, and also by mammography, which is the examination of the breast by special x-ray techniques. Significant progress has been made over the past few years in endoscopic examinations of the esophagus, stomach, colon, and bladder as a means of early detection of cancer. An immunologic test for early cancer detection has been reported in the past year. This test involves a fetal antigen, called carcino-embryonic antigen (CEA), which is present in the blood of patients with cancer of the colon and disappears from the blood of patients treated successfully. Several other such fetal antigens are being investigated for their diagnostic significance in primary cancer of the liver and other human cancer.

Cancer treatment

Surgery

Today surgery offers patients with many types of cancer more chance for a cure than any other known treatment and is the most widely used form of cancer therapy. To cure, surgery must take place before the cancer has spread and become established beyond the tissues that can be removed. Good surgical treatment is now able to cure by the five-year criteria at least 95 percent of patients with skin cancers, about

70 percent of women with cancer of the uterus, 60 percent of women with breast cancers, and 40 percent of patients with cancers of the colon and rectum. Relatively few patients, however, with cancer of the lung, stomach, or pancreas are being cured. In virtually every category, there is a striking difference in cure rate between patients with localized cancer in whom the regional lymph nodes have not become involved and patients in whom regional lymph node involvement is present. Organ transplantation may allow wider surgical excision and so increase the possibility of cure where cancer involves vital organs. The potential of this technique in cancer control is just beginning to be realized. Surgical techniques, organ preservation, tissue typing, and immunosuppression now have been developed to the point where replacement of vital organs irreparably damaged by cancer is occasionally possible, but better techniques of immuno-suppression are urgently needed.

In the future, prevention of cancer by the removal of precancerous growths may eventually be surgery's greatest contribution to the control of cancer. Another major frontier in the surgical therapy of cancer is in reconstruction and rehabilitation. There is a need for a greater number of qualified cancer surgeons. Large medical centers are usually staffed with excellent cancer surgeons but this is not so often the case in less populated regions. Increasing the number of competent cancer surgeons can improve the treatment of cancer and, therefore, increase the number of lives saved.

Radiation therapy

Radiation therapy is second in importance only to surgery as a modality for the curative treatment of a wide range of cancers arising in various parts of the body. In addition, it has an important role in palliative treatment of cases that are too far advanced for cure. It is used for cure or for palliation in 50 to 60 percent of all cancer cases at some stage of their evolution. Radiation therapy makes use of the ionizing radiations—including x-rays and electrons produced by man-made machines, and gamma rays which emanate from naturally or artificially radioactive elements—to destroy cells by injuring their capacity to divide. Since the single most important attribute of cancer cells is their sustained, uncontrolled, lawless proliferation, inhibition of such proliferation is precisely the therapeutic goal which is desired.

Remarkable strides have been made in the past 20 years, owing largely to the technologic development of machines producing beams in the multimillion electron volt energy range. Such beams can deliver a greater dose to deep-seated tumors without the serious skin reaction that formerly limited treatment with lower energy x-ray beams. Thus, five-year survivals of patients with early Hodgkin's disease have been increased from approximately 35 percent to 75 percent, cancers of the cervix from 40 percent to 60 percent, and embryonal cancers of the testis from 25 percent to 60 percent. Modern megavoltage radiotherapy apparatus is expensive and requires major capital investment and centralized facilities, as well as specialized personnel. These complex requirements are most readily satisfied by centralization of radiotherapy departments in major cancer hospitals or in university medical centers.

An integral component of many of the larger radiotherapy departments is an active fundamental research program in cellular and molecular radiobiology, biophysics and radiologic physics. Thorough

comprehension of the complex processes by which ionizing radiations damage vital macromolecules is likely to reveal a variety of approaches whereby the efficiency of cell killing can be enhanced or modified with potential therapeutic benefit. The recovery of cells from the lethal effects of radiation can be blocked by chemical agents—such as Actinomycin D. The principal target in the cell is its deoxyribonucleic acid (DNA); DNA, the prime genetic determinant, is a double-stranded molecule and in this molecule, radiation produces breaks in one or both of the two strands. Two compounds, 5-bromodeoxyuridine (BUdR) and 5-iodo-deoxyuridine (IUdR), have been shown to increase the radiosensitivity of cells by increasing the number double-stranded breaks in DNA. Preliminary results of BUdR infusion and radiotherapy in brain tumors have been encouraging. Single-strand breaks produced in DNA by ionizing radiation are much more numerous but rapidly undergo repair and thus are not lethal under ordinary circumstances. An exciting new line of investigation has recently been opened up by the discovery that several chemical compounds are able, when added to cell cultures soon after radiation, to block single-strand repair. There is an urgent need for intensified investigation of this new approach, since some of these compounds are known to be relatively nontoxic in man.

It has long been known that cells are nearly three times more sensitive to radiation when oxygen is present than when it is absent, as it is in some areas of tumors. Certain nuclear particles—notably neutrons and negative pi-mesons—have attracted attention as substitutes for x-rays and electrons because they are much less dependent on oxygen and thus could effect the hypoxiccancer cells. The optimum dose schedules for radiation are not known and their determination will presumably depend on more information on cell population kinetics of a given type of tumor and their normal tissue homologues.

There is a critical shortage of manpower in the field, not only of radiation therapists but in all of the relevant paramedical areas as well. If all radiation therapy were administered by highly-trained, full-time radiation therapists, it is estimated that four times the present number available would be needed. Many medical schools and large community hospitals remain without either megavoltage equipment, radiotherapists or supporting facilities. There is thus an urgent need to bring the general level of staff, equipment, and facilities throughout the nation up to standards which are acceptable by today's criteria and to offer to all patients the benefits of the many advances made during the past 15 to 20 years.

Chemotherapy

In contrast to surgery and radiation therapy, chemotherapy can be used effectively for disseminated as well as localized cancer. Although chemotherapy had long been considered largely a palliative procedure, it is now evident that certain types of cancer can be cured by chemical treatment, either with single drugs or combinations of several drugs. Some of these curable cancers are choriocarcinomas and metastatic hydatidiform moles, highly malignant types of cancer which originate in the placenta; Burkitt's tumor, a highly malignant form of cancer which arises in the jaw or abdomen and occurs most frequently in children; and Wilm's tumor, which originates, in the kidney of young children and rapidly spreads to the lungs. In acute leukemia of

children and in advanced stages of Hodgkin's disease, long-term remissions have been achieved in an ever increasing percentage of the patients, and in many of these, presumptive cures have been achieved. Cures of carcinomas of the superficial layers of the skin have been achieved in the majority of patients treated with topical application of certain anticancer drugs. There are many other tumors which are markedly benefitted by chemotherapy, but in some no real beneficial effect can be achieved.

Much research is being done in the basic and developmental aspects of chemotherapy research. In the screening for new agents, in addition to the fast-growing mouse leukemias, the use of mouse tumor systems which screen for activity against the relatively slow-growing human carcinomas is being emphasized. Similarly, cancer cell kinetics are now being explored in the slow-growing solid tumors as well as in the more rapidly-growing leukemias and lymphomas. Research in cancer chemotherapy has made it clear that there is great individual metabolic diversity among tumors, which fact enormously complicates the task of chemotherapy. Most drugs currently available exert their action primarily as growth inhibitors and consequently exert toxicity to rapidly dividing normal cells. Some experimental tumors, however, can be cured by individual drugs or combinations of drugs; such cures imply that there are major metabolic differences between these tumors and normal tissues.

For example, mice with a particular form of experimental leukemia [L1210] can be cured. In order to achieve these cures, *all* of the leukemic cells must be killed. That cures of this cancer can be produced without lethal toxicity to the animal is remarkable and encouraging.

The major classes of effective compounds include: alkylating agents, antimetabolites, antibiotics, plant products, and steroids. There are also some miscellaneous individual compounds. Most of these compounds have been discovered by screening in animals. The mechanism of action of the majority of these compounds involves DNA. The alkylating agents react chemically with DNA and prevent its further replication. Many antimetabolites block DNA synthesis. Many antibiotics bind to DNA or intercalate into DNA and consequently block DNA transcription.

In any tumor, only a certain proportion of cells are actively dividing at any one time. Most drugs that inhibit cancer growth kill only these dividing cells. The degree of killing is often proportional to the growth rate of the tumor. The same effect is observed in cancer patients; acute leukemic cells, for instance, can usually be killed much more effectively than cells of slow-growing solid tumors.

As a result of studies on the patterns of cell division characteristic of a number of experimental tumors, drug regimens have been designed that are much more effective against these experimental tumors than when the same drugs have been given arbitrarily. In studies on human cancer cell kinetics, the problem of extrapolating dosage schedules of cycle-dependent drugs from mouse to man demands a thorough knowledge of the kinetics in man of various types of cancer cells, as well as of the most sensitive normal cells—such as bone marrow or gastrointestinal epithelium. Much progress has been made in the understanding of the cell kinetics of acute leukemia in man, but further work is needed in the leukemias and particularly the more slow-growing human solid tumors.

Cancer cells may eventually become resistant to the drugs, as a result of selection from a mixed cell population. This resistance has been shown to result from the loss of activating enzymes, induction of synthetic enzymes, or from an increase in repair processes. Resistance can be minimized by the use of suitable drug combinations. Use of such combinations has been largely responsible for the improved results in the treatment of the acute leukemias and Hodgkin's disease.

Research to establish optimum dosage schedules and route of administration for chemotherapeutic agents has produced such outstanding achievements as increasing the cure rate of metastatic choriocarcinoma from practically zero to greater than 75 percent, and of Burkitt's tumor from zero to 50 percent. Meningeal involvement is common in acute leukemia. Leukemic cells in the cerebrospinal fluid cannot be reached by most otherwise active systemic chemotherapeutic agents and must be treated by intrathecal administration of the drugs. The use of the Omaya reservoir to instill the drug directly into the cerebrospinal fluid of the ventricles appears to achieve a longer lasting effect.

Cancer chemotherapy appears to hold great promise for the future control of cancer. Indeed, it is concluded that the chemotherapy of cancer is today at a point analogous to that of the chemotherapy of infectious disease in 1937. Not only has it been established that certain types of cancers can be cured by chemotherapy but also the technical knowledge is available to mount a large-scale program for the development of new agents and new regimens of treatment.

Cancer Research

CANCER BIOLOGY

Cancer is a population problem among cells. As in other population phenomena, the interactions prove to be multiple, complex, and often subtle. In spite of these complexities, it is now known that normal cells in a functioning organ sense and respond to one another as well as to the specific chemical messages coming to them from distant body sites. In the course of normal aging and tissue renewal, they replicate and grow in exact relationships to one another—relationships which are compatible with the successful function of that organ—lung kidney, intestine, breast, or other. Not only the number of a particular cell type is regulated, but also the geometry of their growth in relation to other adjacent cell types. They literally count one another!

In cancer cells—whatever their cause—something has gone wrong with this social interaction of cells as they no longer sense or respond properly to the intercellular growth stimuli or restraints. The result is a disproportionate growth and the accumulation of certain cell types—often at the expense of their neighbors. In most cases the losses are only partial defects, and the affected cells retain much of their original character. They may be easily identified by growth pattern, morphology, or function as arising from cells of a particular phenotype. In many cases they respond to pharmacological levels of the normal stimulant or depressant for that cell type. Thus, while differing from the cell of origin, the cancer cell may retain many

properties which could be used to therapeutic advantage, if only it were possible to identify those properties which play important roles in the interactive growth of cancer cells with their normal neighbors.

Accordingly, in order to make maximum progress in the control and treatment of cancer, it is necessary to identify the principles and components which operate between cells in normal organ growth and to clarify the specific defects in the cancer cell which leads to its relative unresponsiveness to these normal regulatory mechanisms. It is important, in particular, to identify the interactions and mediating principles which persist or might be re-enforced in specific neoplastic tissues to correct their disorderly growth pattern.

BIOCHEMICAL PROPERTIES OF CANCER CELLS

With respect to the biochemical properties of cancer cells in relation to normal cells, it is known that although there is unity in the fundamental chemical organization of living cells, there is a fantastic diversity in the detailed chemical properties. Tremendous chemical differences are found from species to species, from organ to organ, among different types of cells within individual organs, and even among the same types of cells at different times. Paralleling the diversity of chemical detail in normal tissues, there is also a great diversity of chemical detail among various types of cancer. Such diversity exists not only among cancers of different organs, such as mammary cancer, liver cancer, and skin cancer, but among different cancers arising from a single cell type such as the major type of liver cell (the hepatocyte).

Just as normal cells can lose a variety of enzymes or can alter the ratios between a variety of different enzymes and still survive, cancer cells can sustain major shifts in enzyme pattern and survive.

The rationale of cancer chemotherapy rests on the assumption—supported by the occasional successes of cancer chemotherapy—that chemical differences between normal cells and cancer exist, despite the many common or identical features that maintain the vital processes in both normal and cancer cells.

There are a number of unresolved problems in cancer biochemistry: First, among the chemical components of cells there is no single one whose alteration can be said at this time to be *essential* to the malignant transformation, although there are a number of biochemical properties that correlate with growth rate. There appears, rather, to be an alteration in the relation between the various components, whether this is referred to as altered control, regulation, feedback, homeostasis, or integration, in relation to the needs of the whole organism.

Second, there is no agreement at this time as to whether or not cancer can occur without alteration in the genome—that is, whether or not alteration in the DNA sequences of the cell, broadly equivalent to somatic mutation, is a universal requirement for malignant transformation. The recent demonstration of the integration of the DNA oncogenic viruses into the cellular genome and the recent findings that the oncogenic RNA viruses can exist as a DNA provirus suggest that a comprehensive explanation of viral oncogenesis and its relation to the somatic mutation theory of cancer may be forthcoming.

Third, there is no agreement at this time as to whether the transformation of a normal cell to a cancer cell is universally irreversible,

occasionally reversible, or potentially widely reversible. It is well known that under a variety of circumstances highly malignant cells can lose their malignancy without, however, becoming normal.

Fourth, the available evidence suggests that in the transformation process there may be an activation of latent genes and a shutting off of other genes, just as in normal differentiation and cell maturation. There is the important difference, however, that the process in the cancer cells fails to proceed to the point of coordination with the needs of the rest of the body.

It is now becoming possible to explore the biology of the tumor cell with much greater precision than was ever previously possible. Technological developments, such as radioisotopes and the electron microscope, enable investigators to examine functions and structures of individual cancer cells. Advances in tissue culture techniques permit the study of carcinogenesis in single cells under controlled conditions. The recent discovery that different types of cells can fuse in tissue culture to form viable hybrids has opened the way to genetic analysis of tumor and normal cells of a sort not even envisaged a few years ago.

ETIOLOGY OF CANCER

The goal of cancer etiology—the study of the causes of cancer—is cancer prevention. Three types of agents have now been shown to cause cancers: chemicals, radiation, and viruses. Of these, two—chemicals and radiation—clearly cause cancer in man and the third, viruses, are, on the basis of present knowledge, highly suspect.

Cancer epidemiology

Cancer epidemiology seeks to correlate differences in the incidence of different types of cancer with differences in the external or internal environments of the persons developing these cancers as has already been established. For example, between cigarette smoking and lung cancer.

Recent investigations have raised some intriguing questions that call for further study. These include the malaria-like distribution of Burkitt's tumor among African children; the sevenfold higher incidence of breast cancer among American than among Japanese women; the much greater incidence of colon cancer in the United States than in Africa and, conversely, the extremely high incidence of liver cancer in certain areas of Africa; and the spotty geographical distribution of esophageal cancer throughout Africa.

The results so far of epidemiological research strongly suggest that variations in exposure to environmental agents and in social practices are largely responsible for variations in the incidence of cancers among different groups of people. Therefore, if such environmental exposures and social practices could be identified and eliminated, the majority of cancers in man might be prevented. Because of the complexity of the relationships between man and his environment and the long latent period of cancer development, the identification of particular cancer-inducing factors is extremely difficult, but there is no other area of cancer research that holds more promise for cancer prevention.

Chemical carcinogenesis

The objectives of research in chemical carcinogenesis are 1) identification and removal from the environment of compounds that cause

cancer in man, and 2) identification of the mechanisms by which chemicals induce cancers, so that such induction may eventually be prevented or reversed.

Testing for Carcinogens: Hundreds of chemicals have now been shown to be carcinogenic in experimental animals. Furthermore, new potential carcinogens are being introduced daily into the environment in the form of air pollutants, food additives, pesticides, etc. Reliable screening methods to assay which of these agents may be dangerous to man are not yet available and are urgently needed.

The screening of such compounds is complex for several reasons. First, the experimental procedures now available, which usually involve tests in laboratory animals, are slow and expensive and the correlations between the activity of an agent under these conditions and its action in man in the natural environment are not always clear. Furthermore, exposures to even very low doses of multiple agents, such as may now be occurring in man's increasingly carcinogenic environment, may have additive effects which could not be discerned by the testing of any one agent alone. Finally, there are indications that certain carcinogenic chemicals may be endogenous—that is, produced within the body of certain persons from naturally occurring organic compounds.

Mechanism of action of chemical carcinogens

It is known that many chemicals are changed by the tissues of the organism into their ultimate carcinogenic form. As a consequence, the varying capacities of different tissues and of different individuals for the activation and deactivation of chemical carcinogens are important factors in determining the susceptibilities of individuals to chemical carcinogenesis. It has been possible, in one instance, to identify the particular enzyme system involved in the activation of a particular carcinogen in mice. Such identifications, if they can be extended to the human population, may make possible the detection of persons who are high cancer risks because of their particular enzymatic machinery, and also, if useful inhibitors are found, may open the way to the blocking of the activities of activating enzymes.

As a class, carcinogens have physico-chemical properties that result in their binding to macromolecules within the cell, including DNA, RNAs and proteins. It is presumed that the cancer-causing activity of the agents is related to their affinity for particular molecules, but it is not known which of these affinities are of crucial importance in the induction of cancer in man or in experimental animals.

Induction by chemical of tumors in the skin of mice and probably in other tissues in other species can be divided into two distinct phases, initiation and promotion. In some cases, cancers can be produced only following exposure to both chemicals and in the "right" order. Knowledge of the biochemical events that occur during initiation and promotion would provide important leads to the understanding of the nature of carcinogenesis.

Radiation carcinogenesis

X-rays and other ionizing radiations have been shown unequivocally to cause cancers of the skin, leukemias, bone cancers, and various other types of cancers in man as well as a broad spectrum of neoplasms in experimental animals. Recently it has also been demonstrated that ionizing radiations can produce cancerous transformation in cells in

tissue culture. Because dose and timing can be precisely controlled, radiation-induced transformation *in vitro* offers a uniquely useful model system for studying the early events of carcinogenesis.

Radiation may cause cancer by producing changes in the genetic material of cells (somatic mutation), by activation of latent viruses, and by disturbing growth regulatory mechanisms. Of these three possible modes of action, the latter two have been well documented in experimental animals. In mice, radiation results in the induction of a leukemia virus. Radiation damage to endocrine glands of experimental animals has been shown to produce hormonal imbalance which leads to cancer.

Whether or not such mechanisms are involved in the induction of cancers in man is not clear but this question is of great theoretical interest as well as practical importance.

VIRUSES AND CANCER

Some one hundred viruses now have been discovered which cause cancer in some species of amphibians, birds, and mammals. Although there is, as yet, no unequivocal evidence of a human cancer virus, most investigators working in this field are of the opinion that viruses will soon be shown to be responsible for some types of human cancer.

Search for human cancer viruses

One of the viruses now under intensive study as possibly carcinogenic in man is the EB virus, which has been observed in electron micrographs of cells of Burkitt's tumor (a tumor of African children whose distribution leads to the suspicion that it may be of infectious origin). The EB virus belongs to a common "family" of DNA-containing viruses known as the herpes group, among which is the virus which causes fever blisters. One hundred percent of the African children with Burkitt's tumor have been found to have significant amounts of antibody against this virus, as compared to less than 50 percent of the other African children tested. Other studies of the EB virus have indicated that it is probably the cause of infectious mononucleosis, a benign infectious disease which involves the same type of cells that are involved in leukemia and induces, in these cells, a phase of very rapid growth.

A group of RNA-containing viruses, which have been detected by the electron microscope in samples of human milk and in human breast cancer cells growing in tissue culture, is also suspected of causing human cancers. These viruses are found more frequently in milk specimens of women from groups with high rates of breast cancer—such as the Parsis, a highly inbred group in India—than among the general population. These viruses resemble the mouse mammary tumor virus, a virus which is transmitted from mother to offspring in genetically susceptible strains of mice. It is not known, however, whether or not the viruses found in humans are associated with any form of cancer.

The third highly suspect group of viruses are the C-type viruses which have been shown to induce leukemias and sarcomas in cats, chickens, and mice and have been detected in specimens of human leukemias and lyphomas.

It is difficult to prove that a particular virus causes cancer in man because it is not ethically possible to inject a suspected virus into human beings and see if cancer occurs. Moreover, such viruses when first isolated from laboratory animals have usually grown only in genetically similar animals and no comparably inbred strains of humans exist. However, some ingenious new detecting systems, involving the growth of cells in tissue culture, have recently been worked out which should help to provide a solution to this critical problem.

Mechanism of action of the cancer-causing viruses

Virus particles are composed of either DNA or RNA, depending on the type of virus, and protein. The protein serves as a protective carrier for the nucleic acid and is discarded before or soon after the virus enters the cell. Once within the cell, the viral DNA or RNA interacts with the biochemical components of the cell. In the case of the common infectious viruses, such as influenza, this interaction results in the production of new viral particles and the death of the host cell. In the case of certain bacterial viruses (the "lysogenic" viruses) and also, apparently, of the cancer-causing viruses, the viral DNA or RNA becomes a part of the cell and the cell, as a result, takes on new properties.

Cancer-causing DNA viruses are thought to persist in the cell as part of the cellular DNA and to be replicated with the cellular DNA at the time of cell division and passed on to daughter cells. As mentioned previously, however, some of the known cancer-causing viruses are RNA viruses. In what form are the RNA viruses perpetuated within the cell? Several years ago it was proposed that the RNA of the cancer-causing viruses is translated into DNA within infected cells and that the viral "information" is stored in this form. Recently an enzyme that translates RNA into DNA was found. This enzyme, known as RNA-dependent DNA-polymerase, is associated with all the cancer-causing viruses so far tested and with only two viruses not known to be cancer-causing. This enzyme has been reported to be present in the cells of some human acute leukemias, but not in normal blood cells. This suggests that the enzyme may be the "footprint" of a hidden virus in these human leukemic cells.

Interferon

Immediately following infection by a virus, animal cells produce a protein substance, interferon, which protects neighboring cells from infection by that virus and by other viruses as well. Each animal species produces an interferon that is active only in cells of that species, and the cells produce it for only a relatively brief time.

Very recently it has been discovered that interferon not only can inhibit cancer induction by known cancer-causing viruses but also delay the progression of or produce regression in some leukemias and transplanted mammary tumors in mice. Microscopic studies of the tumors after treatment indicate that the interferon had specifically destroyed the cancer cells without injuring normal cells. Extensive work on technical procedures for the production of larger amounts of human interferon is clearly needed.

INTERACTIONS BETWEEN ETIOLOGIC FACTORS

As noted previously, a number of environmental agents are known to cause cancers. Yet not all persons exposed to these agents develop cancer. Clearly there are also factors within an individual that determine whether or not he will develop cancer. These intrinsic factors include genetic characteristics, aging, activity of particular enzymes, and hormonal and immunologic status. Most apparently "spontaneous" human cancers are probably caused by a combination of factors, both intrinsic and environmental.

According to one recent new hypothesis of cancer causation, certain types of cancer viruses are transmitted from generation to generation, probably as part of the cell's genetic material. Whether or not an individual carrying such a virus develops cancer is a result of whether or not the cancer-producing viral genes remain "turned off," or repressed, or are activated. Chemical carcinogens, radiation, and perhaps other viruses are among the agents that proponents of this hypothesis suspect of being capable of activating these hypothetical viral genes.

MOLECULAR BIOLOGY

Molecular biology seeks to account for the basic phenomena of life, such as growth and reproduction, in molecular terms. In the last two decades, in a series of brilliant discoveries, the nature of the genetic material, how it is replicated, and how it is translated into cellular enzymes and other proteins has been revealed. Present studies are focused on the molecular events involved in cellular differentiation and in the regulation of gene function. Cancer, whatever its causes, clearly involves a dysfunction at the molecular level in the cellular control mechanisms. Basic research in the molecular biology of cancer is the area of investigation most likely to reveal this fundamental, underlying disorder.

IMMUNOLOGY OF CANCER

The defense systems of the body, by immunologic reactions, defend it against invasions by bacteria and other micro-organisms. It is now clear that cancers also invoke immunologic reactions in their hosts. "Spontaneous" regressions of a few cancers in man are well documented. Lowered immunologic status both in man and in laboratory animals results in increase susceptibility to cancer. Finally, autopsies of persons who died of other causes and were not known to have cancer reveal an incidence of "silent" cancers considerably higher than that of clinical cancer. These data suggest that immune reactions can hold the cancer in check, sometimes completely successfully. This new knowledge is opening the way to the development of wholly new methods of cancer therapy and detection, based on immunologic techniques.

Immunologic means of cancer detection

In the cancer cells of laboratory animals, antigens are found associated with DNA and RNA viruses which are shared by all cancers produced by a particular virus. Cancers produced by chemical carcinogens also have antigens, but these are different in each tumor produced by the carcinogen.

In human cancer, antigens have been found in cells of Burkitt's tumor and this same antigen is associated with infectious mononucleosis. Among other common antigens in human cancers is one which is found in melanomas, which is the first suggestion of a possible viral origin for this type of cancer.

Recently the intriguing discovery has been made of a new kind of antigen, a fetal antigen, associated with human cancer. These antigens, which are released from cells and can be detected in the blood stream, are normally present in embryonic or fetal tissues, disappear in adult life, and only reappear in the cancerous cell. (This reappearance suggests that a gene that is switched on in fetal life and switched off after birth is switched on again during carcinogenesis.)

One of these fetal antigens, CEA, is providing the basis for the new immunologic test previously mentioned. The second fetal antigen is found in the blood stream of about 75 percent of patients with cancers arising in the liver. A third fetal antigen is found in association with a variety of other tumors. All of these antigens may be useful in the development of diagnostic tests.

Studies on cancer prevention

A vaccine has been developed which protects chickens against a herpes type virus which cause an infectious lymphomatosis in chickens. The possibilities of developing a vaccine against the related EB virus associated with Burkitt's tumor and other diseases, are being explored.

Immunotherapy

Because of the indication of an association between cancer progression and a lowered immune capacity, ways are being sought to bolster immune responses in cancer patients. In a few cases of acute leukemia in children, addition to the treatment regimen of an agent known as BCG, which nonspecifically stimulates immune reactions, has been associated with a few very long remissions. Immunotherapy will probably be most useful in combination with other forms of treatment, which will remove or destroy most of the cancer cells. However, in such situations it might be of critical importance whether or not a cure was achieved.

CLINICAL INVESTIGATION

Clinical investigation is essentially the culmination of basic and preclinical studies in cancer research and the development from these of techniques useful in preventing, diagnosing, and treating cancer in man. Among the important achievements of clinical investigation have been the testing of new surgical procedures, new types of ionizing radiation and new chemotherapeutic agents in man and the development of schedules and combinations of therapies that have resulted in great improvement in treatment. For certain types of cancer, cures or presumed cures ("presumed" because insufficient time has elapsed) have been achieved by chemotherapy in patients with choriocarcinomas and Burkitt's tumor and occasionally in patients with widespread Hodgkin's disease, acute leukemia, and other forms of cancer for which the previous cure rate was virtually zero.

Areas of active study in this field include investigations of immunologic status of patients with different types of cancer, studies of groups

of persons, such as heavy smokers, patients with mongolism, etc., who have a high risk of cancer development, to define the factors which determine their susceptibility to cancer, and comparisons of enzyme systems in normal persons and cancer patients. The latter studies have resulted in the detection of an RNA-directed DNA polymerase in the leukemic cells of patients with acute leukemia but not in normal white cells. This suggests that the enzyme may be the "footprint" of a hidden virus in these leukemic cells. If this enzyme could be shown to be an essential component of the leukemic cell, there might be a possibility of treating the disease by blocking this enzyme action.

As the pace and productivity of fundamental research increases, a greatly expanded effort in clinical investigation must be undertaken in order to realize fully the promises and accomplishments of cancer research.

Areas of Greatest Importance

Certain broad areas of cancer research, in the judgment of the Panel, warrant expanded and particularly vigorous exploration.

1) *Epidemiological Research* can identify extrinsic influences (such as chemicals, viruses, and radiation) that may play substantial roles in determining the frequency of cancer. Different types of cancer are known to occur with varying incidence in different geographic regions and under varying circumstances of social, economic, nutritional, and occupational conditions. Epidemiological identification of intrinsic conditions such as genetic predisposition, immunological impairment, hormonal effects, or metabolic differences may elucidate other factors affecting the incidence of cancer which could be clinically controlled.

2) *Chemical Carcinogenesis* is a research area of prime importance because of the variety and quantity of new compounds introduced into the biosphere. Preliminary efficient screening should be used to eliminate or reduce this hazard. The ability to evoke neoplastic change in isolated tissue in culture should accelerate the research in defining hazardous compounds and the mechanisms by which cancer is caused. Further research is needed on:

a) The basic cellular and molecular mechanisms of action of carcinogenic chemicals, viruses, and radiations. There may be several mechanisms (both genetic and epigenetic) for each class of carcinogens.

b) The kinds of interactions of viruses, chemicals, and radiations which result in the production of neoplasms in experimental animals and man.

c) The proportion of tumors in man induced by viruses, chemicals, radiations, and combinations of these agents.

d) The prevention of chemically induced cancer in man by the identification and removal of causative chemicals.

It is known that in most circumstances, a compound is transformed to a proximate carcinogen, which is then directly involved in the carcinogenic process. There appear to be common chemical characteristics of the proximate carcinogens and also common characteristics of the cellular metabolites with which they can react. Further research is called for on how to interfere chemically with the metabolism which produces the proximate carcinogen or to inactivate it once it is formed.

3) *Virology*. The recognition that a variety of neoplastic diseases in domestic and wild animals are due to viruses makes it appear increasingly probable that some types of human neoplasia are due to viruses. Although viruses have been found in human cancers, proof of their etiologic relationship is still incomplete. An expanded study of viruses in cancer should include the mechanisms of a how a virus initiates cancer, b) how a virus may be carried in cells for long periods of time without expressing its cancerous potential, c) how a natural defense against viral oncogenesis occurs, d) how a virus can sometimes cause tumors and sometimes other, non-neoplastic diseases, e) how the presence of a virus can be detected by characteristic and special chemical reactions, and f) how the interactions between viral infection and chemical carcinogens occur which sometimes can evoke tumors.

4) *Tumor Antigens*. The characterization, isolation, and purification of antigens from normal cells and from specific tumor cells are important both to the understanding of differences in cellular chemistry in the neoplastic state and to the development of tools for the study of tolerance or rejection of tumors. Some of these antigens may be identical with those found in embryonic tissues, and some may be specifically associated with virus infection.

5) *Cellular Immune Mechanisms*. Much further work is needed on the chemical, biochemical, and antigenic nature of cancer cell membranes in comparison with those of normal cells, especially in terms of the role of the membranes in coordinating cell activity with bodily needs. Substantial evidence indicates that some, if not all, tumors have surface antigens different from normal cells. These differences allow recognition and possible eradication of the tumor by cells of the lymphoid or reticuloendothelial system of the host. This surveillance mechanism is known to be influenced by genetic, hormonal, chemical, and physical factors, and perhaps by many others. The characteristics of production, distribution, and mechanism of action of the effector cells that participate in the defense against tumors are of great importance. Failure of cellular mechanisms to eradicate emerging tumor populations may be the final step before a tumor gecomes established and begins to grow and so is worthy of intensified study.

6) *Humoral Immunity* is conveyed through the agency of one or more of the five immunoglobulins and perhaps by other proteins in the plasma. Humoral effects on tumor cells span the range from lethality to apparent protection of the cell by interfering with cellular immune mechanisms. The interrelation of the immunoglobulins with other proteins, their relation to receptor sites on the cell, and their influence on cellular immune mechanisms, urgently need clarification.

7) *Immunoprophylaxis and Immunotherapy* have both been shown to be effective in experimental animals, and useful immunotherapy has been reported in some children with acute leukemia. Specific and nonspecific stimuli may enhance immunologic response of the host's own immunocompetent tissue, both *in vivo* and *in vitro*. Techniques of using specific tumor antigens, additional immunocompetent cells, and nonspecific immunologic stimuli, are worthy of intensified study. The timing of immunotherapy with respect to other procedures needs clarification, but this form of treatment would appear to be

most useful after maximal reduction in tumor size by surgery, radiation, or chemotherapy.

8) *Diagnosis.* Precise and highly sensitive techniques of diagnosis prior to the appearance of clinical symptoms or of large masses of cancerous tissue are of major importance to advances in therapy. It is suspected that many, if not most, neoplasms secrete materials into the blood which have remote effects on the patient and which are not yet recognizable with tests available. Immunological assay methods have been successfully used to quantify tumor products in biological fluids. For example, chorionic gonadotropin indicates the presence of trophoblastic neoplasia and its detection allows diagnosis and appropriate therapy before a tumor mass is recognizable. A carcino-embryonic antigen appears in the blood of patients with carcinoma of the bowel, and a sarcoma antigen in patients with sarcomatous tumor growth. Continuing research to seek biochemical and immunological means of detecting cancers early deserves expansion. Automated analytic techniques with comparisons of results against computer-banked data for the healthy population and for the same individual at an earlier date would provide a more precise method for early diagnosis.

9) *Chemotherapy.* Several disseminated human tumors have been cured with drugs alone, giving ample testimony to the proposition that selective toxicity does exist and that a potential for cure is present. Effort must be extended to understand the interaction of drug, host, tumor, oncogenic agent, and host defense mechanisms in this equation. Research on which drugs to give for which tumors, in what combinations, and when in the course of the disease (before operation, after operation, with radiotherapy, after widespread metastasis, etc.) are areas of great importance.

10) *Tumor Cell Kinetics.* Only a portion of tumor cells are in an active growth cycle at any one time, and the synthesis of critical cellular constituents is known to occur during specific phases of that cycle. A much deeper understanding of selective toxicity during different phases of the cell cycle is needed in addition to elucidation of the natural death of cells within the tumor, the fraction of cells in the tumor which can reproduce, and techniques of killing cells which are not in the critical phases of synthesis. These types of information are required particularly for slow growing spontaneous neoplasms in experimental animals and in man.

11) *Sanctuary.* Tumor cells lodged at a distance from the closest capillary or beyond the blood brain barrier may enjoy a pharmacological sanctuary where adequate drug concentrations cannot reach them to exert lethal effects. Experimental and clinical research of this problem is required.

12) *New Drugs.* Chemical syntheses of compounds or polymers designed to interfere with critical steps in cancer cell biosynthesis and metabolism are important. The specific targets and techniques of selecting the compounds for trial must be arrived at by chemists and biologists in coordination, and these synthetic programs must be carried out in close proximity to biological testing, so that rapid feedback occurs. Empiric screening of natural products, particularly plant extracts and antibiotics, continues to provide compounds of major clinical usefulness. Nearly a dozen substances of botanical and microbiological origin are now in clinical use and have demonstrated

therapeutic benefits against cancer. The programs of search for botanical and antibiotic drugs against cancer should be extended together with relevant biological screening in close proximity. Much additional research is also needed on:

a) Metabolic or enzymatic differences between normal and malignant cells, which might lead to new drug design.

b) Sophisticated testing of compounds, particularly antibiotics, against various important biosynthetic enzymes. Drugs which may affect enzymes unique to oncogenic viruses hold particular promise for neoplasms found to be caused by viruses.

c) Pharmacology and metabolic disposition of known active compounds in human cancer patients.

d) Development of growth-inhibiting compounds that are *not* immunosuppressive.

e) Synthesis and structure-activity analyses of series of compounds that inhibit key enzymes, with the aim of discovering species or tissue differences in affinities for the drugs.

f) Attempts to lay the groundwork for the development of new classes of drugs that would affect processes of malignancy other than growth, such as invasion and metastasis.

g) Elucidation of the mechanisms whereby certain drugs potentiate the lethal effects of heat on tumor cells.

h) Attempts to find new and effective drugs to produce a radiosensitization of tumors.

Predictive Testing of Drugs before human use is of major value and must be extended. The optimal dose, route, schedule and conditions of administration may be learned from experimental animals in addition to the toxicities and side effects which may develop. What is more, development of methods by which a drug can be tested in the laboratory for its effectiveness on the patient's own tumor prior to its clinical use is an area where important advance can be made.

Clinical Investigations in chemotherapy provide an essential link in cancer research, since the ultimate goal is prevention and relief of the disease in man. Cooperative group studies bring earlier results of higher precision and validity because of the positive intellectual input of several investigators in planning the research.

13) *Radiotherapy*. Use of new radiation sources may allow avoidance of the problems of anoxia in tumor tissues and so substantially improve radiotherapeutic results. Another area of great importance is the continued and expanded study of the effects chemotherapeutic agents in combination with radiotherapy where the optimal choice of drug, schedule, dose, and radiation regimen needs wide investigation.

14) *Surgical Improvements* can be made which should substantially increase cure rates. These involve the early use of surgery as a prevention of the development of invasive and matastatic cancer by removing precancerous tissues. In addition, the combination of radiation and surgery in the eradication of established neoplasms is subject to major improvement by appropriate design, precise scheduling, and the use of drugs, to take advantage of the small residual tumor populations after the initial major reductions. Organ transplantation should be developed as an aid to the extension of curative surgery.

15) *Fundamental Biological Studies* are of the greatest importance in disclosing information about the causes of cancer and strategy for

its cure and prevention. Molecular biology can eludicate DNA mutations. A change in DNA structure as a random event or as a response to an environmental trauma may lead to cellular death, or, if the injury is compatible with cellular survival, a mutation. Following such a mutation, a series of descendants may appear whose change in the DNA, if unrepaired, may be heritable and could be the cause of neoplastic transformation. In the course of normal growth and maturation, this change in DNA would be reflected in the composition of RNA, which in part provides messages specifying particular protein syntheses which determine the character of the cell's enzymes and thus its whole metabolic machinery. Mutations and derangements in the repression and derepression of genetic material are susceptible of study by a wide range of experimental systems. These studies must be pursued in great detail because of their relevance to cancer. Further work is needed in the area of biochemistry to support both the search for preventive and therapeutic measures:

a) Further documentation of biochemical diversity in cell components and in responses to control factors is necessary to depict the true magnitude of the cancer problem and perhaps to sort out the threads of unity which must exist.

b) Examination of tumors in terms of *isozymes* is particularly important because, if two different enzymes can catalyze the same reaction but possess different three-dimensional structures, they may respond differently to chemotherapeutic agents, and the information may suggest new modes of chemotherapy. Isozyme research is also important because it is relevant to the understanding of gene regulation in differentiation, in transformation of normal cells to cancer cells, and in the understanding of the relation of the two processes.

16) *The Nature of Cell Surface* is imprecisely known. Its chemical and physical composition, its mediation of the cell's antigenic identity, its function in nutrition and in drug intake, are poorly understood. The mechanism by which the surface affects the control of a cell's mobility and the role of how this is distorted to allow the characteristic invasion and metastasis of cancer is not understood and merits continuing research.

17) *Biological Organization.* Intercellular communication in a multicellular animal is an obvious reality. The right number of particular types of cells of given architectural relationships accumulate to make up our characteristic normal organs. Among the most typical features of cancers is a loss of normal architectural arrangement suggesting a major alteration in intercellular communication. Mediators of this intercellular communication must be sought. Tumors evoke a new blood supply and a chemical complex responsible for this activity, known as the angiogenesis factor, has recently been isolated and is worthy of major additional exploration. More research is needed on:

a) The development of assay systems for sensing and measuring the growth-regulating chemicals which operate between different cells in specific growth situations and neoplastic conditions.

b) The isolation and characterization of the active principles.

c) The synthesis of precursors, analogs, and antagonists of these important intercellular regulators.

d) The elucidation of the cell cycles and the manner in which new agents and analogs modify steps in the cell's replication cycle.

e) The use of cell biological and genetic methods for modifying the cell's response to the action of such controlling factors.

f) The mechanisms of action of known hormones and nutrients in the control of cell replication and differentiation.

g) The application of this knowledge in the effective staging of cells for more efficient use of existing chemotherapeutic agents.

In addition to opening new avenues to cancer therapy, the knowledge derived from these approaches to the biology of growth control has the extra advantage of being widely applicable to other growth dyscrasias such as vascular and kidney disease, nerve regeneration or replacement, immune defects, and aging.

REPORT OF SCIENTIFIC COMMITTEE

The Impact of Cancer

In 1969, 323,000 persons died from cancer in the United States. Table I shows how cancer deaths compare in numbers with deaths from certain other causes.

Table I.—*U.S. deaths from various causes**

Cancer deaths (1969)	323, 000
World War II battle deaths	292, 000
Auto accident deaths (1969)	59, 600
Vietnam War deaths (6 years)	41, 000
Korean War battle deaths (3 years)	34, 000
Polio deaths (1952 (worst year))	3, 300

*National Health Education Committee, Inc., New York—22 June 1970.

TRENDS IN CANCER MORTALITY

The American Cancer Society estimates that more than 51 million people of the 200 million now living in the United States will develop some form of cancer, and unless better methods of treatment are developed, 34 million of these will die of cancer. The rate of increase of deaths from cancer is more rapid than the rate of increase in the population (Figure No. 1). The increase in the occurrence of new cases (based on data from Connecticut which are particularly complete) is even more rapid than the increase in the rate of death (Figure No. 2). This increase in new cancer cases is related to the increasing age of the population, but it is not due to increase in age alone (Figure No. 3).

Cancer among adults

If the mortality figures are broken down by sex (Figure No. 4), it can be seen that the increased mortality is occurring among males. There is a decrease in the incidence of cancer deaths among women. (These figures are for white only; reliable data are not available for blacks and other races.

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FIGURE No. 1

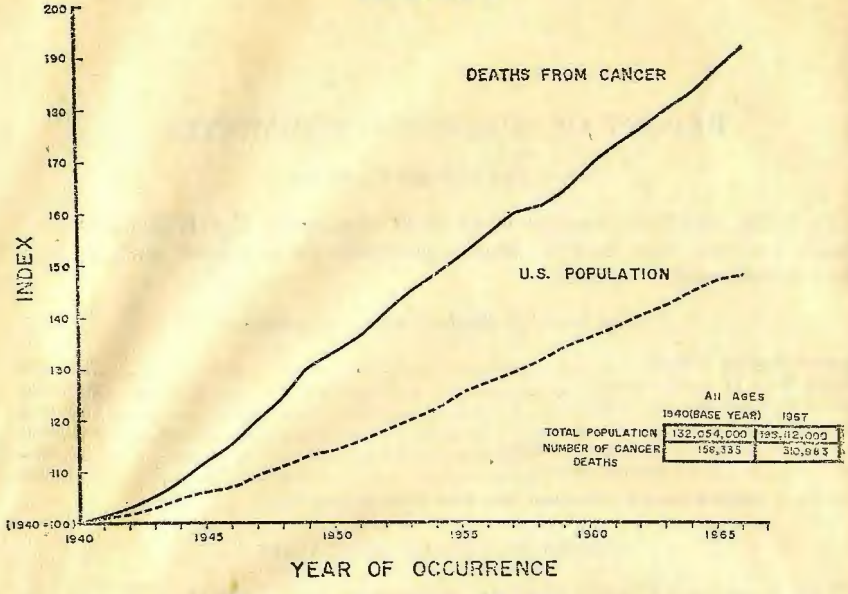


FIGURE No. 2

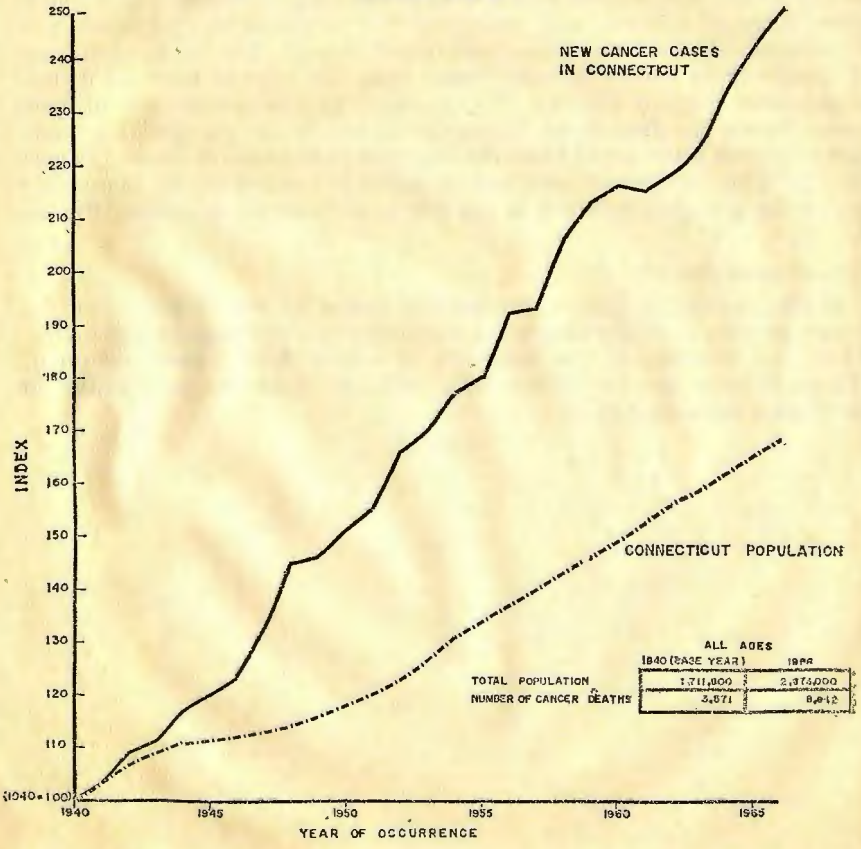


FIGURE No. 3

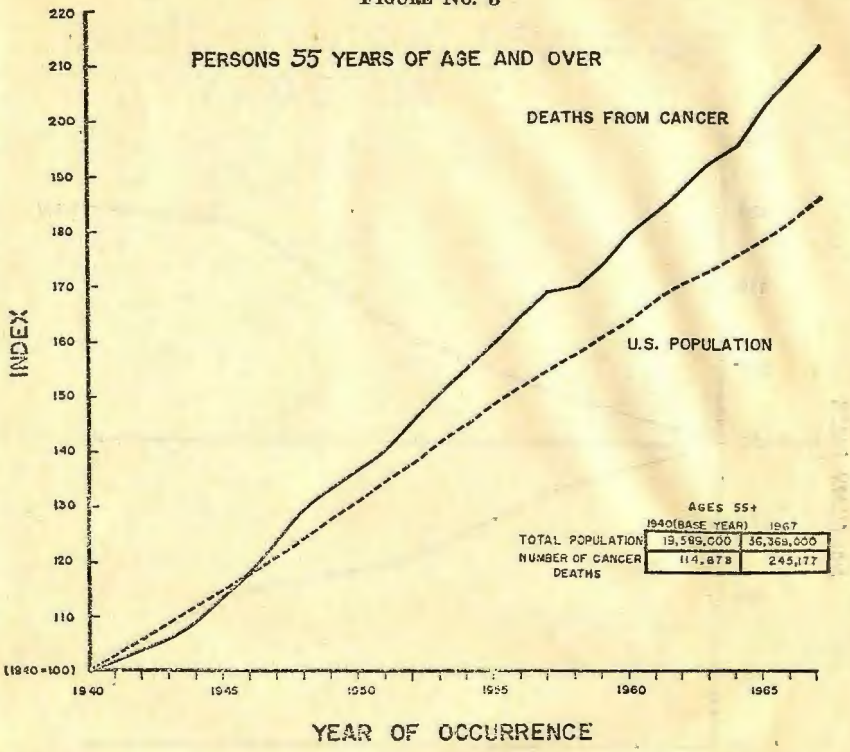

PERSONS 55 YEARS OF AGE AND OVER
DEATHS FROM CANCER
U.S. POPULATION
INDEX
220
210
200
190
180
170
160
150
140
130
120
110
(1940=100)
1940
1945
1950
1955
1960
1965
YEAR OF OCCURRENCE
AGES 55+
1940(BASE YEAR) 1967
TOTAL POPULATION 19,589,000 36,369,000
NUMBER OF CANCER DEATHS 114,878 245,177

FIGURE No. 4

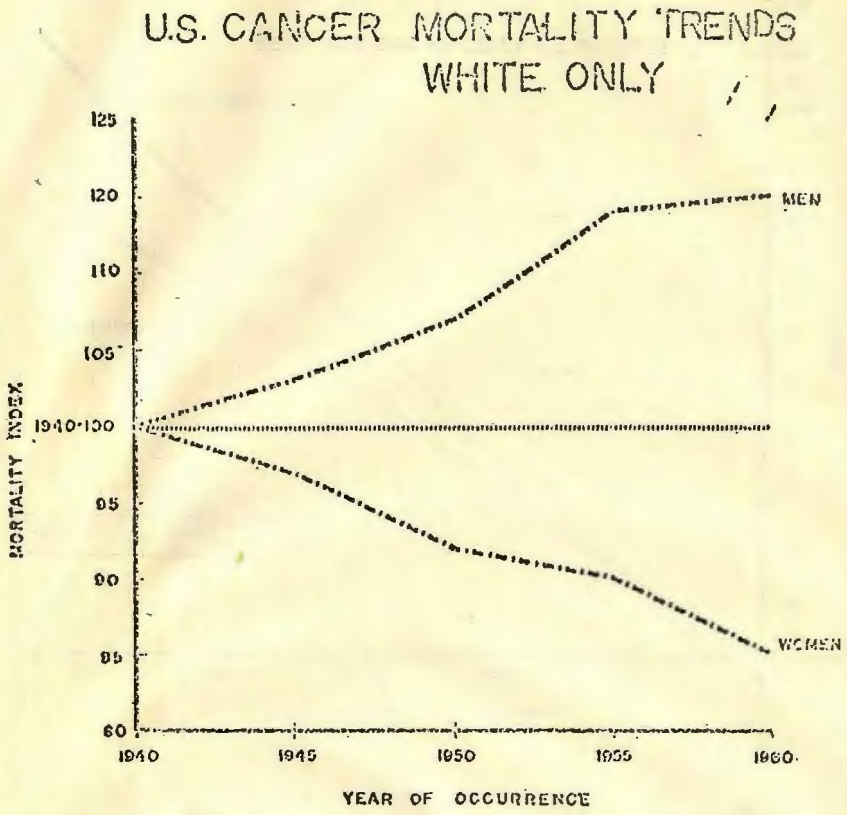

U.S. CANCER MORTALITY TRENDS
WHITE ONLY
125
120
110
105
1940-100
95
90
85
80
MORTALITY INDEX
MEN
WOMEN
1940
1945
1950
1955
1960
YEAR OF OCCURRENCE

FIGURE No. 5

BREAST CANCER

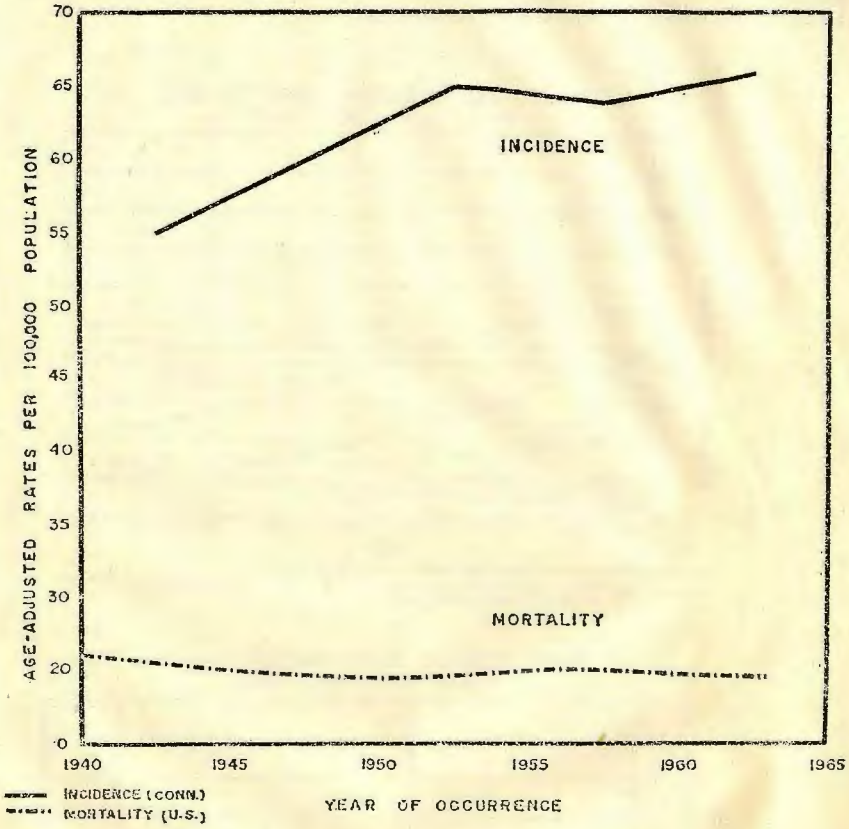
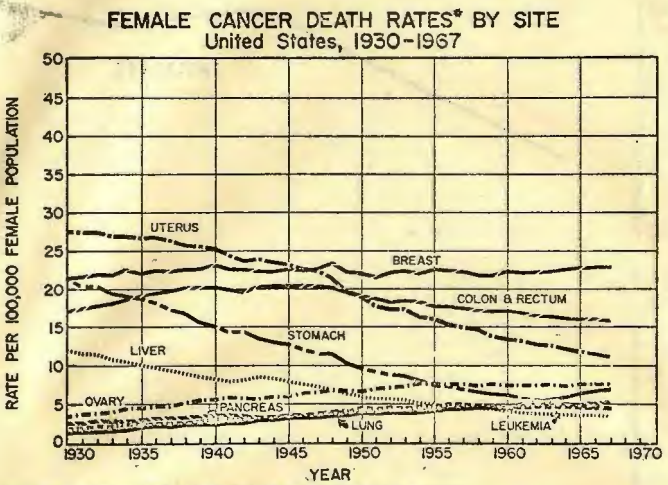


FIGURE NO. 6

Age-Adjusted Death Rates per 100,000 Population of Selected Sites of Cancer, U. S., 1930-1967

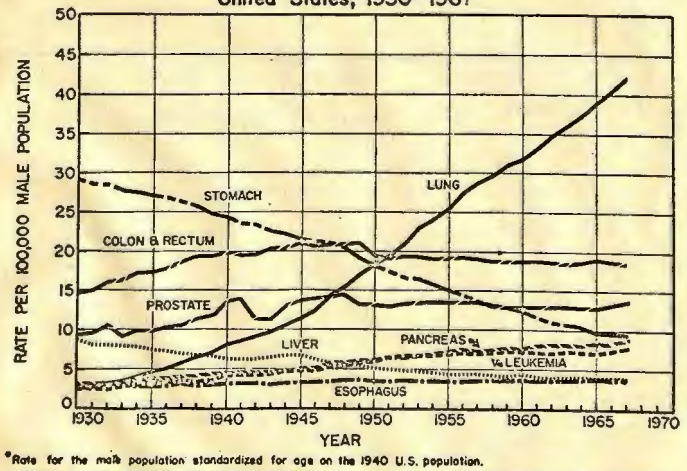
Graphs show the extent of the increase in lung cancer in men and the decrease in uterine cancer in women. The reasons are explained elsewhere. But the sweeping decrease in stomach cancer and steady increase of leukemia remain mysteries of the kind that only further research will solve.



*Rate for the female population standardized for age on the 1940 U.S. population.

Sources of Data: National Vital Statistics Division and Bureau of the Census, United States.

EPIDEMIOLOGY AND STATISTICS DEPT. AMERICAN CANCER SOCIETY, 7-69



*Rate for the male population standardized for age on the 1940 U.S. population.

Sources of Data: National Vital Statistics Division and Bureau of the Census, United States.

EPIDEMIOLOGY AND STATISTICS DEPT. AMERICAN CANCER SOCIETY, 7-69.

Most of the increased mortality among males, during the 20-year period from 1940 to 1960, is a result of the rise in deaths from lung cancer.

The trend in breast cancer among women is of interest. The incidence has been going up, but the mortality has decreased slightly, probably due to earlier case finding and more prompt surgical treatment (Figure No. 5).

The trends for cancer mortality for women and for men are broken down for a number of common sites (Figure No. 6.)

Cancer in children

It is customary to think of cancer as a disease of maturity and old age; however, cancer is also a major cause of death among children and young adults. In fact, it is the largest cause of death, except for accidents, among persons between the ages of 1 and 35. Nearly one-sixth of the deaths which occur between the ages of 5 and 14 are caused by cancer.

Conclusions

It is thus clearly established that cancer is a problem of major scope. It is the second largest cause of death, all ages considered, and undoubtedly more greatly feared than death from heart disease because of the frequently prolonged and debilitating course, often associated with intractable pain, which may occur in the later stages of the disease. Death from cancer is apt to be death with suffering and without dignity.

THE ECONOMICS OF CANCER

There are no complete figures on the economic effect of cancer in this country. Some estimates can be made using the 1963 figures* adjusted to reflect the increases in the cost of the medical services, earnings, and number of cancer patients. According to these estimates, the direct costs for hospitalization, nursing homes, physicians, nurses, and medicines exceeded $1.5 billion in 1969. Similarly calculated, the indirect costs of cancer, including loss of earnings during illness and present worth of loss of earnings during the balance of normal life expectancy, bring the total costs of cancer to over $15 billion for the year.

EXPENDITURES FOR CANCER RESEARCH AND CANCER CONTROL

The National Cancer Institute appropriation for the fiscal year 1969 was approximately $182 million. During this same period, the American Cancer Society expended $24 million for research. Several other smaller voluntary agencies are estimated to have contributed an additional $5 million. The U.S. Department of Health, Education and Welfare, the regional medical programs, and numerous state and local programs of government spent additional sums in cancer training and control. Other government agencies supported research on the periphery of the cancer program. A somewhat larger appropriation for the National Cancer Institute has been recommended by the Administration for the fiscal year 1971; it is estimated that this sum is equivalent to less than two cents a week for each citizen of the United States.

* Health Economic Series—Number 6—Estimating the Cost of Illness—United States Department of Health, Education and Welfare—Public Health, Public Service Publication—Number 947-6—May, 1966.

How do such expenditures compare with other allocations in our national budget? For example, 41,000 Americans died in Vietnam in the past six years but cancer killed 2,000,000 in the same period—35,000 of whom were children. In spite of these appalling facts, in 1969 we spent, per citizen, $410 for national defense, $125 for the Vietnam war, $19 for space investigation, $19 for foreign aid, and *only 89 cents for cancer research.*

Where We Stand Now in Cancer Treatment

It has been estimated that there are now 1,500,000 individuals who are alive and well more than five years after treatment for cancer, and that another 700,000 who have been treated within the past five years are alive and well. It is also estimated that whereas in 1930 only one in five individuals with cancer survived five years after treatment, at the present time one in three of those treated is alive and well five years after treatment, and, under optimal circumstances, this figure may be as high as one in two. This improvement in cure rate is tangible evidence of the beneficial impact of the many advances in diagnosis and treatment which have stemmed from the Nation's investment in cancer research in the past 25 years.

Four Avenues of Advance

There are four chief avenues of advance toward cancer prevention and the cure of more cancer patients. These are:

1) Cancer prevention. The elimination from the external and internal environment of chemicals and other agents that produce cancer.

2) Earlier cancer detection. Improved means of cancer detection will make it possible to apply present and new means of treatment more effectively.

3) Improvements in the availability and application of present means of therapy.

4) The development through research of new methods for prevention and treatment.

It is the judgment of the Panel that the major gains in the effort to eradicate cancer will come from new developments achieved through research. The Panel is persuaded that the breadth and depth of cancer research has matured to a level where clear avenues are seen in need of intensive exploration.

Cancer Prevention

Cancer prevention offers greater possibilities for the control of cancer and the saving of human lives than any other measure presently at our command. Many human cancers can be prevented. Relationships have already been established between the occurrence of certain cancers and exposure to specific environment factors. By application of this knowledge, these types of cancer can be prevented or avoided before the complexities of their cause and development have been solved. Previously prevention has been applicable only to small high risk groups exposed to specific occupational hazards. Today cancer

prevention is entering a new era. Practical preventive measures can be applied to more types of cancer and, most important, to large masses of the population.

Studies in cancer epidemiology reveal that particular kinds of cancer are far more common in particular geographic areas than in others. By identifying the environmental agent that is responsible for the high cancer incidence in a particular region, it may be possible to remove it not only in that area but wherever it is present.

CANCER AND THE CIGARETTE SMOKER

The most important environmental causal agent in the production of internal cancer today is the prolonged inhalation of cigarette smoke, which is now recognized as the major cause of lung cancer. More recently strong evidence has been developed that there is a substantial increase in the mortality rates for smokers, and especially cigarette smokers, from cancer of the oral cavity and pharynx, and that cigarette smoking is a significant factor in the causation of cancer of the larynx. It is tragic that the public has been so slow to recognize that cancer of the lung, which is the largest cause of cancer deaths among men and the most rapidly increasing cause of cancer deaths among both men and women, is largely avoidable. Cancers of the oral cavity, pharynx, and larynx can probably also be prevented. The major cause of these cancers is known—the problem is one of how to convince people to achieve their own survival and to mobilize social forces toward this goal.

OCCUPATIONAL AND OTHER CARCINOGENS

Many environmental factors that promote the development of cancer have been identified, and measures have been taken to eliminate them or guard against exposure to them. The first to be so identified and eliminated was the occupational exposure to coal tar which caused cancer among chimney sweeps. Prolonged over-exposure to sunlight, to ultraviolet lamps, to arsenic, and to certain other oils and chemicals has been shown to cause cancers of the skin. In the chemical industry, cancer of the bladder that developed in workers exposed to betanaphthylamine has been eliminated. Cancer of the lung is being controlled on the Colorado plateau by protecting miners against the excessive inhalation of radon gas. Similarly, other lung cancers are being avoided in workers by preventing the inhalation of dusts from chromate ores. The association of asbestos exposure with an unusual type of lung cancer has led to similar protection against inhalation. Fatal bone cancers in women that resulted from the ingestion of minute quantities of radium over a period of years have been eliminated by stopping their practice of "pointing" their brushes in their mouths as they painted luminous dials on watches and instruments. Cancer of the nasal sinuses occurring in a high incidence in men refining nickel ores is now being avoided by protective measures.

The role of radiation as a carcinogenic agent is much better understood. Effective means have been developed for its detection. Strict regulation and the use of efficient radiation barriers and other protective devices have been instituted to prevent leukemias and thyroid cancers, which can result from radiation exposures.

Identification of these cancer-causing agents and the development of practical methods to reduce or eliminate exposure to them has resulted directly in decreased incidence of the disease in all instances. These results not only save lives now, they confirm that such cancer prevention methods are effective when they can be applied.

AIR POLLUTION

Among the most potent of the carcinogens to which man is constantly exposed are the polycyclic hydrocarbons which result from the combustion of fuels to run our power plants and motor vehicles. The presence of known carcinogenic hydrocarbons in the atmosphere of of large population centers is well established. Although epidemiolog studies of lung cancer make it clear that smoking is by far the most important cause of lung cancer, there are, nevertheless, sufficiently large differences in cancer incidence between urban and rural nonsmokers to indicate clearly that atmospheric carcinogens plan an important role in the development of lung cancer.

OTHER ENVIRONMENTAL CANCERS

Cancer of the penis has been greatly reduced by circumcision and promotion of personal cleanliness. It could be practically eliminated by circumcision prior to puberty.

Oral cancers develop in a high percentage of habitual snuff users. Although this practice is common among certain groups in this country and these cancers can be prevented, there is no organized educational program to achieve this end.

ELIMINATION OF CHEMICAL CARCINOGENS

In recent years, interest has developed in the intricate problems of the carcinogenic potentials of pesticides, herbicides, and food-processing agents—such as colors, flavors, emulsifiers, anti-oxidants, and fungal contaminants. Cosmetics, certain medical preparations, and a host of industrial waste products are under similar scrutiny.

A large number of chemicals have now been identified as capable of causing cancer. Because the possibility of controlling chemical carcinogens is real and the number of potential carcinogens in our environment is so vast, a major program is needed in this area. Such a program, which includes means for screening chemicals and the development of new criteria for their identification, has been developed but awaits execution for lack of resources. Once such agents were identified, it would probably be possible to establish methods for protecting the public from exposure to them.

PREVENTIVE SURGERY

Cancer prevention also includes the recognition and surgical elimination of precancerous conditions in the mouth, chronic ulcers in the stomach, and polyps in the colon. Other examples are chronic burn

scars and, perhaps, other scars which break down constantly, and reheal, nodules in the thyroid gland, particularly solitary nodules, and pigmented moles, particularly on the feet.

The term precancerous lesion is sometimes disputed, because it is difficult or impossible to show that these cancers which arise in such lesions actually represent a transformation from the benign to the malignant state. It is sometimes argued that those that end up malignant were malignant from the beginning and those that are benign will be forever benign; there are no means to distinguish reliably all benign from malignant lesions early in their course.

In either case, however, it has been demonstrated by experience that such lesions are best removed providing that the removal is a low-risk procedure.

Cancer Detection

If cancers are discovered when they are still localized, a majority of them can be cured by surgery and radiotherapy. Early detection followed by prompt, appropriate treatment is the most effective method of reducing deaths from cancer now. Table II compares five-year survival rates among patients with localized cancers with survival rates for patients whose cancers were spread beyond their site of origin at the time of treatment.

It is the capacity of cancer to grow and disseminate that makes early detection of the disease so important. A cancerous tumor of 1 cubic centimeter (approximately $\frac{1}{16}$ cubic inch) in size weighing about 1 gram (approximately $\frac{1}{30}$ ounce) is about the smallest that can be detected by palpation or by x-rays, yet it contains about one billion cancer cells, each perhaps capable of originating a new focus of disease. Discovery of cancers when they are considerably smaller—microscopic in size—should materially increase cure rates.

The past decade has witnessed startling innovations in the development of methods and instruments for cancer detection as well as the perfection of established procedures.

EXFOLIATIVE CYTOLOGY

The cells that line the internal surfaces of the body are constantly shed from these surfaces; each cell has a lifespan of only two or three days. If a cancer, is present—even one that is microscopic in size—cancer cells will appear among the discarded cells. Exfoliative cytology involves the examination of fluids containing such discarded cells in order to detect any hidden, early cancer.

In cancer of the cervix

During the past 10 years, the most notable progress in this field has been in the early detection of asymtomatic cancer of the uterine cervix and the detection of cellular changes which may signal the appearance of such a cancer. In this latter group the lesions are easily destroyed by either minor surgery or radiation and cancer prevented. In the former group, cancers that have not yet invaded extensively, treatment is effective in more than 90 percent of all cases.

The Papanicolaou method, as it is called, is not new, but in the past 10 years there has been great refinement of the technique, education of physicians and the public concerning the availability and usefulness of the procedure, and the training of technicians to make possible the screening of large numbers of women. The importance of these advances cannot be overemphsaized; on a worldwide basis, cervix cancer is the most common form of cancer in women. Among all women in the United States, it is second only to cancer of the breast and, in low income groups, it is even more prevalent than breast cancer.

Mass screening data obtained from selected populations indicate that both the occurrence of cervix cancer and the deaths from cervix cancer can be greatly reduced if every woman is tested annually.

In lung cancer

Although attempts at mass screening of the general population for lung cancer through the cytologic examination of sputum does not seem feasible at this time, the screening of selected high-risk groups has proven to be practical. An example is the screening of uranium miners in the Colorado plateau. Epithelial changes which precede cancer have been detected. In cases in which such changes were detected and exposure was continued, cancer did develop in subsequent years. Other examples of high-risk groups are heavy smokers over the age of 40, asbestos workers, and persons with unexplained respiratory symptons.

In bladder cancer

Cytologic examination of the urine can reveal asymptomatic cancer of the urinary bladder. Routine examination of urine samples of members of high risk groups such as aniline dye workers has proved practical. The technique is also important in following patients with previous treated bladder cancer to detect any possible recurrence of the disease.

In gastric cancer

Cytologic screening for gastric cancer has not proved rewarding in the United States where there is an unexplained and very impressive decrease in the incidence of this disease. The technique has proved to be practical in high-risk groups, such as the Japanese, and in such groups has been effective in detecting the early, more curable, stage of the disease.

In other types of cancer

The development of cytology has been very important in the diagnosis of cancer in many areas of the body (mouth, colon, kidney), although it has not yet been useful in the detection of asymptomatic cases.

DIAGNOSTIC RADIOLOGY IN CANCER DETECTION

Most human cancers, even in their earlier stages, deform normal anatomic structures in characteristic ways. Because the display of normal and deformed anatomy is the essence of diagnostic radiology, it has a major role in the detection and control of many forms of

cancer. Its highly developed techniques serve to detect symptomatic cancer, to determine its extent, and to observe the effects of treatment.

The detection of cancer at a stage when the patient has noted some clue—pain, bleeding, a mass, fatigue, etc.—has been one of the most productive functions of radiology. Methods are also being developed for the use of diagnostic radiology to detect cancers before symptoms appear.

Mammography

Mammography, the examination of the breasts by special x-ray techniques, is an important new development in the control of breast cancer. Mammography is often capable both of detecting tumors so small that they cannot be palpated by the examining physician and also of distinguishing benigh from malignant tumors. The availability of a new diagnostic technique for this extremely common form of cancer has had several effects:

1) A revitalization of the interest of the medical profession in the detection of early breast cancer.

2) A better understanding of the bilaterality and the natural course of the disease.

3) Stimulation of the pathologists to find the small cancers and premalignant changes in the breast by multiple whole organ sections and radiography of the specimen.

4) Earlier and more comprehensive treatment of the patient as a result of teamwork by the surgeon, radiologist, pathologist, and chemotherapist.

X-ray examination of other organs

Shortly after the discovery of x-rays, methods to examine the hollow organs, especially the gastrointestinal tract and lungs, were developed. The GI series and the barium enema, as they are known, today remain the most decisive clinical methods, short of actual tissue examination, for detecting cancer in the esophagus, stomach, and large bowel, and for differentiating it from other types of disease that may cause similar symptoms.

In the air-filled spaces such as the nasal sinuses, the larynx, and particularly the lungs, simple x-rays are similarly important. Moreover, special methods to show in great detail the surface of the larynx and the airways of the bronchial tree are in daily use. Ingenious devices to thread catheters and small brushes into the extensively branching system of the bronchi of the lungs are now available. These devices, combined with fluoroscopically controlled needle biopsy directly through the chest wall, extend the usefulness of chest radiography from simple visualization of tumors to actual nonsurgical tissue sampling.

The nervous system—the brain and the spinal cord—contain spaces that can be made temporarily visible. Uses of special techniques for visualizing the spinal and subarachnoid spaces, of the cavities in the brain, and the blood vessels, and for seeing the lymph nodes, have brought cancer deformity of these structures under clinically useful scrutiny.

TABLE II.—5-YEAR SURVIVAL PERCENTAGES RELATIVE TO NORMAL LIFE EXPECTANCY

	Regional	Localized
1. Ascending colon:		
Male	32	74
Female	45	83
2. Transverse colon:		
Male	36	70
Female	47	70
3. Descending colon:		
Male	39	68
Female	41	76
4. Sigmoid colon:		
Male	38	71
Female	44	74
5. Rectum:		
Male	29	63
Female	32	66
6. Stomach:		
Male	13	36
Female	13	44
7. Nose, nasal cavity, pharynx:		
Male	24	43
Female	38	62
8. Lung and bronchus:		
Male	7	25
Female	8	35
9. Larynx:		
Male	29	76
Female	35	74
10. Breast: Female	52	83
11. Uterine cervix	46	79
12. Uterine corpus	52	84
13. Prostate	47	62
14. Testis	61	84
15. Kidney:		
Male	30	62
Female	36	60
16. Bladder:		
Male	26	69
Female	27	73
17. Melanoma of skin:		
Male	38	75
Female	36	79

Source: "End Results in Cancer," Report No. 3; U.S. Department of Health, Education, and Welfare, 1968.

These relatively new methods, while useful for initial cancer detection, have greater application in evaluating the extent of known cancer so that treatment, whether surgical, radiation, or with drugs, can be rationally selected and delivered. In staging the lymphomas, Hodgkin's disease, and testicular neoplasms, radiography of the lymph nodes has proved to be a very useful development in recent years. And similarly, though to a lesser extent, radiography of the blood vessels is employed to stage uterine and bladder cancer and to determine the feasibility of surgical removal of cancer of the lung and liver. But these special methods are not always required for evaluation of the extent of cancer. In fact, the simple chest film and skeletal survey are indispensible means for establishing distant metastasis to the lungs and bones.

X-Ray screening

Another function of diagnostic radiology is the x-ray screening examination of large numbers of people for symptomless cancer, as in the use of mammography in this country or gastric cancer in Japan. Similar efforts to detect curable lung cancers by x-ray examinations have been less encouraging.

Diagnostic radiology is helpful to the cancer patient not only in primary diagnosis and evaluation of extent of disease but in appraising the effectiveness of treatment. All its methods from simple fluoroscopy and radiography to highly specialized procedures are available for the

problems such as the response to radiation or drugs, the question of local recurrence, the management of complications of treatment, etc. Diagnostic radiology is essential throughout the course of most cancers whether or not they are successfully controlled.

ENDOSCOPIC EXAMINATIONS

Significant progress has been made over the past few years in the endoscopic examinations of the esophagus, stomach, and colon, largely due to the development of special techniques for photographing these areas and, in particular, the perfection of flexible fiber optic instruments. These instruments permit a complete examination of the esophagus and stomach with excellent photographic documentation. Biopsies and cytologic washings can be obtained in about 90 percent of the malignant lesions that are seen.

Fiber optics have been adapted to sigmoidoscopy, and instruments have been developed that can reach as far as the cecum. However, they have not proven to be practical for large-scale use because of their cost and because they are only slightly more effective in the detection of cancers above the 25 centimeter level than the standard sigmoidoscope and barium enema examination.

ULTRASONIC TECHNIQUES

Pulsed echo-ultrasound is a relatively new medical diagnostic tool for the detection of cancer. By this method, cross-sectional pictures (ultrasonograms) of a tumor can be obtained, outlining the extent of the lesion as well as other physical characteristics. The ultrasonic method is capable of outlining soft tissue structures in detail without distortion of the measurements or of the outline of the tumor. The widest applications of this technique have been for the detection and location of tumors in and around the eye.

Ultrasonography has proved to be a practical technique for demonstrating a midline shift when a space-occupying lesion may exist in the brain. This procedure has also been used for the detection and evaluation of gynecological cancers as well as in the differentiation of kidney cysts from cancers. Its possible usefulness in the detection of breast cancers is also being explored.

CHEMICAL TESTS AND MEASUREMENTS—AUTOMATED MULTIPHASIC SCREENING PROGRAM

The screening of large segments of the public in order to detect asymptomatic cancers offers the best possibility of improvements in cancer diagnosis. One of the obstacles to mass screening programs is the shortage of personnel trained in such procedures. One solution involves the development of devices for the self-collection of material for tests. For example, a simplified test has been developed for cancer of the gastrointestinal tract in which a patient takes his own stool samples, using a special kit, and forwards them to his physician who examines them for occult blood.

Chemical measurements in the detection of cancer are largely limited at present to hormone and enzyme assays in serum and in urine. These include urinary tests for chorionic gonadotropin in

choriocarcinoma and tests for acid phospatase in carcinoma of the prostate. It is impossible to predict with precision the outcome of the research to extend such methods to the detection of other forms of cancer. The goal of such research will be the development of precise high-speed chemical and physical measurements of cancer growth far superior to those obtained by the present methods of physical diagnosis, and this seems to be within the bounds of reasonable expectation.

Automated multiphasic screening examinations have become a reality in almost every major hospital today. They utilize automated and semiautomated electronic and mechanical equipment to rapidly provide comprehensive quantitative and precise testing for a large number of diseases. This procedure can incorporate a statistical method which automatically determines the likelihood of selected diagnoses, according to predetermined sensitivity and specificity criteria. It is now being used in pilot programs which will include cancer detection examinations for certain cancers and for the recognition of high-risk individuals.

IMMUNOLOGICAL TESTS FOR CANCER DETECTION

Last year it was reported that a fetal antigen (called CEA, for carcinoembryonic antigen) is present in the blood of patients with cancer of the colon. This fetal antigen disappears from the blood of patients treated successfully but not from the blood of those in whom the cancer has spread.

The presence of another such fetal antigen, alpha-fetoprotein, has been demonstrated in the blood of patients with primary cancer of the liver.

Still another fetal antigen system, unrelated to the previous ones, has been discovered to be associated with a wide variety of human cancers from the breast, colon, lung, and ovary.

These fetal antigens can be detected by a radioassay test that is capable of identifying the CEA in as little as one-billionth part in the serum. This promises to provide both an accurate screening test for cancers of the bowel and also a means of determining whether a cancer has been removed completely by surgery. Patients who are found to have the antigen in their blood can be given barium x-ray examinations and other diagnostic tests to locate the cancer and confirm the diagnosis.

If the CEA test proves to satisfy the rigid protocols of biological testing and verification, an important goal will have been achieved—a means for the earliest possible detection of a major cancer. Similarly, the other two antigenic systems have great promise as a method of early detection of the presence of cancers of these sites. In addition, all three systems should provide much needed precise techniques for the evaluation of the results of therapy.

Cancer Treatment

Surgery

Today surgery offers patients with many types of cancer more chance for a cure than any other known treatment. For this reason, surgery is the most widely used form of cancer therapy. Surgical procedures have inherent limitations in the total cure of the cancer

patient, but their great importance in the management of early cancer now and in the foreseeable future makes it imperative that all areas of possible improvement be exploited.

CURATIVE SURGERY

To cure cancer by surgery, the surgical treatment must take place before the cancer has spread and become established beyond the tissues that can be removed. During the past century, increasingly extensive operations for cancer were devised and evaluated, and such procedures are now carried out wherever good cancer care is practised.

Despite the increasing extent of the procedures, the risk of surgical operations for cancer has been progressively reduced and is still being reduced. This has permitted application of surgical methods to more and more persons whose cardiovascular status, kidney function, or general metabolism is seriously impaired. Such concomitant handicaps are of course frequent in the older age groups. This lowering of the operative risk is absolutely crucial to the application of preventive surgery, the removal of suspicious tumors or ulcerations, or the removal of certain pathological areas which predispose to cancer.

Recent attempts to establish even more extensive surgical procedures have not resulted in increased rates of cure, and there now appears to be relatively little prospect of major advances from a more extensive removal of tissues.

Organ transplantation

It is possible that perfection of techniques of organ transplantation may extend the possibilities of cancer cure. The potential of organ transplants in cancer control is just beginning to be realized. Surgical techniques, organ preservation, tissue typing, and immunosuppression now have been developed to the point where replacement of vital organs irreparably damaged by cancer is occasionally possible, but better techniques of immunosuppression are urgently needed.

Total hepatectomy with orthotopic liver homografting offers future hope of possible cure to patients with hepatoma, gall bladder cancer, or bile duct cancer spread beyond the level of partial hepatic resection. It might also be useful in certain instances of metastatic cancer (particularly of the colon) where the liver is the only apparent site of metastases.

An equal or greater need exists for lung transplantation. Primary and metastatic lung cancer is a common problem in cancer patients and accounts for a high proportion of cancer deaths.

The ability to transplant intestine is essential to control some abdominal cancers. Sacrifice of the superior mesenteric vessels with death of small intestine is necessary if some cancers are to be surgically encompassed.

Research in transplantation biology for application in cancer patients must seek immunosuppression which is precise and unattended by risks now present. Techniques of tissue typing, and alteration of tissue antigenicity are promising areas for extended research.

Even without transplantation, however, surgery will continue for some time to be the most important form of cancer therapy. Additional major gains to the cancer patient from surgery are expected as better public education, and better education of physicians, dentists, and

other persons consulted about health problems lead to detection of more and more cancers before they have spread. If prompt and thorough surgery by well trained and qualified cancer surgeons were available to all persons today, there would be an immediate and striking rise in the rate of cure of many types of cancer.

IMPORTANCE OF EARLY DIAGNOSIS

Good surgical treatment is now able to cure, by the five-year criterion, at least 95 percent of patients with skin cancers, about 60 percent of women with breast cancers (virtually all of those in which the disease remains localized), about 40 percent of patients with cancers of the colon and rectum, and 70 percent of women with cancers of the uterus. However, relatively few patients with cancer of the lung, stomach, or pancreas are being cured. In virtually every category there is a striking difference in cure rates between patients with localized cancer, whose regional lymph nodes have not become involved, and patients in whom regional lymph node involvement is present. (Such involvement is indicative of probable further spread of the disease.) (See Table II.)

Clearly one of the reasons surgery is not universally more successful is the continuing failure to diagnose cancers early. By the time the surgeon sees the patient, the cancer has metastasized and is beyond his reach. This failure is due to several factors: the natural biological behavior of certain cancers which do not cause symptoms until they have spread; fear, indifference, or a lack of alertness on the part of the patient; insufficient knowledge, training and equipment of some physicians; the overwork or carelessness of competent physicians. Almost all of these reasons reflect in some way the shortage of properly trained doctors and health educators.

PREVENTIVE SURGERY

Surgery is increasingly more valuable as a prophylactic procedure in cancer therapy. For instance, at one university hospital, 13 percent of the operations done on the surgical service were preventive in nature in 1935, 23 percent in 1945 and about 30 percent in 1955 and in 1965. This vital role is not reflected in the survival figures since by its application, cancer has been prevented. Preventing the future development of cancer by the timely removal of precancerous growth may eventually be surgery's greatest contribution to the control of cancer.

RECONSTRUCTIVE SURGERY AND REHABILITATION

A major frontier in the surgical therapy of cancer is in reconstruction. The plastic and reconstructive surgeons are increasingly finding ways to restore either function or appearance or both after extensive surgical procedures. The knowledge that such reconstruction is possible often determines whether or not a patient will accept an otherwise disfiguring or incapacitating procedure. Perfection of reconstructive surgical techniques performed immediately after cancer removal has markedly shortened the convalescence time of patients with sometimes major surgical losses. By the use of new graft techniques and new anatomical understanding of the source for tissue flaps, it has been possible to reconstruct parts of the head and neck in the same

operation at which the cancer is removed. Such speedy reconstruction markedly aids in rehabilitation, allows earlier additional treatments to the cancer, when necessary, and shortens hospital stay.

PALLIATIVE SURGERY

In addition to the role of surgery in saving lives by eradicating cancer, the surgeon is also frequently called upon to improve the remaining months or years of a patient whose cancer cannot be eradicated. Much judgment must be exercised, however, because the objective is to prolong enjoyable and productive life—not to prolong agony.

For those patients in whom the growth of cancer leads to pain, the neurosurgeon can often bring great relief by dividing the nerve pathways which carry the painful sensations. In the lower two-thirds of the body, this can usually be done with minimal impairment of other functions. Numerous other surgical procedures, which go under the general designation of palliative surgery, are of great help to the individual patients. They often contribute significantly to longevity and, if they can bring comfort and usefulness back to the patient, they are worth the considerable effort which is often involved.

NEED FOR CANCER SURGEONS

Greater numbers of qualified cancer surgeons are needed. Large medical centers are usually staffed with excellent cancer surgeons but this is not so often the case in less populated regions. Increasing the number of competent cancer surgeons can improve the treatment of cancer and therefore increase the number of lives saved.

The training of surgeons in cancer surgery can better be done, however, in a cancer center, where large numbers of patients with many types of cancer can be cared for during the years of a surgeon's training. Here it is possible for the surgeon to learn to understand the importance of extensive and thorough procedures for cancer cure and also the roles of preventive and reconstructive surgery. The trainee can also have broad exposure to the problems and roles of radiation therapy and chemotherapy. He can most easily follow the advances in fundamental biology and immunology. The surgeon trained in a cancer center should have a more thorough and practical insight into the various methods of early detection and understand their possibilities and limitations.

ROLE OF SURGERY

In summarizing our consideration of the role of surgery in cancer, one of the Panel of Consultants wrote:

"The long-term future may belong to the immunologist and the geneticist, the intermediate future to the chemotherapist, but the present and the immediate future belong in the main to the surgeon and to some extent to the radiologist.

To state the matter another way, to the extent that curative chemotherapy is developed first, surgery will be needed less, but in those fields in which early detection is developed first, it will be largely surgery which will convert these gains in information into cured patients by extirpation of the cancer before it has spread.

It therefore becomes a vital part of the attack on cancer to provide the physical facilities, the incentives to attract able persons, the educational programs to prepare excellent surgeons, and the incentives to achieve a broad delivery of their services to all who require them."

Radiation Therapy

Radiation therapy is second in importance only to surgery as a means for the curative treatment of a wide range of cancers. In addition, it has an important role in palliative treatment of cases that are too far advanced for cure. It makes use of the ionizing radiations—x-rays and electrons, produced by man-made machines, and gamma rays, which emanate from naturally or artifically radio-active elements—to destroy cells by injuring their capacity to divide. Since the central attribute of cancer cells is their sustained, uncontrolled, lawless proliferation, injury to this property is precisely what is desired. Although some rapidly dividing normal cells are also killed during the radiotherapeutic eradication of a cancer, the large reservoir of similar normal cells outside the irradiated field is readily able, in most instances to replenish the supply and to repair the irradiated tissues. Thus, in favorable cases, the cancer gradually disappears completely during treatment, the acute radiation reaction in the normal tissues then slowly subsides, and one or more years later, the patient may present no external evidence whatever of having been treated. Currently, radiation therapy is used either for cure or for palliation in an estimated 50 to 60 percent of all cancer cases at some stage in their evolution. However, the development of radiation therapy in the United States has been hampered by a serious shortage of medical and paramedical manpower, lack of research funds and of capital funds for major equipment and facilities, and ineffective organizational relationships.

ADVANCES IN TECHNOLOGY

Despite these continuing problems, radiation therapy has made remarkable strides in the past 20 years, largely due to the technological development of machines producing beams in the multimillion electron volt (megavoltage) energy range. Such beams can deliver a greater dose to deep-seated tumors without the serious skin reaction and discomfort that formerly limited treatment with lower energy (kilovolts) x-ray beams. Large areas can be irradiated with more precise definition of the margins and the alignment of the beam, less side-scatter and thus better protection of adjacent vital structures, and minimization of the volume of normal tissue encompassed within the high dose region. Differential absorption of radiation in bone relative to the soft tissues, a serious problem with kilovoltage x-rays, is almost nonexistent at megavoltage energies, with the consequence that dosage is more homogeneous. Finally, the greater versatility of such machines has made possible the development of highly sophisticated treatment techniques employing multiple intersecting beams, rotational therapy, large fields elaborately shaped to the contours of particular organs, and pinpoint beams for cancers in the eye and the larynx. For all of these reasons, megavoltage x-rays and electrons from linear accelerators and betatrons and gamma rays from radio-

active cobalt 60 teletherapy apparatus have become the standard modality of radiation treatment for deep-seated cancers today. An increasing number of scientific reports testify to their efficacy, as indicated by substantially improved survival rates of patients with a variety of types of cancer. Comparative five-year survival rates from represenative series of cases treated with kilovoltage x-rays 15 or more years ago, and with megavoltage x-rays or gamma rays in recent years, are presented in Table III.

TABLE III.—IMPROVED SURVIVAL OF SEVERAL TYPES OF CANCER WHEN TREATED WITH MEGAVOLTAGE RADIOTHERAPY

Type of cancer	Representative 5-year survival (percent) with kilovoltage X-rays	Representative 5-year survival (percent) with megavoltage X-rays
Hodgkin's disease [1]	30–35	70–75
Cancer of the cervix	35–45	55–65
Cancer of the prostate	5–15	55–60
Cancer of the nasopharynx	20–25	45–50
Cancer of the bladder	0–5	25–35
Cancer of the ovary	15–20	50–60
Retinoblastoma [2]	30–40	80–85
Seminoma of the testis	65–70	90–95
Embryonal cancer of the testis	20–25	55–70
Cancer of the tonsil	25–30	40–50

[1] A cancer arising in lymphatic tissues. Formerly believed inevitably fatal, it is now being permanently cured in a high proportion of cases.

[2] A cancer of 1 or both eyes which occurs in young children. In addition to improved survival, megavoltage radiotherapy has permitted preservation of good to excellent vision in many cases and is now being used instead of surgical removal to treat the 1st involved eye.

EVALUATION OF THERAPIES

Randomized clinical trials have been introduced into clinical investigations in the field of radiotherapy during the past 10 years. They provide a statistically powerful and rigorous method for the objective assessment of new techniques and concepts of treatment. Patients are allocated at random to one of two or more alternative methods of treatment in accordance with carefully planned, controlled protocols which also stipulate all relevant medical safeguards. For those types of cancer that are not sufficiently abundant to permit a single hospital center to develop a statistically valid series, a group of centers or hospitals follow the same protocol and pool their data. Such cooperative clinical trials, though costly and organizationally complex, have the advantage of greatly accelerating the accumulation of data on sufficient numbers of cases to provide definitive conclusions. Among the clinical trials currently in progress or recently completed are tests of the efficacy of preoperative radiotherapy in lung cancer, of localized vesus total lymphoid radiotherapy in early stages of Hodgkin's disease and other malignant lymphomas, of radiotherapy versus combination chemotherapy in advanced Hodgkin's disease, of radiotherapy versus surgery in localized cancer of the prostate, of combined chemotherapy and raeiotherapy in advanced cancers of the oral cavity and pharynx, and of radiotherapy alone versus combined radiotherapy and surgery in cancer of the urinary bladder. As these studies reach completion, they will provide indisputable new standards of excellence in treatment which may be expected to be adopted by the medical profession throughout the nation.

RADIOTHERAPY AND THE CANCER CENTER

Modern megavoltage radiotherapy apparatus is expensive and requires major capital investment in specialized facilities to house such equipment. Moreover, the effective utilization of such facilities and equipment requires a large and diversified staff, including not only radiation therapists but also such paramedical personnel as physicists, dosimetrists, radiotherapy technicians, and social workers. These complex requirements are most readily satisfied by centralization of radiotherapy departments in major cancer hospitals and university medical centers. Radiotherapy departments have become an essential component of multidisciplinary cancer treatment programs in all major cancer centers, state cancer hospitals, and many of the mation's university-based medical centers. The development of these departments and the increasing efficacy and scope of megavoltage radiation therapy have in turn improved the status of radiation therapists, enhanced the recognition accorded to radiation therapy as a medical specialty, and attracted increasing numbers of outstanding young medical graduates to careers in this field. By concentrating the radiotherapeutic care of large numbers of cancer cases, such centers have greatly expedited and facilitated the early assessment of end results of new techniques of radiotherapy used alone or in combination with other modalities of treatment.

RESEARCH IN RADIOBIOLOGY

An integral component of many of the larger radiotherapy departments is an active fundamental research program in cellular and molecular radiobiology, biophysics, and radiologic physics. Indeed, these are now the areas of greatest promise for the future of radiation therapy. It is imperative that the complex processes by which ionizing radiations damage vital macromolecules and lethally injure the cells in which they reside be fully elucidated. Thorough comprehension of these phenomena is likely to reveal a variety of approaches whereby the efficiency of cell killing can be enhanced or modified, with potential therapeutic benefit.

Radiobiological investigations on mammalian cells and tumors have made dramatic advances in the past 15 years. It is now established that the killing of cells by ionizing radiation, both *in vivo* and in clonal culture, is an exponential function of dose, beyond an initial low dose range in which the survival curve exhibits a "shoulder." When the same total dose is split into two fractions separated in time by an increasing interval, survival increases for the first two to four hours, then decreases during the next three to four hours, and finally increases again, with concomitant restoration of the "shoulder" to the survival curve. It is thus apparent that the "shoulder" represents a recovery process, which is operative at low doses but becomes overwhelmed as the dose increases. Split-dose recovery is exhibited by normal and tumor cells alike. The fact that recovery can be blocked by low temperature, certain chemical agents such as Actinomycin D, and at least in some cells by hypoxia suggests that it is an energy-consuming enzymatic process, but neither the identity of the molecular lesion nor the mechanism of its repair are yet known.

DNA breaks

The principle macromolecular target in the cell is its DNA. In bacteria, it has been shown that ionizing radiation produces both single and double-strand breaks in DNA, and several lines of evidence suggest that the double-strand breaks account for most of the lethal events. The halogenated thymidine analogs, 5-bromodeoxyuridine (BUdR) and 5-iododeoxyuridine (IUdR) may be incorporated into DNA in place of thymidine. The powerful radiosensitizing effect which they exert when thus incorporated has been shown to be due to an increased yield of double-strand breaks in DNA. Since the actively dividing cells of most cancers would be expected to synthesize DNA more often, on the average, than many normal tissues, it was quickly appreciated that the halogenated pyrimidines might be therapeutically useful as differential radiosensitizers of cancer cell populations. Clinical trails of radiotherapy in conjunction with BUdR, infused intra-arterially into advanced cancers of the oral cavity and pharynx and into highly malignant glioblastomas of the brain, have been in progress both here and in Japan. They are currently in abeyance in this country, however, for lack of clearance by the Food and Drug Administration for additional supplies of the drug. Preliminary results of BUdR infusion and radiotherapy in the brain tumors have been extremely encouraging.

Single-strand breaks produced in DNA by ionizing radiation are much more numerous but rapidly undergo enzymatic repair and are thus not lethal under ordinary circumstances. However, the fact that certain bacterial mutants which lack one or another of the multiple enzymes involved in the repair process are also exquisitely radiosensitive suggests that single-strand breaks, when unrepaired, may also be lethal for the cell. An exciting new line of investigation has recently been opened up by the discovery that several drugs and chemical compounds are able, when added to cell cutlures soon after irradiation, to potentiate cell killing and to block single-strand repair. Among the agents already shown to have such dual action are acriflavine, chloroquine, quinacrine, and an unidentified derivative of hydroxyurea. A possible clue to their mechanisms of action may reside in the fact that several of them are known to bind to DNA and may thus interfere or compete with the binding of a repair enzyme to a site of injury. There is an urgent need for intensified investigation of this new approach, since some of these compounds are known to be relatively nontoxic in man. Moreover, one of them has been reported to exhibit a higher uptake and concentration in the cells of certain tumors than in normal cells; this is exciting because such differential effects in tumor cells are a requisite attribute for any clinically useful chemical radiosensitizer.

OXYGEN AND RADIOSENSITIVITY

It has long been known that cells are nearly threefold more radiosensitive in the presence of oxygen than in its absence. Many cancers outgrow their blood supply, and the presence within them of necrotic foci strongly suggests that those cancer cells that have come to lie more than 150 to 200 microns from the nearest capillary are severely hypoxic or anoxic. This has suggested that the recurrent growth of

many cancers after an initially good radiotherapeutic response may be due to the survival of microscopic foci of such hypoxic, radioresistant tumor cells which, after shrinkage of the tumor, are once again brought into proximity with blood vessels, can obtain oxygen and nutrients, and thereupon resume active proliferation. Since the central problem is the differential impairment of oxygenation within parts of the tumor, as contrasted with the rather uniformly complete oxygenation of the adjacent normal tissues, it is clear that it can be circumvented either by supplying more oxygen to the hypoxic tumor cells or by reducing the level of oxygenation of the normal cells ot that prevailing within the tumor. The former can only be accomplished by treating patients within pressure chambers in which they breathe pure oxygen at three to four atmospheres of pressure; the latter can be achieved by the application of tourniquets during radiotherapy of certain relatively infrequent types of cancers which occur on an extremity. Unfortunately, after several years of clinical investigation, the initial promise of this approach has not been sustained by the results of carefully controlled clinical trials. A plausible explanation for this is the recent discovery that many types of experimental tumors exhibit reoxygenation of the initially hypoxic subpopulation of cell during the interval between successive daily fractionated radiation exposures. Fractionated radiotherapy, which has been used empirically for more than forty years because it was found to yield superior results, may owe its efficacy, at least in part, to the fact that reoxygenation occurs between treatments, thus obviating or minimizing the otherwise serious consequences of the presence of hypoxic tumor cells. The fact that reoxygenation failed to occur or did so only partially and sluggishly in certain experimental tumors suggests the need for careful studies of a much broader spectrum of tumors. If failure to reoxygenate is indeed a predictable characteristic of certain types of tumors, then radiotherapy in conjunction with hyperbaric oxygen may still have an important, though selective, place in their treatment.

ESTABLISHMENT OF OPTIMUM DOSE SCHEDULES

It is widely recognized that the empirically derived daily (five times per week) fractionation schedule which has been almost universally adopted is unlikely to be optimal for all types of cancer. There is an urgent need for detailed data on the cell population kinetics of a broad spectrum of tumors and their normal tissue homologs. With the aid of such data, together with information now being developed concerning other relevant parameters, it should be possible to generate mathematical models of optimal fractionation patterns for different types of tumors and then to test them against conventional daily fractionation in a series of randomized clinical trials. It is entirely reasonable to anticipate that substantial advances in cure rates for selected cancers would emanate from this approach. Meanwhile, one proposal has already come forth which may have value in disseminated lymphocytic lymphomas, lymphatic leukemias, and seminomas. Radiation-induced cell death for these highly radiosensitive cell types is known to differ from that for most other cells; instead of occurring during or after attempted mitosis, it occurs during interphase, prior to entering mitosis. Moreover, the survival curve for these cells is essentially exponential, with little or no "shoulder" and thus no split-dose recovery.

From this, it follows that more frequently spaced treatments, perhaps at four to six-hour intervals, utilizing very small doses in the "shoulder" region of the curve for most other cells, should cause lethal injury to be cumulative in the tumor cells, but not in critical normal cells such as those of the bone marrow or intestine, which have the capacity to recover repeatedly during the intervals between doses. A trial of this concept in experimental animals is now in progress.

NEUTRONS AND PI MESONS

The potential contributions of physics and technology to radiotherapy are by no means exhausted. Certain nuclear particles, notably neutrons and negative pi mesons, have attracted attention as substitutes for x-rays and electrons because they are much less dependent on oxygen and, in the case of pi mesons, have unique properties which would make possible a much more precise and selective localization of the beam energy to the volume occupied by the cancer. For these reasons, the technological advances represented by the pi-meson-producing proton accelerator under construction at Los Alamos Scientific Laboratory and the superconducting medical pi meson generator under development at Stanford are of great interest. The successful solution of the many technical difficulties which currently beset the development of neutron generators with a flux sufficiently great for medical use would also permit the experimental testing of the promise for radiotherapy which neutrons are believed by many radiotherapists to hold.

No discussion of the present status of radiotherapy in the United States would be complete if it dwelt only on the achievements and the potentialities of this modality, to the exclusion of the many serious problems which now confront it. There is a critical shortage of manpower in the field, not only of radiation therapists but in all of the relevant paramedical areas as well. If all radiation therapy were administered by highly-trained, full-time radiation therapists, it is estimated that 1,500 to 1,700 would be needed now and 2,000 by the year 1980. Yet, there are only some 400 full-time radiation therapists in the entire nation at present. Although special training programs, supported by the National Cancer Institute, have increased the total number of trainees from about 25 in 1960 to about 200 in 1970, recent cutbacks in funding have prevented the establishment of additional programs in several departments newly staffed and equipped to offer excellent training opportunities. The gap between the number of full-time radiotherapists available and the number needed has been filled until now by general radiologists, whose training in radiation therapy is much more limited and who are obliged to devote most of their time and energy to the practice of diagnostic radiology. It is thus not surprising that the level of competence for the delivery of radiation therapy to cancer patients exhibits such disparity. One to two dozen of the larger departments are equal or superior to the best anywhere else in the world, yet most of the nation lacks first-rate radiotherapy and suffers from its excessive decentralization among a large number of small community hospitals without adequate staff or facilities. One-fourth of our medical schools do not have a single full-time radiation therapist on their faculties. Although radiotherapy is well represented in the curricula of a few medical schools, it has grossly

inadequate time in most of the schools and none at all in several. Meanwhile, training in general radiology is being deemphasized in favor of specialized, separate training programs in therapeutic and diagnostic radiology. Although this is a highly desirable trend, there is cause for serious concern that, unless additional support is provided urgently for radiation therapy training, natural attrition among the large number of general radiologists now practising radiation therapy on a part-time basis will further aggrevate the existing manpower crisis in this field. Nor is the problem limited to physicians; there is an equally grave shortage of the paramedical members of the diversified team—the radiologic physicists, radiotherapy technicians, and dosimetrists—who are essential to the proper staffing of all modern megavoltage radiotherapy installations today.

Finally, many medical schools and large community hospitals remain without either megavoltage equipment or supporting facilities and are unable to obtain the major capital funds with which to procure them. There is thus an urgent need to bring the general level of staff, equipment, and facilities throughout the nation up to standards which are acceptable by today's criteria and to offer to all of our people the benefits of the many advances made during the past 15 to 20 years, while the major research-oriented radiotherapy departments continue to work, both in the laboratory and in the clinic, for further gains in the radiotherapeutic management of cancer.

Chemotherapy

In contrast to surgery and radiation therapy, chemotherapy—the treatment of cancer with drugs and with hormones—can be used effectively for disseminated as well as localized cancer. Chemotherapy has become a reality only in the past 30 years. Each of these three decades has seen important advances in the number of compounds available and in the spectrum of their usefulness. Within the last decade, it has become clear that, although chemotherapy had long been considered largely a palliative procedure, capable of extending but not saving lives, certain kinds of cancer can now be cured by chemical treatment. A major goal of current cancer chemotherapy is to achieve cures by prompt and vigorous treatment of such cancers.

Combinations of chemotherapeutic agents have been used with substantial success, particularly when each of the drugs used acts on the cancer cells in a different way. Major improvements in the treatment of certain types of cancer have been achieved both by using several of the active drugs simultaneously and by the use of different drugs in sequence.

In patients whose cancers are limited to a body region which has an accessible separate blood supply, the introduction of chemotherapeutic agents into an artery which supplies the cancer tissues has provided substantial benefits.

Cancers which can be cured by chemotherapy

Chemotherapy now constitutes a major and indispensable therapeutic approach, capable of producing cures, in particular kinds of widespread cancer.

Choriocarcinoma

Choriocarcinoma is a highly malignant cancer which originates in the placenta. It can be cured by treatment with a single drug in 25 to 50 percent of women, and by combination chemotherapy administered, either simultaneously or sequentially, in 75 percent of women. These cures can be achieved even when the cancer has spread to distant organs.

Metastatic hydatidiform mole

Metastatic hydatidiform mole is a more benign disseminated neoplasm of the placenta. It can be cured in 95 percent of patients by a single drug and, moreover, it can be prevented by prophylactic treatment, as demonstrated by recent international study planned, organized, and supervised in the United States, but conducted primarily in Asia, where the frequency of hydatidiform mole is much greater.

In a study undertaken to determine whether this form of tumor could be prevented by chemotherapy given prophylactically, 55 women were given three weeks of drug treatment after evacuation of hydatidiform mole. None of these developed metastases. In 65 women who were identically treated except for the chemotherapy, 8 developed evidence of metastatic or presistent neoplasia. These data provide clear evidence that prevention of spread of this neoplasm can be accomplished by drug treatment.

Burkitt's tumor

Burkitt's tumor is a highly malignant form of cancer which arises in the jaw and abdomen and often grows very rapidly to a large size. It occurs most frequently in children and, in some areas of Africa, it accounts for 50 percent to 70 percent of all cancer in children. It is especially common in the rain forest areas of the tropics, and it is also seen, although less frequently, among children in the United States (See: Viruses and Cancer). During the early 1960s, the occasional cure of this disease by chemotherapy with methotrexate or various alkylating agents was achieved by several groups. More recently, much improved results using massive intermittent doses of cyclophosphamide have been reported by a unit of the National Cancer Institute working in Africa. Present data demonstrate that the disease has been cured in half of all children treated and in 90 percent of these in whom the disease was treated early.

Acute leukemia of children

Acute leukemia of children is the most common form of cancer in childhood in the United States. Before chemotherapy, the median survival of children with acute leukemia was four months and long-term survivals were virtually unknown. By progressive improvements in the availability of drugs and in their use, the median survival of the children treated in many clinics now is three years, and some children have been living without evidence of disease for more than five years. A survey of the world's hematologists indicates that 52 children who had acute leukemia have survived for more than 10 years and 50 of these have no evidence of disease. Therefore, presumptive cures have been achieved. Moreover, the increasing proportion of treated children who have long survival periods without recurrence of the disease demonstrates that better treatment is already at hand.

Hodgkin's disease

Hodgkin's disease is a unique and specialized form of cancer affecting the lymph nodes, spleen, liver, lungs, and bone marrow in various stages of its spread. It is possible to cure 75 per cent of patients with early Hodgkin's disease by aggressive radiotherapy but, until recently, there was little hope for patients with the advanced stage of this disease. Now a high rate of remission can be achieved with combinations of drugs. Some recent active treatment programs employ four and even five drugs given simultaneously or sequentially. Remarkable regression of advanced disseminated disease has occurred, and a high proportion of patients so treated still remains without evident disease activity, despite the cessation of treatment up to three years ago. Combinations of radiotherapy and chemotherapy have also provided preliminary evidence of striking improvement.

Embryonal carcinoma of the testis

Embryonal carcinoma and choriocarcinama of the testis can usually be treated successfully with surgery and radiotherapy when they are still localized. In patients with disseminated cancers of these types, chemotherapy has produced remissions in one third of patients with long-term freedom from disease in 7 per cent.

Adenocarcinoma of the utrine corpus

Adenocarcinoma of the uterine corpus, even when widely metastatic, responds to treatment with progestational hormones in approximately 20 per cent of patients. In some of these, freedom from evidence of active disease has persisted for as long as seven years.

Carcinomas in the superficial layers of the skin

Carcinomas in the superficial layers of the skin have disappeared in the majority of patients and have not recurred after topical application of high concentrations of cancer chemotherapeutic drugs such as 5-fluorouracil.

Other cancers benefitted by chemotherapy

Chemotherapy has provided therapeutic benefits but not cures in a number of other types of cancer. Chronic myelocytic leukemia and chronic lymphocytic leukemia, lymphosarcoma, reticulum cell sarcoma, multiple myeloma, polycythemia vera, mycosis fungoides, malignant melanoma, and neuroblastoma are other forms of cancer in which palliation and clinical improvement can be achieved by chemotherapy, and for which, in the disseminted state, chemotherapy is usually the preferred means of treatment.

Several types of carcinoma are also significantly benefited by drug treatment. Clinical improvement occurs but long-term freedom from the disease has not been observed in any appreciable fraction of the patients. Carcinomas of the breast, the large intestine, the stomach, the pancreas, the prostate, the epithelium of the head and neck, the thyroid, the ovary, and the adrenal gland are all subject to palliation in a proportion of patients when the appropriate cancer chemotherapeutic drugs are used. The drug regimens differ widely for the several different types of cancer mentioned.

Some cancers are notably insusceptible to the chemotherpapeutic agents which have been used so far. Although the tumors may decrease

in size, major clinical benefit has not ordinarily accompanied this regression in patients with carcinomas of the lung, the cervix, the kidney, or the bladder.

SUCCESS AND FAILURE IN CHEMOTHERAPY

Chemotherapy can occasionally produce cures in a broad spectrum of cancers. The cancers that can be cured by chemotherapy are not always those which grow most rapidly. Not all are from the same primitive embryonic tissue; many organ classes are represented. Furthermore, curative chemotherapy is not usually a product of a single drug nor a single technique of drug use. Rather, many different drugs are involved, sometimes in combination with each other, sometimes with surgery and radiation. This high degree of specificity between a particular tumor and a particular drug or drug combination implies that no universal chemotherapeutic cure is to be anticipated. The complexity of special drugs and regimens for special tumors can also be construed to mean that a drug that fails in the treatment of one kind of cancer may exhibit major activity against another.

The sensitivity of cancer cells to drug action is dependent on a variety of possible factors, not all of which are known. The drug must reach the cancer cell surface, enter the cell, remain active or undergo necessary activation, reach a critical target site, and combine with it at a time when chemical processes on which the cell depends for its viability or reproduction are in progress. Furthermore, competing and bypass pathways in the cell must not be able to compensate for the chemical injury which the drug inflicts. Failure of the whole chain of events can result from the failure of any one step. Thus, any drug given by the wrong regimen to a mass of cancer cells which are biologically insusceptible to its action at that time is a failure of chemotherapy. Better drug design and better understanding of the biological characteristics of each cancer type, and indeed of each cancer, will advance the effectiveness of chemotherapy.

REGIONAL PERFUSION WITH CHEMOTHERAPEUTIC AGENTS

Perfusion of individual limbs or body regions with blood, sometimes heated, containing cancer chemotherapeutic agents has been the subject of extended research. The perfusion technique allows exceptionally high concentrations of drug to be used for relatively short periods during operation. Conceptually, success depends on sufficient anatomical localization of the cancer to be encompassed in the perfused region, sufficient circulatory isolation to avoid leakage back into the general circulation, and on the absence from the perfused bed of vital organs which have chemotherapeutic sensitivity greater than that of the cancer. The best results have been reported for the treatment of melanoma and sarcoma of the extremities.

COMBINATIONS OF CHEMOTHERAPEUTIC AGENTS

Combinations of drugs may allow potentiation of their effect on the cancer while distributing their toxic impact over several different normal tissues. Because all drugs do not affect the same normal tissues,

their use in combination, although it may produce a broader spectrum of toxicity, usually results in less toxicity for any particular tissue.

Resistance to treatment may develop as a result of the emergence of a cancer cell population no longer susceptible to the effect of a given drug. This may occur if a whole new mutant population of cells inherently resistant to the drug arises from a rare mutant cell in the original population, or if the cells acquire some new chemical characteristic which allows them to circumvent the effect of the drug. An example of the latter is the induction of the enzyme asparagine synthetase, which renders the previously sensitive leukemic cells resistant to the therapuetic effects of L-asparaginase. Combinations of drugs may diminish or eliminate the opportunity for resistance of the cancer cell to develop. Major progress has been achieved in acute leukemia, Hodgkin's disease, and breast cancer with combination chemotherapy. Broader studies should be carried out of the possible usefulness of such agents in other types of cancer.

HORMONE-DEPENDENT CANCERS

Certain hormone-dependent cancers, such as breast and prostatic carcinomas, are particularly susceptible to hormone manipulation. For this reason, studies on the mechanisms of action of hormones on human cancer are extremely important. In addition, more emphasis should be given to studies on the action of cycle-dependent and cycle-independent nonhormonal drugs alone and in combination with hormones on these cancers.

CENTRAL NERVOUS SYSTEM LEUKEMIA AND LYMPHOMA

Leukemic cells in the brain substance and cerebrospinal fluid cannot be attacked by most chemotherapeutic agents which are otherwise active against systemic disease because of differences in drug penetration across the blood vessels. Certain drugs given intrathecally, however, such as methotrexate and cytosine arabinoside, are temporarily very effective. The use of the Ommaya reservoir for intraventricular drug administration appears to achieve more long lasting effects.

IMPORTANCE OF SUPPORTIVE CARE

Temporary sustenance of the cancer patient by the use of specialized supportive measures may allow interim survival, despite effect of the disease or temporary side-effects of cancer chemotherapeutic compounds, until full benefits can be obtained. Refinements in the transfusion of blood platelets and of leukocytes have demonstrated their value in patients with critical deficiencies of these elements of the blood, caused either by leukemia or by drug toxicity. During periods of abnormal and deficient leukocyte counts, decrease in the threat of bacterial infection by major reduction or elimination of the bacterial content of the intestine, and isolation in a sterile atmosphere has provided protection against fatal sepsis. Further evaluation of the various techniques for protecting against infections is needed to determine which would be the best from the points of view of efficacy, patient tolerance, nursing requirements, etc.

The transfusion of large numbers of human leukocytes has been of value in supporting patients with nonfunctioning marrows against infections. More studies are required, however, to develop better and less costly techniques of separating leukocytes and delivering them to the patient.

Techniques of preventing or reversing the effect of cancer chemotherapeutic compounds on normal tissues have been recognized. Thus, critical end products of chemical reactions can be supplied to the patient, averting some of the toxic drug effect.

HOW ANTICANCER AGENTS WORK

The essential premise of chemotherapy is that cancer cells differ in some way from normal cells and so can be destroyed by chemicals that will not produce equivalent injury to normal cells. The fact that some cancers can be cured by chemotherapy provides definitive proof that this basic premise is valid. By understanding more previsely how effective chemical agents act, it will be possible to uncover such differences and, perhaps, to design new chemicals to take advantage of them.

Considerable advances have been made in the understanding of cancer chemotherapeutic compounds as a result of the availability of sophisticated techniques of biochemistry, biophysics, and pharmacology. Isotopically labeled drugs can be studied in their distribution, excretion, metabolism, and in their activation within the cancer cell. Studies of isotopically labeled precursor compounds have been able to pinpoint the effects of drugs on specific biochemical pathways, thus allowing for their more rational use alone and in combination.

The mechanisms of action of cancer chemotherapeutic drugs are complex. In order for any cells, including cancer cells, to multiply there must be new synthesis of DNA, the genetic material which controls the cell function. The mechanism of action of most cancer chemotherapeutic compounds involves effects on DNA. The alkylating agents for example, react chemically with DNA and prevent its further replication. Many antimetabolites block DNA synthesis; these include 5-fluorouracil, 5-fluorodeoxyuridine (FUDR), cytosine arabinoside, methotrexate, hydroxyurea, and 5-hydroxypicolinaldehyde thiosemicarbazone (5-HP). Many antibiotics bind to DNA or intercalate into DNA and consequently block DNA transcription; these include actinomycin D, daunomycin, mithramycin, and mitomycin. Some compounds are incorporated into DNA and thus cause defects in DNA function; these include 5-iodo-2′-deoxyuridine, trifluorothymidine, and thioguanine. Many drugs, particularly purine and pyrimidine analogs, must be converted by target cells into the active form of the drug, usually a nucleotide. Some plant products such as colchicine, vinblastine, vincristine, and podophyllotoxin act as mitotic poisons to prevent cell replication.

For the cell to function, RNA must be produced under direction from the DNA. RNA serves not only to provide an encoded message for protein structures, but also to allow assembly of amino acids for protein synthesis. Some anticancer agents interfere with the function of DNA as a template for the transcription of RNA. Some cancer chemotherapeutic agents combine directly with certain enzymes or other constituents of cancer cells. Others deplete cells of specific

nutrients required by the cancer cells. In the case of many agents, including, in particular, several hormones, many plant products, and some antibiotics, the mechanism of action is unknown.

CANCER CELL KINETICS

Cellular reproduction occurs by a series of different chemical steps known as the cell cycle. An understanding of the order of the steps has allowed the identification of the phase of cellular activity in which specific drugs act. Bioassay of tumor-killing capacity has identified major differences in the effectiveness of drugs, or of a single drug when given by different methods of administration, and has prompted a total reorientation in the techniques of administering cancer chemotherapy. It is now evident that a drug for cancer must be defined not only by its chemical composition but also by its specific dosage, specific route, and by schedule of administration, each of which significantly modifies its action activity.

Studies by means of the spleen colony assay have enabled quantitative comparisons of individual drugs to be made against normal bone-marrow cells and against leukemic cells. These assays have been further refined to show which part of the cell cycle different drugs affect: BCNU, for example, is cycle-independent, cyclophosphamide kills cells in any part of the cycle (cycle-specific), whereas cytosine arabinoside kills only during DNA synthesis (phase-specific). Such knowledge permits more intelligent design of drug combinations.

Different growth rates of cancers may not imply discrete differences in the duration of different phases of the cell cycle but rather differences in the fraction of cells in cycle at any one time. Recognition of this fact has brought greater understanding of the theoretical needs in cancer chemotherapy. Thus, cancers which grow slowly as a mass may not necessarily contain slow-growing cells. On the contrary, it has been found that many cancers have large numbers of cells which are not actively growing at all, whereas those cells which are growing have a short rapid growth cycle. Research attempts are in progress to return the resting cell to the growth cycle thus increasing its vulnerability to cancer chemotherapy.

The problem of extrapolating dosage schedules of cylce-dependent drugs from mouse to man demands a thorough knowledge of cancer cell kinetics in man. Much progress has been made in understanding the kinetics of acute leukemia in man, but further work is needed in the leukemias and particularly in the more slowly growing human solid tumors.

CELLULAR SPECIFICITIES

Some types of anticancer agents act specifically against certain cancers regardless of their rates of growth in relation to other tissues in the body. These include drugs active against squamous cell cancers of the skin and cancers of the adrenal gland as well as the hormones active against cancers of the secondary sex organs, the breast, and the prostate gland. Particular efforts should be made to discover how such agents act, and to find others with such specific toxicities.

DEVELOPMENT OF COMPOUNDS FOR TESTING

In the last three decades, more than 30 compounds with clinical usefulness for the cancer patient have been developed from natural

sources such as plants and microbiological filtrates and by synthesis. Most cancer chemotherapists believe that they have just scratched the surface in finding the anticancer drugs needed for effective control of all disseminated human tumors. Therefore, there is need to continue the search for improved drugs. Such drugs will most likely derive from the collaborative work of tumor biologists and aggressive, intelligent, and dedicated organic chemists. These workers isolate and identify natural products from plant and microbiologic sources with the intent of chemical modification and improvement. They also synthesize new compounds, often designed as structural analogs of molecules of known biological importance. Completely new organic chemical structures never previously tested in cancer are also of interest and importance. Search for new activities should be given high priority.

Most chemotherapeutic agents for infectious disease or cancer have been discovered by empirical screening. In the past two decades, the screening programs have discovered many compounds active against the fast growing leukemias and lymphomas. It is now particularly important to add techniques that screen for agents active against the relatively slow-growing carcinomas which make up the great majority of human cancer as well as continuing the screens which have produced drugs of value for the more rapidly growing tumors and leukemias.

Plant materials have been the mainstay of materia medica for centuries, and even today a large proportion of the effective medicines used in a variety of diseases come from plants. Examples include digitalis, ergot, alkaloids, quinidine, belladonna, morphine, codeine, and many others. Other medicines in wide use, although now produced synthetically, were first discovered in plants. Salicylates (aspirin) are perhaps the outstanding example. Consequently, there is sound logic when searching for a possible treatment for cancer, to devoting a substantial part of the effort to plant materials. Two plant derivatives, vinblastine and vincristine (from the periwinkle), have now been shown to be very valuable and occasionally, when used in combination with other drugs, have cured some cancers in man. Hence interest in plant extracts has increased.

Furthermore, it has been demonstrated that many other plant extracts are effective in some experimental animal cancers. The best known are camptothecin, and lapachol, but there are dozens of others. Many have not been studied further after the initial demonstrations of anticancer activity in some experimental animals. Some of the plant materials already described in the basic science literature may prove to have important clinical value against cancer. Consequently, it is recommended that studies be expanded of the effects on cancer of drugs derived from plants. An effective program should include evaluation of the extracts already reported to have significant anticancer effects, as well as a search for new materials.

The use of active drugs for cancer may bring success even before there is a clear understanding of the malignant process, just as the success of other medications, many derived from plants came long before a clear understanding of the nature of the diseases for which they are used.

PREPARATIONS FOR CLINICAL TRIAL

After the development of an idea and synthesis of a new drug, or after the first evidence of activity of a compound isolated from a

natural source, several steps are necessary before the drug can be used in cancer patients. Confirmation of the antitumor activity in the same screening system is essential as well as a broad-scale study in other known biological screening systems. These include transplanted carcinomas, sarcomas, leukemias, both those sensitive and those resistant to conventional chemotherapeutic agents, and miscellaneous tumors in mice and rats and sometimes in hamsters, guinea pigs, and rabbits which allow comparison of the biological effect with that of other known compounds which have previously been tested in these systems. The spectrum of activity may indicate types of human tumors which might be particularly susceptible or insusceptible to action of the drug. After the activity of the compound is confirmed, two general areas of study ordinarily follow. One is undertaken to determine what the compound does and how it does it, and the other to test its effects on animal species, which may predict some of its effects in man. Investigation of the mechanisms of action involves studies in cells and biochemical systems *in vitro* as well as studies in the whole animal. The objective of such investigations is to determine whether the specific focus of action of the compound can be pinpointed since such understanding allows much more rational use of the drug. Such programs of study usually involve an assessment of whether interference is produced in the biosynthetic pathways for DNA, for RNA, for protein, and for other cellular chemical events. Studies may also seek evidence of chemical transformation of the drug to a more active or less active product within the cell, and influences on the changes in the drug are sought. Studies of the kinds here detailed usually require radioisotopic techniques and measurement of microquantities to determine the pathways of chemical reactions.

PRECLINICAL PHARMACOLOGY

In preparation for use of a compound in man, it is critical to determine its general characteristics of metabolism and effect in other species. For this purpose, the drug is tested in mice to determine that amount of compound which is lethal, and the relative ratio of the lethal dose to the therapeutic dose for several different tumor systems. Similar observations are made in rats and sometimes in other small animals. A compound is administered by several routes, either orally, intravenously, subcutaneously, intramuscularly, or intraperitoneally to determine differences in effect on the animal from differences in rate of achieving high concentrations in the blood and duration of drug activity. Several schedules of administration are usually studied to determine the frequency of dosing (which influences the concentrations of drug in blood and tissues) and relative effects of different dose schedules on normal organ function and survival. After determination of these parameters, studies are ordinarily made in dogs and/or monkeys. The compound is given in doses predicted from therapeutic and toxic activities in rodents. Assessment of the different routes of administration is made, as well as determinations where possible of the concentrations achieved in biological fluids. Chemical tests of normal organ function are obtained before and after drug administration to determine where the impact of the drug is exercised. Studies of blood formation and destruction are also conducted to recognize possible toxic effects of the compounds on bone marrow

function. The first evidence of an adverse effect of the drug on normal animals is determined so that one can have warning of undesirable activities of the drug should similar chemical or hematological abnormalities appear in man.

Thus, careful preclinical pharmacology and toxicology are necessary to arrive at the optimum dose and route of administration of a new agent. It should be emphasized, however, that much less extensive preliminary toxicology is needed for a chemotherapeutic agent against cancer, for example, than for a headache remedy or a tranquilizer. Patients with advanced cancer have a finite life expectancy in the absence of effective treatment. Preclinical pharmacologic study should be extensive enough to indicate the proper techniques of initiating clinical use of the drug. Preclinical study should not be so complete and elegant a pharmacologic assessment that it produces information on minor chronic toxic effects which are not life-threatening to the affected cancer patient. One thousand cancer patients die in the United States every day on the average. If a new compound shows appreciable activity against cancer, extensive pharmacologic studies can be made concomitantly with the subsequent clinical trials.

SPECIAL TEST SYSTEMS

While these studies are in progress, experimental therapy with unique animal systems is often in progress. Such studies as combinations, drug treatments in tumors known to be resistant to particular drugs with relevance to the compound under study, treatment in spontaneous tumors, combination treatments with surgery or x-ray in animals, and different selected schedules of drug administration all may contribute information of value for clinical extrapolation.

STAGE I TRIALS AND CLINICAL PHARMACOLOGY

The first study of a new compound in man requires major skill in clinical pharmacology. Thorough familiarity with the preclinical findings and the test systems in which abnormalities were found is required. Furthermore broad and specialized knowledge of cancer is required so that a critical observer may determine if changes that occur in the patient are ascribable to the neoplastic disease or to effects of the drug. Critical observation of chemical parameters of hepatic, renal, and other tissue functions are made as well as clinical observations of cardiac, pulmonary, brain, nerve, muscle, skin, gastrointestinal, and other vital functions. Appropriate critical measurement techniques, such as electrocardiograms, electroencephalograms, X-ray examinations, and similar studies, are conducted to assess function in these organ systems. Frequent determination of the morphologic status of the blood and bone marrow is ordinarily necessary since many compounds affect this rapidly growing tissue. Where possible, leterminations of blood levels and excretion levels of the drug are made, sometimes using isotopically labeled compounds and sometimes using chemical determination of the drug per se. Gradually increasing doses are given to successive patients to assess the potential therapeutic and possible toxic effects of the compound. It is critical that the clinical pharmacologists start at levels expected to be nontoxic from the preclinical studies and proceed with caution, deliberation,

and expertise to escalate the dose until evidence of therapeutic effect or of toxicity occurs. Upon attaining a tolerable treatment regimen, which may require studies of several routes and schedules of administration, a broader scale investigation is undertaken to detect therapeutic activity in diverse types of neoplasia. The choice of patients and the types of their neoplasms depend, in part, upon the tumors affected in the preclinical species and upon knowledge of the mechanism of action of the drug. Some human tumors grow very rapidly, whereas others have an indolent and slow progression. The schedule, duration, frequency, and similar considerations of drug dosing may influence the approach to these neoplasms. The time required to deliver a drug from its conception or first recognition in a testing system to clinical usefullness is dependent not only on all the biological factors above enumerated, but upon resources for making compounds in sufficient quantity and of reproducible purity for clinical testing. A minimum time of two to three years is required, and if evidence of clinical activity is forthcoming, more extended investigations in the optimal method employing a drug must be undertaken, and decades may be required to perfect its clinical application.

STAGE II TRIALS AND COMBINATION THERAPY

Stage II trials are concerned with the establishment of optimum dosage schedules and combinations for chemotherapeutic agents. This type of clinical investigation has produced such outstanding achievements as increasing the cure rate of metastatic choriocarcinoma from practically zero to greater than 75 percent and of Burkitt's tumor from zero to 50 percent. By the use of more rational dosage schedules and combinations, the median survival in acute lymphoblastic leukemia has been increased from 4 months to 30 months and the rates for five-year survival with no evidence of disease from zero to as high as 20 percent in some series. These results emphasize the need for further imaginative studies in this area.

STAGE III TRIALS

These are large-scale trials to compare the efficacy of different applications of the various therapeutic modalities. Each of these requires extensive evaluation by critical observations in many pateints with proper controls in different institutions. Fore example, one such study, presently underway, has been undertaken to compare surgery plus 5-fluorouracil with surgery alone in surgically resectable large bowel cancer.

MANPOWER

There is a serious shortage of pharmacologists to work on experimental and clinical cancer chemotherapeutic agents. The proper investigation of candidate compounds requires the full participation of scientists with such training in the effect of drugs on normal and neoplastic tissues. There is a serious lack of physicians with clinical training and skills in the use of cancer chemotherapeutic compounds. This is evident by the clinical progress which has been made in cancer centers, and the rarity of physicians with appropriate skills in most

countries. Cancer chemotherapy, like any highly technical field of intellectual endeavor, requires special training and experience. Most cancer chemotherapy can no more be performed by the general physician than can radiotherapy or cancer surgery. Fellowship programs designed to provide an expanding base of cancer chemotherapists will be required to deliver optimal care to the patient who can be benefitted by chemotherapy.

COMPARISON BETWEEN THE CHEMOTHERAPY OF INFECTIOUS DISEASES AND OF CANCER

It appears from the data previously cited on cures of cancers by chemotherapy that cancer chemotherapy stands today about where infectious diseases therapy stood in 1937, when it was established that chemotherapy could cure certain systemic streptococcal infections. There are now drugs that will cure better than 50 percent of the patients with a few types of disseminated tumors. As in 1937, when the infectious disease chemotherapist had nothing effective against staphylococci, gram negative bacilli, rickettsiae, or viruses, so now the cancer chemotherapist has only palliative rather than curative therapy available for most of the carcinomas and other slow-growing tumors. Once the curative potential of chemotherapy against a few infections was understood, however, a massive program was mounted, and it was only a relatively few years until the majority of bacterial infection were brought under chemotherapeutic control. Similarly, the significant percentage of cures achieved in the few previously mentioned metastatic tumors and leukemias demonstrate the potential curability of widespread neoplastic disease by chemotherapy.

Now, as was the case in bacterial infections in 1937, the potentiality of chemotherapeutic cure of cancer has become apparent and there is sufficient technical knowledge to begin to amount a large-scale successful chemotherapeutic program against other forms of widespread neoplastic disease.

IMMUNOTHERAPY

The fourth and least developed technique for the treatment of cancer is immunotherapy. Since the immunologic defenses are frequently impaired in the patient with cancer, and since surgical and chemotherapeutic attacks on infection are relatively unsuccessful in the absence of an adequate immune response, it seems likely that immunotherapy will, in the future, become an essential part of the successful management of cancer. Immunotherapy has been shown to be effective in some experimental animal systems and has been reported to be of benefit in some children wtih acute leukemia. In this type of therapy, the patient's own defense mechanisms are stimulated in an effort to produce an immunologic response against the few leukemic cells remaining after intensive chemotherapy. In addition, the use of immunologically active cells from other persons is being explored as well as injections of large quantities of the patient's own blood cells grown outside the body and irradiated. Active research is needed to determine the most effective natural or synthetic stimulus, and to establish techniques for increasing the patient's immune response against cancer. The timing of immunotherapy with respect to other

therapeutic procedures needs clarification. It appears likely to be most effective after maximal reduction in tumor size by surgery, radiation, or chemotherapy.

COMBINED THERAPY

Various combinations using two or three of the available therapeutic modalities of surgery, radiation therapy, and chemotherapy have produced increased cure rates for Wilms' tumor, retinoblastoma, and other solid tumors.

Wilms' tumor of the kidney is a highly malignant form of cancer which occurs in newborns, infants and young children. After surgical excision it may recur locally, or spread by the blood stream to the lungs. The tumor is sensitive to radiation and to dactinomycin chemotherapy. When the two treatments are combined with surgery, cure rates now reach 75 percent. Retinoblastoma, which can be cured in a majority of patients by radiotherapy, appears to respond more favorably when radiation therapy is combined with treatment with an alkylating agent. Preoperative radiation therapy in head and neck cancer has improved the five-year cure rates from subsequent surgery. Some observers have also reported improvement in survival from combinations of chemotherapy and radiotherapy in particular types of head and neck cancer.

The results of studies now nearing definitive analysis indicate that combinations of chemotherapy, with radiotherapy and with surgery will also yield better results for other types of cancer.

Cancer Research

The Biology of Cancer

WHAT IS CANCER?

Cancer has been with man since his earliest time. Indeed evidence of cancer has been found in fossil bones, and a few written descriptions of the disease appear in Egyptian papyri. Tumors, today, are still classified according to their appearance, behavior, and location—very much as they have been for the last 2,000 or 3,000 years.

With the development of microscopy, tumors came to be described also according to their microscopic appearance, which is determined by their tissue of origin. Then it became clear that tumors, like normal tissues, are composed of living cells, that cancer cells derive from normal ones, and that there are many different kinds of tumors, just as there are many different kinds of cells in the body.

Each kind of normal cell can potentially give rise to a tumor, through a change called *carcinogenesis*, which endows the cell with *neoplastic* ("new growth") properties. Neoplastic cells transmit to their descendants both their neoplastic properties and some of the attributes of the tissue from which they originated. They sometimes carry out functions of normal cells, but they are often a simplified, more primitive version of the normal cell, and they also display more variability and often give rise to cellular monstrosities. Through repeated cycles of cell division, they form lumps of neoplastic cells—

tumors—whose main characteristic is unlimited growth, a growth that never reaches a state of equilibrium, as does the growth of normal tissues.

Whether rapid or slow—and sometimes tumors grow more slowly than some normal tissues—the growth of a tumor has no limit other than that imposed by the death of its host. Thus a tumor is essentially a tissue that does not respond to normal growth controls.

Malignant tumors, usually referred to as *cancer*, have additional characteristic properties: they are *invasive* and they can *metastasize*. Furthermore, they exhibit a certain irregularity in the structural arrangement. These properties of invasiveness, and capacity to form metastases may be related to each other; they both probably result from the fact that malignant cells can behave in an aggressive way with regard to surrounding cells and so are able to penetrate biological barriers that stop normal cells or benign tumors. Cartilage, or elastic and dense fibrous tissues—such as tendons and ligaments—seem to present obstacles to the spread of cancer; bone, however, is as readily invaded as soft tissues. At the growing edge of malignant tumors, accumulations of active lymphoid cells can be seen; these represent the body's attempt at defending itself. (See: Cancer Immunology.)

Metastases

A metastasis is a secondary growth which occurs at a distance from the primary tumor and which is made of the same type of cells. It is derived from live neoplastic cells which originate in the primary tumor and travel to other parts of the body; this usually occurs after the primary growth has reached a fair size. Different types of tumors vary as to the likelihood of their forming metastases and the stage of the disease when this may occur. In advanced cancers, metastases may form by the hundreds, all over the body; it is this tendency to spread that so often makes cancer incurable. In fact, metastases are the most important clinical feature of cancer, because they usually determine whether the patient can be cured or not.

The purpose of early treatment is, therefore, to eliminate the primary tumor before metastases have had a chance to develop.

Effects on the host

The presence of a cancer can have different effects on its host, depending on the kind of cells it is derived from and upon its location. For example, cancers derived from endocrine glands often may continue to secrete hormones. Even small primary tumors may be fatally dangerous to their host if they disrupt the functioning of vitally important organs. However, many aspects of the effects of cancer on the body are still poorly understood, and there is often no explanation for the lack of correlation between a tumor's size and the disturbances it may cause in the physiology of its host, or the time of his death. What all cancers have clearly in common, however, particularly when they grow rapidly, is a priority in the body's economy and thus, a tendency to grow even when the rest of the body suffers from relative starvation. The loss of weight and anemia so produced cause "cachexia," the typical debilitation of patients with advanced cancer.

By the time cancer can be diagnosed, its cells have broken free of the body's own constraints and defense mechanisms. Moreover, they are

even capable of dictating the behavior of normal tissues. Most tumors have a "stroma," which is a supporting network of connective tissue cells; in it, normal blood vessels supply the tumor with oxygen and nutrients and remove metabolic waste. It is the tumor that dictates the growth and development of the stroma and its network of blood vessels. Thus, normal cells actually serve the malignant ones, which could not otherwise survive in large masses if their metabolic needs were not continuously provided for.

Since cancer cells within a given tumor are functionally very much alike, the properties of the tumor mass represent the collective properties of all cancer cells that compose it. Cancer research is essentially concerned with the study of the properties of tumor cells, because the basis of the disease—cancer—lies in the cancer cell itself.

THE CANCER CELL

A major goal of cancer research is to find specific and constant chemical differences between normal and cancer cells, differences that are essential to the development and maintenance of the properties of malignancy. If such differences were found, it would be possible to interfere with them, and destroy cancer cells specifically through the use of chemicals without inflicting damage to normal cells. Even controlling only the growth or only the capacity to metastasize would similarly offer a possibility to control cancer.

Advances in methodology

Until recently, biophysics and biochemistry offered only primitive tools to cope with the extraordinary complexity of living matter and many things which are now taken for granted—radioisotopes, fluorescent markers, electron microscopes, and the like—did not exist. Cancer research was a difficult task and it is to the credit of its pioneers that, nevertheless, most of the present-day basic concepts of cancer were formulated by them.

The development of many different inbred strains of laboratory animals, apparently genetically identical within a strain, has been of momentous importance for the study of the behavior of cancer cells. Of similar importance was the discovery of antibiotics, which prevent the infection of cell cultures with microorganisms. Now, systematic experimentation can be carried out with many kinds of cells, both normal and cancerous, in systems that are much simpler than whole animals and in which even single cells can be observed directly.

Thus, it has become possible to induce and observe the neoplastic change *in vitro* with all agents that can induce cancer *in vivo*—chemical carcinogens, viruses, and radiations—and to observe the structural and behavioral changes produced in the cells. The process of carcinogenesis in cultured cells is called *cell transformation*. Its most prominent feature is a change in cellular growth patterns. Normal cells from connective tissue grow in single-layered, parallel bundles and, usually, stop growing when they crowd against each other. *Transformed* cells tend to be more irregular in shape, to grow in a criss-cross fashion, and to pile up over each other. Altered properties are exhibited both by cells transformed *in vitro* and cancer cells of the same type which are taken from laboratory animals or man and propagated in culture flasks. Cells transformed *in vitro* are usually able to produce a tumor when they are inoculated into an appropriate host.

Momentous advances in biochemistry and biophysics over the last decades have also enabled the properties of normal and cancer cells to be studied very thoroughly at the subcellular level.

Basic research in physics, chemistry, and molecular biology has been responsible for the development of new methods and techniques which have greatly facilitated progress. Among these are chromatography, electrophoresis, ion exchange columns, counter-current distribution, and high speed centrifugation methods, as well as spectrophotometry, all of which are valuable for the separation and identification of cellular substances. The use of radioactive isotopes to trace metabolic pathways in cells and follow substances throughout the body has demonstrated for the first time the chemical complexity and dynamic nature of living matter.

Differences between normal and cancer cells

Extensive comparative studies of normal and cancer cells have allowed a large number of important observations to be made. It has been learned that living matter is fantastically complex in its organization. Certain basic constituents are common to all cells, since they are always formed by the same kinds of molecules. Although these kinds of molecules may be alike in their overall chemical composition, they are different by reason of the way their subunits are ordered, which in turn, affects how the molecules are shaped. Such structural differences have great importance for their function. There is also great variability at the subcellular and molecular levels in the constitution of living cells, not only from species to species, but from organ to organ, among different types of cells within individual organs, and also the same type of cell at different times. There is similarly a great chemical diversity among the various types of cancer, not only among cancers of different organs but among different cancers that may arise from the same type of cell. Furthermore, like normal cells, cancer cells are capable of adapting to new conditions through changes in their metabolism.

Certain specific metabolic peculiarities of cancer cells have been discovered that can be exploited therapeutically. For example there is a certain type of leukemia whose neoplastic cells have a need for the amino acid L-asparagine because, unlike most normal cells, they are incapable of synthesizing it for themselves. L-asparagine is an essential component of cellular proteins. Thus, the administration of L-asparaginase, an enzyme capable of destroying free L-asparagine in the body fluids, is therapeutic because it deprives the leukemic cells of an essential nutrient. Treatment with L-asparaginase has produced good remissions in pateints with "L-asparagine-dependent" leukemia. However, there usually seem to be some leukemic cells present that do not share this dependence. Such cells can multiply preferentially during L-asparaginase treatment and may replace the dependent ones, so that this form of therapy becomes ineffective with time. It seems clear now that the L-asparagine requirement is no more than a chance association with the neoplastic state of leukemic cells and not an essential, nor even a constant characteristic of it.

The biochemical differences that have been found thus far between normal and cancer cells are differences that do not distinguish *all* cancer cells from *all* normal cells; they involve, at best, differences between one type of cancer cell in relation to its corresponding normal cell. Almost always such differences are quantitative, involving larger or smaller amounts of a component, such as an enzyme, or differences

in the rate at which certain metabolic processes occur. These quantitative differences can be exploited, however, and though no "magic bullet" is available as yet, certain chemotherapeutic drugs do, in some cases, kill cancer cells preferentially and in some instances produce cures.

So, despite the fact that no consistent and general biochemical characteristic has been detected so far in cancer cells, the search continues. Better methods of analysis are becoming available, which can be applied to isolated and purified cell fractions rather than to whole tumors or even whole cells. Suspensions of purified cell nuclei and other cell organelles can now be studied in great detail. New methods are capable of distinguishing between enzymes of normal and cancer cells that catalyze the same chemical reactions but differ in their chemical structures. Beside suggesting new modes of therapy, subtle changes of this kind, such as those already found in the liver in premalignant lesions, can provide valuable information on the relationship between enzyme modification and carcinogenesis.

Important tools other than, or in combination with, the purely biochemical ones, are now being extensively used to pinpoint the areas of cellular metabolism where critical events are likely to take place. Such tools include radiation, viruses, and carcinogenic chemicals. With their use, the cancer cell seems finally close to yielding the kind of information biochemists have been searching for so long, the thread of unity which must link all cells that have neoplastic properties.

Genetic or epigenetic change?

The sudden and persistent cellular change that accompanies the cancerous transformation has lent great credence to the "somatic mutation theory" of carcinogenesis. This theory postulates that the neoplastic state is an expression of a very specific change of the cellular genetic material itself.

This question of whether the cancerous change is due to an alteration of the hereditary material—*a genetic change*—or whether it is due to an alteration of a non-genetic biochemical process which somehow affects the hereditary material—*an epigenetic change*—is of paramount importance. If the basic change is *genetic*, then it is essentially irreversible and susceptible only to selective destruction or to therapeutic approaches in the still remote area of genetic engineering. However, if it is *epigenetic*, it is theoretically reversible and more readily controllable.

Much recent evidence bears on this crucial question. In one way or the other, the genetic material is implicated in *all* cellular functions, both normal and neoplastic, since none can occur unless originally specified, directly or indirectly, by genes. So, in the last analysis, any function of the neoplastic cell will implicate genetic material, whether originally present or introduced by a virus (a possibility that will be discussed in greater detail elsewhere in the report). The crucial question is, therefore, not whether or not the genetic material is implicated, because it clearly is implicated, but whether or not there is a *change* in the genetic material.

There is no evidence that cancer is due to a mutation. Even in retinoblastoma, a rare hereditary cancer of the eye, due to a mutation in a single gene, the presence of the gene does not *cause* the cancer since the mutated gene is present in *all* cells of the organism but does

not produce a generalized carcinogenic stimulus. It affects only certain specific cells of the eye at a specific stage of their differentiation. Thus the direct, immediate cause of retinoblastoma is to be found in specific epigenetic, metabolic events. These events may be favored by general metabolic activities determined by the mutated gene, but the mutated gene is not in itself sufficient.

Despite the fact that the genetic material is implicated in the expression of malignancy, there is no evidence of the existence of a "cancer mutation," and it can be argued convincingly that genetic mutation, in the strict sense, has not been shown to be required for cancer causation.

Reversal toward normal

Important evidence in support of this hypothesis comes from experiments with plant tumor cells. Plant tumors, like animal tumors, may be initiated by diverse physical, chemical, and biological agents both *in vivo* and *in vitro*. Whatever the initiating cause, plant cells, as they become neoplastic, acquire the capacity to synthesize substances that normal cells also require but cannot make themselves. Plant tumor cells also manufacture an essential division-promoting hormone that all cells need for growth. The capacity to synthesize such substances is *not* the result of a genetic change but rather the result of a change in the expression of genetic information, since under proper conditions plant cancer cells can revert back to normal. Thus, the nuclei of plant tumor cells are *genetically equivalent* to those of normal cells. These experiments point, therefore, to an epigenetic mechanism concerned with the expression of genetic potentialities present in all cells, as a cause of cancer.

Reversal from the neoplastic state to one much closer to normal is not unique to plant tumor cells. Such reversibility has been found in widely different tumors in a wide spectrum of animals under certain defined conditions. Examples of tumor reversal have been shown in amphibians. In mammals, two cases have been particularly well documented. The tetratocarcinoma of the mouse is a tumor that contains, typically, a variety of cells representing a number of differentiated cell types and some undifferentiated cells. The least differentiated cells are the only malignant ones. However, their malignant potential is such that a teratoma can be induced in mice by the transplantation of a single undifferentiated cell. In each of the 42 cases of successful "takes" with single cells, highly malignant tumors grew which contained, in addition to the undifferentiated cell type, as many as 15 types of differentiated cells. These differentiated cells had originated from the single cell inoculated, proving that the malignant cell was multipotential—capable of giving rise to various different cell types. When the most differentiated cells were transplanted, even in large numbers, they proved nonmalignant.

The squamous cell carcinoma of the rat is a tumor of the basal layer of the skin. The basal undifferentiated skin cells multiply then in an uncontrolled, typically neoplastic way. They give rise both to new basal neoplastic cells and to differentiating cells which do not divide and which become capable of synthesizing keratin. Basal undifferentiated cells, transplanted into animals, for typical squamous cell carcinomas, whereas transplanted differentiating cells do not grow to form tumors.

Neuroblastoma is a highly malignant tumor which usually occurs in very young children. It is derived from neuroblasts, undifferentiated cells of nervous tissue. Spontaneous reversals of this tumor are unusual but are less rare than in other types of cancer. They have been attributed to strong immune reactions to the tumor (See: Cancer Immunology), however, spontaneous changes from immature neuroblasts to mature ganglion cells also occur in long-term cultured cells, in which the effect of an immune reaction is excluded.

Recently, highly malignant pigmented cells from a tumor called melanoma were observed to revert to an appearance close to normal under chemical treatment, *in vitro*, and to lose their malignant properties in the process. The mechanism underlying this last case of reversion is being investigated but still remains unknown.

Spontaneous as well as induced reversions from the typical "transformed" growth pattern of cells transformed by oncogenic viruses, have been observed with several cell and virus combinations. In all cases, the loss of neoplastic growth characteristics *in vitro* was accompanied by loss of malignancy in the test animal.

The multipotentiality of the cancer cell nucleus is convincingly demonstrated by experiments in which nuclei of a frog kidney tumor are transplanted into eggs from which the nuclei have previously been removed. The reconstituted eggs are capable of normal division and develop into normal larvae.

These examples show conclusively that the genetic material of malignant cells can express multipotential properties. Not only are highly malignant, poorly differentiated cells capable of further and even normal differentiation, but they are also able to lose their malignant properties. This strongly argues against the irreversible nature of cancer. Evidently the mechanisms responsible for the maintenance of the malignant state are not genetic, and those causing it are involved with gene expression rather than gene mutation.

Gene expression, differentiation, and cancer

It may at first seem surprising that heritable, permanent properties affecting basic cellular functions might not be due to changes in the genetic material but may be caused and maintained entirely by "epigenetic" changes. However, the process of normal differentiation—the progressive specialization of cells and tissues that occurs throughout development—is itself a striking example of just such phenomena.

When the fertilized egg divides, each daughter cell receives an identical set of chromosomes and hence an identical set of genes. Parceling out of exact copies of these same genes takes place at each cell division until the adult stage is reached. But the adult organism is made up of bone, muscle, blood, nerve, and other cells. All these cells differ from each other biochemically, physiologically, and structurally, but each still contains the same set of chromosomes and genes as the original egg cell. What has occurred is *differentiation*. Different specialized cells have different arrays of enzymes and other proteins, and protein synthesis, it is now known, is under direct genetic control. So, the occurrence of a different range of enzymes in cells which have a common origin is most easily understood on the assumption that *not all genes function in all cells at the same time*—i.e., that genes are expressed differentially.

Just as normal cells differ from one another, tumor cells also differ from normal cells in the kind and relative amounts of their enzymes. These differences could be due to mutations which cause elimination, dysfunction, or duplication of certain genes. However, the fact that certain tumors undergo reversion and differentiation does permit this interpretation. As is the case with differentiating cells, changes in the patterns of enzyme synthesis of these cancer cells must take place in the presence of an *unchanged* genetic complement, by the "masking" and "unmasking" of particular genes.

Differentiation and growth

Differentiation results from the progressive sorting out of specific patterns of gene expressions. It occurs normally in developing cells, and, apparently, abnormally, in cancer cells. This process seems to be closely linked to the mechanisms that regulate cell proliferation. It is failure in these mechanisms that results in uncontrolled growth.

In very young embryos, cell divisions occur rapidly and more or less synchronously for all cells. During differentiation, cell types emerge which diverge progressively, both in the rate at which they multiply and in their physiological properties. Finally, in the adult, complete specialization is reached, together with a state of balanced growth.

Although most adult cells are potentially capable of dividing, as they do in cell cultures, in the body some of them cease division completely once they are differentiated; others continue dividing but in a precisely controlled manner, with the number of new cells that arise perfectly balancing those lost through cell death. Growth control mechanisms must, therefore, exist in the body that are not obeyed by cancer cells.

It has recently become possible to observe, in cell cultures, the process of cell differentiation in differentiating embryonic muscle cells. The appearance of multinucleated myotubes (which precede the differentiation of muscle fibers) concides exactly with a fall in the overall rate of proliferation of the differentiating cells.

Cancer arises spontaneously more often in mitotically active tissues. Although nonproliferating cells are less susceptible to becoming transformed by viruses or radiation, they resume DNA synthesis—an event usually preceding mitosis—when infected by an oncogenic virus or irradiated. This happens, for example, when maturing muscle fibers, that would otherwise synthesize no more DNA, are infected with Rous sarcoma virus. In the well-documented case of the small DNA viruses (see: Viruses and Cancer), the induced DNA synthesis is accompanied by the synthesis of several enzymes, most of which must be specified by the cell, since the virus does not carry sufficient genetic information to do so.

A number of similar observations of changes in the pattern of gene expressing occurring in conjunction with new DNA synthesis, has led to the conclusion that, for either cell transformation or a major cell differentiation process to occur, the cellular genome must undergo a round of replication. It is now believed likely that it is during this process that new genes are "switched on" (or de-repressed) and others "switched off" (or repressed).

The cell cycle

The period that elapses between one cell division (mitosis) and the next, can be divided into different phases, each clearly distinguishable biochemically by characteristic metabolic activities. The all-important DNA synthesis takes place in a particular phase of the cell cycle. Large amounts of special proteins are needed to build the mitotic spindle apparatus. Pools of appropriate precursors are taken in or synthesized, in preparation for each of these activities. A large number of gene inductions and repressions must therefore take place each time a cell prepares to divide and during division itself. To achieve an orderly sequence of events, a large number of internal control mechanisms must operate. Of these exceedingly little is known.

Control of growth and differentiation

With few exceptions, once DNA synthesis starts, the whole cell cycle goes through all its phases until the one immediately preceding the next round of DNA synthesis. Normal tissues seem to maintain their integrity, as well as proper balance of growth with other tissues, by regulating the number of cells permitted to begin DNA sysnthesis. Control over the initiation of DNA synthesis seems to be the main point at which cell proliferation is regulated. It is thus clearly important to basic biology and cancer research to understand the mechanism which controls cell division by initiating DNA synthesis. Although the mechanism itself is totally unknown, four different factors seem to influence rather specifically the onset and continuation of DNA synthesis. These are: 1) a cytoplasmic factor, 2) the immediate cellular environment, 3) systemic factors sush as hormones, and 4) DNA itself.

Cytoplasmic control

The existence and importance of a cytoplasmic factor in the control of cell division have been demonstrated by nuclear transplantation experiments. For example, not more than 1 percent of differentiated frog cells synthesize DNA when they are in their original tissue. However, when the nucleus of such a cell is implanted in the cytoplasm of an egg cell, DNA synthesis begins immediately. Ninety percent of mouse liver nuclei react similarly when they are transplanted to frogs' eggs. Even isolated nuclei, *in vitro*, can be made to synthesize nucleic acids actively if they are exposed to cytoplasmic fractions taken from actively growing cells. The stimulatory factor is not specific; it exists in both normal and tumor cells. Its activity seems to correlate with the level of mitotic activity in the cells in which it originates.

Aberrations in such a potent factor could produce very significant departures from normal proliferation patterns.

Intercellular controls

Groups of cells organized into tissues are, in turn, subject to external controls that affect their proliferation. Some control factors are produced by neighboring cells. Observations of differentiation in cell cultures will be of great value in determining the number, role, and nature of such factors. For example, it has already been observed that differentiating muscle cells release substances capable of affecting both cell behavior (cell fusion) and division.

Mouse skin cells produce a tissue specific substance (a "chalone") which inhibits skin cell mitosis in the presence of the hormone adrenaline. The epidermal chalone has been partly characterized chemically. The adrenaline-chalone interaction might be considered as exerting a direct effect on gene expression.

A number of chemical factors, which can be isolated from normal serum, are required to induce mitosis in cells prepagated in culture. Some of those factors, when purified, are highly active in stimulating mitosis in normal cells, for which they are a necessary requirement. Virally transformed cells, however, have been shown to require lesser amounts of these stimulating factors for mitosis.

An "overgrowth stimulating factor" (OSF) has recently been found to be released into tissue culture media by cells transformed with the Rous sarcoma virus. OSF can cause normal cells in culture to adopt the appearance and growth habit of transformed cells. Stimulated cells revert to normal, however, when OSF is removed.

OSF seems to be a combination of different enzymes capable of breaking down proteins, because its effect can be mimicked by trypsin. It has also been reported that many transplanted tumors show a high content of similar enzymes in or near their most actively growing cells. Apart from its interest as a growth-stimulating factor, isolation of OSF is of great interest because this factor may be responsible for the aggressive properties of tumor cells. Cancer cells are not only invasive but are capable of stimulating the growth of their host's connective tissue. OSF could contribute to, or be the cause of, cancer's invasiveness and also be the messenger that orders normal tissues to proliferate.

The existence within a tumor mass of normal blood vessels that actively support its metabolic needs has long remained a puzzle. Cancer cells transplanted into transparent chambers in rabbits' ears, can be kept under microscopic observation. As soon as such cells form a mass a few millimeters in diameter, the surrounding normal capillaries start to grow towards the tumor. In the process, the cells of the walls of the blood capillaries divide actively, with the wave of mitotic activity starting, at first, a relatively long distance from the tumor cells themselves. The phenomenon, named "tumor angiogenesis," is one of genuine induction due to a stimulant released by tumor cells. Once adequately supplied with blood, the tumor cell mass can go on growing; however, if angiogenesis does not occur, the tumor's growth is self-limiting. Recent successful attempts to isolate the "tumor angiogenesis factor" (TAF) have shown it to be a combination of RNA and protein. Pure TAF, implanted under the skin of any animal in a small container out of which it can diffuse, induces an extremely violent reaction which results, within a few days, in a voluminous and intricate local network of blood bessels. TAF is a poor antigen but attempts at modifying it appropriately so as to produce a specific antibody against it are now being made. Its neutralization by an antiserum would deprive solid tumors of the network of blood vessels on which they depend for further growth and so provide an effective means of controlling these tumors.

Hormonal Controls

Hormones represent important components of the body's "homeostatic" methanisms, those mechanisms that can either stimulate or restrain mitotic activity and regulate cellular metabolism and gene

expression. It is not known if hormones other than adrenaline need to combine with a "chalone" type of molecule in order to exert their effects on cells. Such a requirement has been postulated, however, because hormones are often highly specific as to their "target" tissues, and combination with a tissue chalone would provide an explanation for the tissue specificity of hormone action.

There is ample experimental evidence both for the all-important regulatory role of hormones and for the hypothesis that continuous and excessive hormonal stimulation can so disrupt cell function as to induce cancer. For example, tumors appear in ovaries grafted in the spleen, because of intense gonadotropic stimulation of the ovary, and pituitary tumors may result for the continuous administration of antithyroid drugs. Both procedures disrupt "feed-back" control mechanisms. Similar disruptions resulting from irradiation have produced the same results (See: Radiation Carcinogenesis).

In the case of hormone dependent cancers (See: Interactions among Etiologic Factors), it seems clear that the cells neoplastic properties do, at least, partially revert upon removal of the hormonal stimulus and/or the restoration of the proper feed-back mechanism. It appears, therefore, that the tumor's malignant properties rest to a large extent in gene expression.

After a long period during which studies of hormone activity and chemistry were a prominent part of cancer research, interest in them waned, mainly because it has proved very difficult to understand how hormones exert their effects at the cellular level. Recently however, a new compound known as cyclic AMP, was discovered which may be the intracellular mediator of hormonal controls, and as a consequence, entirely new avenues have been opened to the study of the regulatory role of hormones in normal and cancerous tissues.

It is now known that hormones travel from their tissue of origin to their target cells on whose membrane they react with an enzyme, adenyl cyclase. This enzyme is a discriminator for environmental signals, capable of amplifying them through mediating the production of cyclic AMP (3′, 5′ adenosine monophosphate). The cyclic AMP then acts as the intracellular messenger, capable of modifying patterns of biosynthesis through the repression or de-repression of genes.

Cyclic AMP has already been found to mediate the effects of several hormones. The recent discoverv of a second chemically related compound with similar functions gives reason to believe that others will also be discovered. These molecules apparently exist widely in nature, as indicated by the fact that cyclic AMP was found also in microorganisms. There is no doubt that study of such agents will provide much knowledge concerning the intracellular events that result from extracellular control factors, and that some of this knowledge will be of great importance to the understanding of how hormones influence the development and maintenance of cancer.

The Cell Surface

The surfaces of cells are highly diversified chemically, structurally, and functionally. Many cellular functions are actuallv surface functions or are regulated by surface properties; the absorption of nutrients by the intestine, the secretions of glands, the excretion of salts and water by the kidneys and lachrymal glands, nerve excitation and transmission of stimuli, are all surface phenomena.

Studies of the properties of components at, or near, the surface of both normal and cancer cells have revealed that here, profound changes in function, composition, and structure accompany the neoplastic state.

For example, electron microscopy of virus-transformed cells reveals that the surfaces of transformed cells exhibit visible structural changes. Functionally, changes in membrane permeability result in greater autonomy of the cells in relation to their environment, making it possible for them to grow in media that do not support the growth of normal cells. A similar phenomenon, also determined by new membrane properties, has been observed in plant tumor cells. In these cells, greater permeability to certain ions is directly responsible for the maintenance of malignancy. These ions, it has been shown, are the inducers of enzymes which confer metabolic autonomy on plant tumor cells.

Many cancer cells readily agglutinate with an agglutinin derived from wheat germ, whereas normal cells agglutinate with wheat germ only following treatment with trypsin, which apparently exposes previously hidden binding sites. Cancer cells show differences in movement and behavior that may be of great significance to their invasiveness and ability to metastasize *in vivo*. These differences are, in all likelihood, mediated by altered surface properties.

The surfaces of cancer cells have often been described as less "sticky" than normal ones; they detach easily from the surfaces they grow on *in vitro*. This lack of adhesiveness might well be related to their ability to separate from a tumor mass and to be transported to another part of the body where they continue to proliferate as metastases.

The community of cells in an organism acts in a coordinated way. Coordination and order imply communication. Signals from distant cells, such as those mediated by hormones, are first received by cell surface receptors, among which is the enzyme adenyl cyclase. Communications between adjacent cells have been found to occur through membrane junctions. These junctions allow intracellular substances, some of which may be regulators of growth and function, to flow from cell to cell. It is of interest that membrane junctions were found to exist between cells of all normal tissues examined but not between cells of most tumors, nor between normal and tumor cells.

Antigenic differences that may exist between normal and neoplastic cells (See: Cancer Immunology) stimulate immune reactions when located on the cell surface.

Thus, many cellular properties, including some of the most important ones, that distinguish normal from cancer cells, are expressed as surface properties. For that reason, methods have now been devised for the isolation and separation of a cell fraction which contains almost entirely materials from the surfaces of cells. Studies of the "plasma membrane" fraction should prove very fruitful. The knowledge so gained will undoubtedly be important to an understanding of the unregulated growth and invasiveness of cancer cells, including their critical capacity to metastasize.

Of almost immediate practical interest would be the development of substances that bind preferentially to cancer cell surfaces. These might allow the use of chemotherapeutic agents which have too narrow a margin of safety for systemic administration.

Similarly, a modification of the cancer cell surface could be envisaged that would increase the immunogenicity (but not alter the specificity) of its antigenic components.

The study of the nature and properties of cell surfaces must now have high priority in cancer research.

SOMATIC CELL GENETICS

The processes of differentiation and of carcinogenesis both introduce variation among somatic cells.

Extensive experiments in developmental biology, coupled with the demonstration of the versatility of at least some cancer cellnuclei, have led to the conclusion that both types of cell variation are likely to occur against the background of a constant cellular genome. Unequivocal data regarding the basis for somatic cell variation have been difficult to obtain, because until now, methods have not been available for the genetic analysis of somatic cells.

Classical genetic analysis—"Mendelian genetics"—can only be applied to organisms that reproduce sexually, because it is based on the separation of genetic determinants that occur during the formation of germ cells and their recombination in the progeny.

Somatic cells, whether normal or malignant, reproduce by simply doubling the amount of their genetic material before each cell division and distributing it equally to each daughter cell. Thus, there does not seem to be any way for somatic cells to separate and recombine their genetic material. Therefore they have not lent themselves to methods of classical genetic analysis. This is why it has not yet been possible to establish unequivocally whether cancer is genetically determined or not. However, because cancer cells usually have abnormal chromosomes, it has been hoped that detailed studies of such cells would provide evidence on the possible implication of genetic changes in malignancy.

Cytogenetics

With the development about 15 years ago of improved methods for the visualization of the chromosomes of dividing cells, studies in "cytogenetics" became possible. They confirmed that each species has chromosomes of constant number and characteristic sizes and shapes. Cancer researchers reasoned that any departure from chromosome normality, if it were found regularly in cancer cells, would suggest a specific genetic lesion as the basis of the disease.

Cancer cells, however, proved to be as varied in their chromosomal content as in their biochemical make-up. They often show large variations in numbers, shapes, and integrity of chromosomes, with, as a rule, no single change that is common to all cancer cells, nor even to all types of cancers caused by the same agent or derived from the same cell.

Furthermore, even in reports that suggested a high incidence of specific chromosomal causes in certain tumors, it could still not be determined whether the changes seen had causal significance, were a consequence of the neoplastic state, or merely incidental to it. The latter now seems to be most likely, since several highly malignant tumors are known to have perfectly stable, normal, chromosome

complements. Thus, one has to conclude that if a specific chromosomal change does exist, its detection must be beyond the power of the light microscope.

Research on the chromosomes of cancer cells, however, has contributed some findings of great interest. Studies of variability within cancer cell populations have greatly contributed to the understanding of the dynamics of tumor growth. On the basis of variations in chromosome patterns within the cells of individual tumors, it was concluded that the former constitute one or more "clones"—groups of cells that are descendants of a few or even of a single cell. One of these clones is the tumor's "stemline"—the group of cells which, at a given time, is the most vigorous and best adapted. From this group, variants constantly arise that may either be selectively eliminated or else, in time, supplant the original stemline. Sequential examinations of the chromosome complements of cells within a growing tumor reveal them as a highly dynamic and variable population, showing a much increased degree of variability as compared to the constancy of the chromosome complements of normal cells.

Only one chromosomal abnormality has been consistently observed in association with a particular type of neoplasm—the so-called "Philadelphia" chromosome. It is present in the leukemic cells of a majority of patients with chronic myelogenous leukemia. Named after the city where it was discovered, it is a small chromosome which lacks a fragment of its chromatin. It is difficult to know what the association means, because 1) not all patients with this type of leukemia show the Philadelphia chromosome in their leukemic cells; 2) it is present in a few people without leukemia; and 3) it seems to be present also in the bone marrow cells other than those from which the leukemic cells are derived. As is the case with many other associations between chromosome defects or even gene mutations and cancer, the Philadelphia chromosome could merely predispose cells to cancer.

However, far from having exhausted its possible contributions to cancer research, the study of chromosomes seems at this time to offer unexpected new possibilities.

Cell fusion

The field of somatic cell genetics has been recently stimulated greatly by a discovery which now makes possible the accurate genetic analysis of somatic cells.

The co-cultivation of different cell lines results in the "fusion" of some of their cells, with the result that the nuclei from different cells are able to co-exist within a common cytoplasm or even to fuse with one another. Under these conditions, the two sets of chromosomes then carry out both their replicative and synthetic activities side by side.

Such odd findings offer an extraordinary opportunity for new experiments in the fields of somatic cell genetics as well as of cancer research. Cell fusion, which was first observed as a rare spontaneous occurrence, has been found to be much more easily achieved in the presence of an inactivated and therefore noninfective virus. By agglutinating cells, the virus facilitates the coalescence of their cell membranes. By this method, "hybrid" cells now can be produced almost at will.

One of the most remarkable properties of the "hybrid" cells thus obtained is that no matter how distantly related the cells are—as, for example, from mouse and man—the cellular functions of both cytoplasms and sets of chromosomes seem to be carried out in complete coordination. All the intracellular signals that regulate the sequence of events of the cell cycle are apparently "understood" by the cytoplasmic and nuclear components of both the cells; this is one of the most eloquent illustrations of the fundamental unity of the basic processes of life.

As is often the case in all fields of science, the development of new methodology has allowed the use of cell fusion for systematic and far-reaching investigations. The methods are based on the production and isolation of two different "mutant" cell lines, each having a specific defect such as, for instance, an inability to survive in a particular medium. If the two mutants are mixed under conditions where they can fuse, hybrid cells will arise in which the two different sets of chromosomes function cooperatively, within a common nucleus and cytoplasm. Since one set of genetic determinants is able to make up for any deficiency in the other partner, they "complement" each other. Each set of chromosomes supplies the missing function of the other, so that the hybrid cells survive while the parental cells die. The phenomenon of "complementation" opens a new era in the field of somatic cell genetics.

Following fusion, hybrid cells start out with two full sets of chromosomes. However, hybrids between cells of different species begin to lose chromosomes, which suggests that genetic redundancy is not tolerated under such conditions. However, if one chromosome set carries a genetic deficiency in one of its members, which affects survival in the medium used, and the second set happens to carry a chromosome capable of compensating for the deficiency, the chromosome with the useful gene is not discarded along with the others. Thus genetic analysis of somatic cells is becoming possible, because progressive chromosome loss achieves separation of groups of parental genes, and the preservation of one or more chromosomes, or parts thereof, that continue to function in association with a new genome, is the analog of recombination. Neoplastic characteristics can now finally be put to a genetic test.

Preliminary experiments have already been carried out using fusion between pairs of malignant and nonmalignant cells. At first the hybrids all proved malignant upon inoculation into susceptible animals. However, more recently, another set of hybrids made with other pairs of parental cells were nonmalignant or remarkably less malignant than their parent cancer cells.

Chromosome studies of such nonmalignant hybrids showed both parental chromosome complements to be present. However, upon continued transfer in tissue culture, the usual chromosome loss took place and, subsequently, the cells were found to have regained the original parental malignancy when inoculated into test animals. Concomitant chromosome studies suggested that in the nonmalignant hybrids, one or more chromosomes of the normal parental cell had been able to suppress malignancy.

These experiments represent only very preliminary explorations of the possibilities offered by cell fusion in cancer research. Expanded use of this technique will require extensive and precise chromosome

studies; should they be carried out with present techniques, full exploitation of the new possibilities for investigation will be slow. Automated methods have recently been developed that will greatly facilitate all cytogenetic studies once they become generally available.

Much information on the part played by the genetic material in the etiology and the maintenance of cancer can be expected soon to be at hand.

Drug resistance

Study of the resistance of cells to drugs and radiation is of great importance both to the understanding of somatic cell variation and to the improved use of chemotherapy and radiotherapy in man. In contrast to the stable responses to environmental changes shown by normal cells *in vivo*, tumor cell populations show great adaptability. They are able rapidly to become adjusted to, and to survive, high levels of toxic drugs and irradiation. They display, in this way, efficient adaptation both *in vivo* and *in vitro*, a property that normal cells show only when propagated *in vitro*.

The phenomenon of adaptation as displayed through progressive acquisition of resistance to environmental factors, particularly drugs and radiation, is one of the major obstacles to efficient long-term chemotherapy and radiotherapy in patients and seems to be basic to tumor progression.

Adaptive evolution is a prominent characteristic of populations of microorganisms. This adaptation is the result of the action of selective forces following direct genetic change. The emergence of new bacterial variants occurs with a frequency similar to that expected for spontaneous mutation, and resistance is not only inherited but may also be transmitted from cell to cell by the various mechanisms involved in genetic recombination. Thus resistance is clearly genetically determined in bacteria.

In somatic cells, the respective contributions of genetic and epigenetic mechanisms to adaptation are not known. However, it has been established that, within permanent cell lines, drug resistance occurs occasionally as a result of a sudden, rare, and random variation. By analogy with the development of resistance in microorganisms, the phenomenon has been assumed to be genetic and such resistant variants have been termed "mutants."

Drug resistance in somatic cells usually develops through a succession of incremental steps. In one case, a series of changes followed by clonal selection *in vitro* resulted in cells that were resistant to a particular drug (amethopterin) at levels 100,000 times greater than that tolerated by unadapted leukemic cells. Reversion of resistant cells to sensitivity to a drug sometimes occurs when the cells are kept growing in culture in the absence of the drug. Interesting observations have been made that suggest interactions between populations of mixed resistant and sensitive cells. For example, single clones from such a mixed population may reveal a high proportion of resistant cells, despite the fact that the total population behaves as though it were sensitive. A form of interaction between cells is suggested by such findings, although the mechanisms are still unknown.

In microorganisms, irradiation or treatment with mutagenic chemicals prior to selective cloning for the isolation of resistant mutants increases the overall frequency of mutations including the appearance of resistant mutants. With somatic cells, similar treatments decrease

the rate of spontaneous mutation. This effect can be attributed, in part, to the fact that these cells—unlike microorganisms—are diploid. Thus genetic changes may be produced that affect only one of the two genes present for each genetic character, the unmutated gene masking the expression of the mutated one.

Only very recently genuine mutations have become identified through cell fusion experiments. Such new methods now open up vast new possibilities for the study of drug and radiation resistance in cancer cells. More complete understanding of these phenomena may lead to a much more effective and rational application of both radiation-therapy and chemotherapy.

CELL CULTURE

Cell culture has been fundamental to most of the recent advances in knowledge of the cells of higher organisms and its use offers the best hope of acquiring new information concerning the mechanisms regulating gene variation and expression. It is now possible to propagate cells from almost any tissue, normal or malignant, in culture and, in many cases, to grow c ones from single cells. Use of genetically homogeneous cell clones has permitted quantitative assessment of agents capable of inducing cell variation or cell death. Determination of precise nutritional requirements for cells can now be made at the molecular level. The induction of stable chromosome changes and, recently, the isolation of stable single-gene mutations have been achieved. Now complementation analysis by means of cell fusion provides a way to analyze cellular properties genetically. Applied to cells with similar phenotypes, this method is already making it possible to detect changes as subtle as those occurring within single genes. Not only can the end products of a gene's activity be identified, but also the precise metabolic steps leading to complete gene expression.

The all-important study of the processes of growth and differentiation depends on developing appropriate and simple *in vitro* systems in which cells can differentiate and maintain stable differentiated functions. Attempts could then be made to regulate cellular functions by the use of chemical stimuli of the sort which operate in the intact organism. At the same time, the ability to culture cells with specialized synthetic functions would allow the isolation of regulatory substances.

So far, it has been almost impossible to maintain normal cells with specialized functions *in vitro*, because they are usually at a disadvantage as compared with unspecialized cells (fibroblasts) of the same tissue. It has also been impossible to stimulate these unspecialized cells to develop specialized functions. However, the proper conditions for studying at least some forms of tissue differentiation and organogenesis *in vitro* are becoming available. It is also becoming possible to study *in vitro* the events stimulated in cells by steroid hormones; following exposure to the hormones, cells synthesize several new components without any disturbance of their general metabolism or growth.

Such very specific inductions can only be studied under the simplified and controlled environmental conditions of cell culture.

Cultured cells propagated on a very large scale are already in demand but will be needed much more in the future. Their spohisticated synthetic activities will be indispensible to the production of

materials for biochemical studies; they can provide a source of effector molecules and other products, such as interferon, viruses, antibodies, and antigens, both for experimental purposes; the eventual production of vaccines, and new therapeutic substances.

The diversified techniques of the discipline of cell culture have made possible the direct study of cells, as well as the use of their synthetic capabilities. It is vital that progress should continue in cell culture—which is a field of research in itself. Otherwise, it is likely that future advances in cancer research and the application of methods for the control of human cancer will be greatly hampered.

A careful consideration of the many faceted-attacks on the cancer problem now under way suggests that the following studies offer great promise of hastening the control or prevention of this disease:

AREAS OF PROMISE

1) Relationships between mutagenesis, carcinogenesis, and teratogenesis in mammalian cells.

2) Nutritional requirements of human normal and cancer cells.

3) Mechanisms of reversion, and/or differentiation of cancer cells, and the relationship of such events to malignancy, in "spontaneous" tumors of plants, animals, and man as well as in tumors induced by viruses, chemical carcinogens, or radiation.

4) Relationships between cell proliferation and the development of specialized cellular activities.

5) Relationships between DNA synthesis and the establishment of new patterns of protein synthesis.

6) The cell cycle and its controls, including methods for synchronization of division in cell populations.

7) Intracellular communications; their modalities and the substances mediating them; differences in production of, and response to, such substances, by normal and cancer cells.

8) Angiogenesis induced by tumors.

9) The induction of cancer by hormones and production of hormone-dependent tumors.

10) Intracellular mediators of hormone action and their role in the development and maintenance of hormone-dependent tumors.

11) Structures of cell surfaces including analyses of changes in structural, chemical, and antigenic composition as related to cell movement, agglutinability, adhesiveness, and permeability.

12) Correlations between surface properties of cancer cells and the latter's ability to form metastases.

13) The enzymatic profiles of normal cells, cells of premalignant lesions, and cancer cells at different degrees of neoplasia.

14) Development of methods for the preparation and purification of cell fractions and the study of metabolism at the subcellular level.

15) Development of automated methods for the cytogenetic study of cell populations.

16) Genetic analysis of carcinogenesis and its characteristic components of unregulated growth and invasiveness, in order to establish the role of the cellular genetic material in the production of cancer.

17) The use of somatic cell hybrids for genetic analysis.

18) New methodology leading to the possible use of cell hybrids in the clinic.

19) Mechanisms leading to resistance to drugs and radiation, of reversion to sensitivity, and of complementation between mixed sensitive and resistant populations of cells.

20) Development of cell culture methods permitting the maintenance of normal differentiated cells *in vitro*.

21) Study of differentiation and organogenesis *in vitro*.

22) Developments and improvements of methods for the large scale culture of cells.

The Etiology of Cancer

Etiology is the study of causes. Knowledge of the causes of diseases makes it possible either to eliminate them from the human environment, to interrupt the etiologic process, or, to increase the resistance of the human body to withstand their assault. In this way, many infectious diseases were conquered by prevention, often without knowing the precise pathologic processes that caused them. Prevention of cancer is a major goal of the studies described in this section of the report.

Cancer Epidemiology

Cancer epidemiology is the study of the incidence and distribution of human cancers in relation to a variety of environmental and intrinsic factors. It seeks correlations between the occurrence of the disease and particular conditions in the environment or in the characteristics of the people affected.

The relationships between man and his environment are immensely complex; each type of cancer is a relatively rare occurrence and appears generally independently from the occurrence of others. To study the epidemiology of cancer requires long-term observations of large numbers of people and is therefore not only arduous but costly.

CONTRIBUTIONS OF EPIDEMIOLOGY

Epidemiological studies have made it clear that cancer is not caused by a simple infection or a single genetic factor. They have strongly suggested that variations in exposure to environmental agents and in social practices are largely responsible for variations in the occurrences of cancers in different populations. Thus, they have indicated that cancer is, to a large extent, preventable. And, indeed, on the basis of epidemiological evidence, specific causes of cancer have been identified and eradicated.

PROTECTION FROM RADIATION

Ionizing radiation is perhaps the single carcinogenic and leukemogenic agent most thoroughly and intensively studied at the epidemiological level and perhaps, for that reason, the one for which the most effective and vigorous preventive measures have been introduced. Its role is clearly established in the induction of chronic myeloid and acute leukemia in Japanese atom bomb survivors, in patients repetitively treated over the spine for rheumatoid spondylitis, and in radiologists of a generation ago, and these observations have provided the basis for crude estimates of the dose-response function in adults.

Radiation has also been shown to be carcinogenic for a wide variety of tissues and sites in man; the skin, the lung, and the skeletal system are the most important of these. However, such radiation-induced

tumors account for an insignificant fraction of all cancers, and many of them have been effectively eliminated by the protective measures universally adopted during the past two decades.

However, despite the effective protection already advised and the extensive work already completed in this field, the amount of data available on the dose-response relationship for adult occupational and environmental exposure to radiation is still far from adequate to provide sound and reliable guidelines to the rapidly emerging nuclear power industry, and additional intensive prospective efforts in this field are urgently needed.

OTHER ENVIRONMENTAL HAZARDS

Although a large number of occupational hazards, other than radiation, have already been eliminated the possible contributions of cancer epidemiology in this area are far from exhausted (See: Occupational and Other Carcinogens).

Striking geographical variations in the incidence of different forms of cancer have been found. For example, primary cancer of the liver may be as much as 500 times more frequent in the African populations of Mozambique than among black people in the United States or the United Kingdom. The search for a causative environmental factor present in Africa has led to the implication of a mold product, aflatoxin, a potent carcinogen produced by a mold that can grow on peanuts or cereal grains.

The Africans living in the Transkei area of the Republic of South Africa (on the other hand) have the highest incidence of esophageal cancer in the world. American women have about seven times more cancer of the breast than Japanese women.

Cancer of the colon and rectum is the leading internal cancer in the United States, but is infrequent in Mexico, Latin America, Africa, India, and Japan. Cancer of the esophagus is ten times more frequent in Puerto Rico than in upper New York State.

The high incidence of stomach cancers in some countries and its decreasing incidence in the United States has provoked much interesting speculation. There are a number of other striking differences in the distribution of cancer which require further study. All these sharp discrepancies in cancer incidence seem to be related to environmental and not genetic factors since they hold true for people with similar genetic constitution in different environments. Now is the time to identify causative factors before migration, intermingling, and uniformity of living habits become established, and such factors, as a consequence, become far more difficult to isolate.

The analysis that established the correlation between cigarette smoking and lung cancer is one of the most outstanding contributions of cancer epidemiology. The studies of cigarette smoking identified the main cause of the form of cancer with the greatest increase in incidence of all cancers and one of the poorest five-year survival rates following treatment.

IDENTIFICATION OF HIGH-RISK GROUPS

Epidemiology of cancer makes it possible to recognize sometimes not only *what* but also *who* is unduly susceptible or resistant. It is already possible to predict increased levels of risk for certain groups

of individuals, the highest risk existing in the identical twin of a child who has developed leukemia before the age of six. Many other human diseases have been found to be associated with a high incidence of cancers. Down's syndrome (mongolism) for example, a genetically determined congenital abnormality, is associated with a high incidence of leukemia. Among these children, moreover, the peak mortality appears three years earlier than among leukemic children without Down's syndrome. Rare but striking family clusters of cancer have been found, which suggest an occasional genetic predisposition. The identification of cases of high susceptibility to cancer has great importance for the people at risk, since, for them, the most careful medical supervision is clearly indicated in order to achieve life-saving early diagnosis.

Recent experiments have suggested one potential predictive test for high-risk individuals. Simian oncogenic virus, SV40, is added to cultured cells taken from suspected susceptible individuals. It has been shown that the proportion of cells that can thus be transformed is 20 to 100 times higher if the cells come from individuals with Down's syndrome or Fanconi's anemia (also known to be highly susceptible to cancer) than if they come from normal individuals. The applicability of this test for the evaluation of predisposition to cancer is now under study.

Epidemiological methods lend themselves to investigating other topics of great potential importance. There are strong reasons to believe that a defect in cellular immunity (the mechanism responsible for the rejection of grafted foreign tissues) may be a predisposing element in cancer. (See: Interactions between Etiologic Factors). Evidence is now emerging that the incidence of cancer is higher among those people in whom the immune response is impaired.

Variations, among normal people, regarding susceptibility to a wide variety of environmental agents certainly also exists. It is necessary to refine "safe" exposure levels, those that ensure adequate protection

The requirements for protection and hence the formulation of safeguards, regulation, and laws must rest on epidemiological and experimental information. Although absolute thresholds probably do not exist, practical ones can be estimated and levels of safe exposure enforced for a large number of present hazards.

EPIDEMIOLOGY OF CANCER VIRUSES

The first human virus to be suspected of causing a human cancer was isolated as a result of epidemiologic observations made in populations of tropical Africa. Burkitt's tumor occurs relatively frequently among the children of certain areas where malaria is also endemic. The particular distribution of the tumor from the geographic and ecologic standpoint, as well as its age distribution, typical of an infectious disease, have led to the suspicion that Burkitt's tumor might be caused by an infectious agent. This led to concerted efforts to isolate a virus from Burkitt's tumor cells and resulted in the successful identification and propagation *in vitro*, of a virus (EB virus) presently under intensive study (see: Viruses and Cancer). Meanwhile, seroepidemiological studies are being carried out in the affected population as well as elsewhere.

With most viruses, antibody is absent prior to disease, shows a peak during disease, and declines during convalescence. Such a pattern of antibody production has been generally used to incriminate specific viruses for specific diseases. An evaluation of the distribution of antibodies against EB virus in the affected and nonaffected populations can provide valuable information as to the relationship to cancer.

The recent discovery of another virus, observed in breast cancer tissues and in human milk, may soon call for similar investigations. The agent has not yet been grown successfully in tissue culture and so serological studies are not yet possible. It is likely, however, that sufficient supplies of the appropriate antigen will soon be available, and large surveys should then be launched. Such a need could arise within the next two years.

Epidemiological studies of the distribution within the population of pre-invasive cervical carcinoma have disclosed the fact that the disease is found more frequently among women who have had multiple sex partners at an early age. This evidence has clearly raised the question of the possible infectious origin of this type of cancer. A virus, Herpes simplex type II, has been found to be consistently associated with this type of tumor. Although association does not prove casuality, this interesting observation has stimulated further study of the relationship between the disease and the virus and has also spurred intensive investigations on the oncogenic potential of the herpes virus (see: Viruses and Cancer).

Since the discovery that many animal cancers are induced by viruses, which include some isolated from pets (dogs, cats, parakeets, etc.) and cattle, it has become important to examine whether associations between animal cancers (such as bovine lymphoma) and human disease can be found. There have been for example, cases of leukemia developing in children who had been bitten by cats, or appearing in people who had been in contact with diseased cattle or fowl. It is not clearly established yet whether such associations are more than coincidental. However, suspicion is in order since several animal viruses are capable of infecting human cells in culture and of "tranforming" them. With many oncogenic animal viruses, the classical pattern of antibody production does not occur in the animal, and it may not occur in humans either. There is, therefore, need of epidemiological evidence for a positive correlation between incidence of animal and human cancer.

Adequate epidemiological studies need careful planning, involve very large numbers of people and require long-term follow-up. This is particularly important in cancer owing to relatively rare occurrence of each type and the fact that the time elapsing between contact with any possible etiologic agent and the appearance of the disease may be measured in decades. Thus, studies in cancer epidemiology must be always of long duration and require continuing long-range support.

These inherent difficulties have discouraged the undertaking of epidemiological studies in the field of cancer. Thus, at present, there is a lack of adequate cancer registries containing accurate and comprehensive data, recorded in a standardized, universally accepted form and of modern automated systems of information storage and retrieval applied to cancer information.

Because of its past contributions and the promise it holds for the future control of cancer, epidemiologic studies are to be facilitated without delay and given consistent support so that factors responsible for the marked variations in cancer incidence observed today are clearly identified.

AREAS OF PROMISE

Given appropriate support and encouragement, the following epidemiological studies hold great promise:

1) Geographic variations in the incidence of certain cancers and the identification of environmental hazards responsible for these variations.

2) Occupational and industrial hazards.

3) Nutritional factors, whether dietary regimens, bowel habits, or carcinogens in the form of food preservatives or contaminants that may be associated with certain cancers.

4) Relationship of certain cancers to hormonal status.

5) Human susceptibility to different carcinogenic agents, in order to determine the levels of "safe" exposure for most people.

6) Health impairment which increases susceptibility to cancer so that individuals at high-risk might be identified and given appropriate preventive and diagnostic care.

7) Combined epidemiological and serological studies on the distribution of specific antibodies against EB virus suspected of causing some human cancers, as well as on other viruses that become similarly inplicated.

Chemical Carcinogenesis

AIMS OF RESEARCH IN CHEMICAL CARCINOGENESIS

The principal aims of research in chemical carcinogenesis, in terms of cancer prevention, are:

1) To identify the chemical compounds that can cause, or are causing cancer in man or experimental animals, and to find means of preventing their entry into the human environment or of removing them from the environment.

2) To elucidate the mechanisms involved in the processes of the induction of cancer by chemicals, as a basis for the prevention, interruption, or reversal of such processes, possibly by means of specific chemicals.

THE POTENTIAL HUMAN HAZARDS FROM CHEMICAL CARCINOGENS

There is now no doubt that chemicals play a role in the development of cancer in the human population. Knowledge of this role dates from the early findings of Percival Potts (1775) on the increased incidence of skin cancer among chimney sweeps who underwent prolonged exposure to soots, and the observations of Rehn (1895) on the unusually high incidence of cancer of the urinary bladder among men working the dyestuffs industry and, as is now known, who were exposed to certain aromatic amines.

In 1915, two Japanese workers succeeded in producing cancer by repeated application of coal tar to the ears of rabbits. In 1933, a cancer-producing chemical, benzpyrene, was isolated from coal tar.

Since that time benzpyrene and related polycyclic hydrocarbons have been isolated from polluted air, automobile exhaust, cigarette smoke condensates, and many other sources in the human environment.

Hundreds of chemicals of diverse structures and types have been shown to be carcinogenic for experimental animals, in which they produce many types of cancer prevalent among humans, including cancers of the lung, mammary tissues, and stomach. Most of these chemicals are laboratory products, but knowledge of the carcinogenicity and varied structures of these compounds raises the question of what carcinogens might be present now or potentially in the human environment.

The importance of this question is underlined by the conclusion of epidemiologists that a high proportion of human cancers are of environmental origin. The question is further highlighted by the sophisticated technology of modern life in which chemicals are used in a myriad of ways to control or alter the environment. The list of manmade products that are recognized as carcinogens is long and is growing rapidly. It is clear that we are living in a cancer-inducing environment brought about by steadily advancing technology.

POLLUTANTS AND ADDITIVES

Among the most potent of the carcinogens to which man is constantly exposed today are the polycyclic hydrocarbons which result from the combustion of fuels and which are present in steadily increasing amounts in the air of urban centers. The present epidemiologic data probably do not reflect the present-day hazard from air pollution because studies on the relationship of smoking to lung cancer indicate that 25 years may be required for the development of this type of cancer.

Thus the full impact of prolonged exposure to environmental pollutants may not be realized yet. Such pollutants include, for example, food additives, chemicals used in food processing, pesticides, herbicides, packaging materials, drugs, and cosmetics. Such substances may enter the body by various routes such as the lung the mouth, and the skin and although the total exposure to any single chemical is likely to be very small, their cumulative effect, to which the population is constantly exposed, may constitute a serious hazard (See: Interactions between Etiologic Factors). Present knowledge of the carcinogenic hazard they represent, either alone or in combination, is far from adequate.

NATURALLY OCCURRING CARCINOGENS

In addition to the manmade carcinogens, the population is exposed to toxins produced by living organisms. An example of the latter is aflatoxin B1, which is produced by certain strains of the fungus *Aspergillus flavus*, which can be a contaminant of foods such as peanuts, when stored under warm, moist conditions. Aflatoxin B1 is a very powerful carcinogen for the liver of the rat and is suspected to be one of the causes of cancer of the liver in certains parts of Africa (see: Cancer Epidemiology).

Cycasin occurs in cycad plants found throughout the tropical and subtropical areas of the world. It bcomes a potent carcinogen, when acted upon by normal intestinal micro-organisms, which convert the

natural compound into an active product. Another powerful natural carcinogen has been found in bracken. It induces tumors in a range of animal species, including cattle, and apparently causes cancer of the bladder in man. Bracken may be consumed by livestock as well as by people in certain areas, such as Japan and Turkey, when little other food is available.

POSSIBLE ENDOGENOUS CARCINOGENS

It has been known for some time that a number of synthetic chemicals, the N-nitroso compounds, can induce cancer in a range of different organs and animals. Also, the production of such compounds by a simple reaction between secondary amines and nitrites (both rather simple and common chemicals) in an acid medium had been known for many years. It was recognized only recently, however, that N-nitroso compounds can be formed in this way in the natural environment and even within a living body. This discovery was made in Norway when a flock of sheep was found intoxicated by fish meal containing sodium nitrite as preservative. The feed was found to contain dimethylnitrosamine, a powerful carcinogen, which had presumably resulted from chemical reactions between secondary amines, naturally occurring in fish, and the nitrite preservatives. Nitrites are commonly used to preserve human food, and analytical work on the possible formation of carcinogenic nitrosamines in human foods is being attempted. Such analyses are proving a very difficult problem for the chemist, but the possible risks to humans must be clearly determined. The outcome of such studies may have great significance not only for public health but also economic consequences for the food industry.

More recently, it has been established that the same simple chemical reaction which produces nitrosamines in an acid medium in the laboratory can also take place in the acid content of an animal's stomach. In fact, simultaneous administration of secondary amines and nitrites to rats induces cancers in a variety of organs. The potential significance to man of all these findings can be only conjectured at this time but, at present, there is no reason to believe that nitrosamines could not be formed under similar circumstances in the human stomach.

THE ONCOGENIC PURINES

The purine N-oxides, which constitute the oncogenic purines, are of particular interest because they are chemically closely related to the naturally occurring purines present in all cells as components of the nucleic acids and of energy-transfer molecules such as ATP and so might be examples of endogenous carcinogens.

A number of such purine derivatives of variable carcinogenic potential has been synthesized. Studies of their metabolism have been made. Like other chemical carcinogens, they form complexes with negatively charged groups in various molecules. In the rat, they are as strongly carcinogenic as some of the most powerful carcinogenic hydrocarbons. There is no evidence yet that they are formed in living cells, but the chemical reactions needed to their formation could take place in the intracellular chemical environment.

Thus, man is continually insulted by a wide variety of chemicals, and the kinds and amounts of these chemicals vary with the individual's location, diet, personal habits, and the technological state of his immediate environment. It has been estimated that a major proportion of all cancers could be eliminated—prevented altogether—by the eradication of chemical carcinogens from the environment.

An important practical aspect of studies in chemical carcinogenesis is the assessment of the carcinogenic activity of chemicals which are, or may be, present in the human environment. Much research in this area has already been carried out, but very much more is needed, especially in view of the rate—estimated to be about 10,000 new compounds per year—at which new and hitherto unknown chemical compounds are entering the environment. Of these new compounds, probably only 500 to 2,000 come into contact with enough people and in sufficient amounts to warrant testing for carcinogenicity.

One of the assay methods used at present involves, for each chemical, some 200 newborn mice, each of which has to be observed throughout its lifespan—i.e., for approximately two years. Total cost per experimental mouse is $120 to $150, so up to $30,000 and two years are required to test a single chemical.

The present assays, which are carried out in animals at high dose levels so as to potentiate carcinogenic effects, do identify carcinogenic activity, but provide only a relative estimate of dose-response. Since in the human situation, most contacts with carcinogens occur at a low dose, it is important to be able to extrapolate from effects at high dose to effects at low dose. The number of animals required to apply the rigorous pharmacological models of dose-response measurements to carcinogenicity testing is very large and therefore make such experiments very costly. This problem is urgent, however, since it has great importance for the legal regulation of chemicals of great economic importance, such as pesticides, cyclamates and other artificial sweeteners, as well as oral contraceptives.

Another difficulty is to extrapolate to man results obtained in laboratory animals, but the comparison of the metabolism of carcinogens in tissue slices from biopsies or necropsies of human and animal tissues may help in solving this problem.

A further shortcoming of the present testing methods is that they overlook the decisively hazardous effect weak carcinogens may have when they are combined with other weak carcinogens in the environment. The process of carcinogenesis in the skin of mice—and probably in many other tissues and species—can be divided into at least two identifiable stages: "initiation" and "promotion." It is known that many chemicals act more efficiently either as "initiators" or as "promoters" and that initiator and promoter reinforce each other's effect. It is time-consuming and costly to test the many compounds suspect of carcinogens in various combinations.

NEED FOR NEW TESTING METHODS

The pressing need for better, faster, and cheaper testing methods is obvious, and the search for new ones is in itself a critical area of cancer research. Faster methods available *now*, even if they gave results that were only preliminary or indicative, would be extremely useful.

The use for testing purposes of cultured cells is now being explored. Cell transformation can be induced with chemical carcinogens in cultured animals and human cells. (See: Cancer Biology.) Cell transformation *in vitro* occurs in a shorter time than is required for cancer to develop in an animal. It is possible, however, that transforming activity in the homogenous populations of cultured cells does not accurately reflect activity in the whole organism.

Recently, however, it was observed that cells from individuals belonging to groups known to be highly susceptible to cancer undergo viral transformation much more readily *in vitro*. (See: Cancer Epidemiology). Stable cell lines derived from highly susceptible individuals might allow the development of faster and less expensive *in vitro* testing systems of exquisite sensitivity.

Other even more radical approaches involve application of the knowledge that many carcinogens can also produce mutations. Mutagenic activity has already been used to identify hazardous chemicals. Testing for mutagenesis in bacteria or viruses, rather than in animal cells, for preliminary screening, would further reduce both the cost and the time required. However, the exact relationship between mutagenesis and carcinogenesis remains to be established.

HOW CHEMICAL CARCINOGENS INDUCE CANCER

Research workers have long been aware of the importance of learning what happens when a chemical carcinogen enters a susceptible organism, reaches a target tissue, and interacts in some manner to produce cancer. Detailed information on the process of cancer formation by chemicals is the soundest basis on which to develop means of protecting the human population against the carcinogenic effects of chemicals in the environment and of inhibiting the processes once started. These investigations have been difficult because carcinogenic substances vary widely with regard to many properties such as structure, molecular size, and solubility, as well as in degree and kind of chemical reactivity.

In spite of the difficulty, investigations in this area have been fruitful, and knowledge of the nature of the carcinogenic processes induced by the chemicals has increased greatly in recent years. Much of the advance in knowledge has resulted from the development of better model systems. Thus, by the appropriate combinations of chemical carcinogen, of species, and of route of administration, the investigator can now induce tumors in almost any tissue at will. Further, in some cases cancer can be induced by chemicals in cell culture, and the effects of chemical carcinogens on specific systems can also be studied at the subcellular level. Thus, model systems can be designed in relation to the particular problem under study.

METABOLISM OF CHEMICAL CARCINOGEN

Only a very small fraction of a dose of a chemical carcinogen administered to an animal is involved in the induction of a tumor. In most cases the major share of the carcinogen is converted (usually enzymatically) in the body to substances which are no longer carcinogenic and which are eliminated from the body. To the extent that these reactions occur, the deleterious effects of the chemical are reduced.

However, another fraction of the administered carcinogen (usually only a small portion) is metabolically converted into the actual carcinogenic form; this chemically reactive derivative, which has been designated as the utlimate carcinogen, is directly responsible for the induction of the tumor. In some cases, notably with a class of chemicals called the alkylating agents, the carcinogen is an ultimate carcinogen in the form administered and thus requires no metabolic activation.

The cancer-inducing activity of chemical carcinogens varies from species to species, from organ to organ, and probably from person to person. These differences may reflect variations in the cellular enzymes that metabolize carcinogens. An enzyme system of this sort which metabolizes polynuclear hydrocarbons has been identified. Its level of activity correlates well with the sensitivity of the cells to the toxic effect of polynuclear carcinogens. This system also catalyzes the binding of the ultimate carcinogen to DNA. An inhibitor has been found that blocks its action.

Measurements of the levels of these activating enzymes systems may lead to the identification of individuals who may be highly susceptible to certain specific carcinogenic exposures and the enzyme system's inhibition by drugs may lead to a marked decrease in cancer risk.

TARGET MOLECULES

Recent studies have shown that the known ultimate carcinogens share a common chemical property in that they are all strong electrophilic reactants (i.e., they contain electron-deficient atoms). As a consequence of their electrophilic character, these ultimate carcinogens, as a class, react chemically with nucleophilic sites (i.e., atoms with excess electrons) in tissues. The information-containing macromolecules of the cells (DNA, RNA, with protein) are relatively rich in nucleophilic sites and, in those cases which have been adequately studied, derivatives of chemical carcinogens have been found to be firmly bound to the DNA, RNA, and protein of target tissues. Further, in some cases, susceptibility to cancer formation by chemical carcinogens has been correlated with the kinds and amounts of these macromolecule-bound carcinogens. Since cancer cells divide to give rise to more cancer cells in the absence of the original transforming agent, the chemical induction of alterations in the information-carrying macromolecules offers one of the most plausible explanations for the induction of tumors by chemicals.

Complexes of covalently bound carcinogens and DNA, RNA, or proteins have been found in extracts of precancerous and cancerous tissues and in cell-free extracts treated with chemical carcinogens. On the basis of present evidence, such bindings appear to be an essential state in the carcinogenic process.

Evidence that ultimate carcinogens bind to a variety of macromolecules is an important step forward in the understanding of the mechanism of chemical carcinogenesis. Since each kind of macromolecule involved has a variety of important functions, however, it is still not known which of the many interactions is the critical one. At this point, it is essential to demonstrate that the carcinogen-bound macromolecules are *functionally* modified and that such functional changes are consistent with a rational theory of carcinogenesis. This is where research in this area stands today.

Two possibilities remain: RNA and proteins. Epigenetic mechanisms for tumor induction, akin to those proposed for normal cellular differentiation, are equally attractive. The activities of a cell appear to depend on the selective expression of segments of its DNA. Which parts of the DNA are expressed is determined by a complex system of repression and derepression in which other macromolecules, proteins and RNAs, play key roles (See: Molecular Biology). The altered affinity for critical sites on the DNA of chemically modified repressors or derepressors could greatly modify the responsiveness of a cell and its descendants to homeostatic mechanisms.

Some studies have suggested that chemicals may trigger the development of cancer through facilitating the expression of latent oncogenic viral genomes in the "normal" cells. Other investigations have emphasized the action of certain chemical carcinogens—which appear to be generally "immuno-depressive"—in decreasing the immunological capacity of the host and in thus providing a more suitable environment for the proliferation of abnormal cells.

The critical question of what is the crucial change involved in chemical carcinogenesis thus remains to be answered. There is no reason at the present time to conclude that the induction of cancer occurs by only one of the above mechanisms. Each mechanism may play a role in the induction of neoplasms in specific cases, and it is important that a broad perspective be maintained.

CO-CARCINOGENESIS

In at least some cases, the induction of tumors is a multistage process, in which each phase is facilitated by specific chemicals that may not be active at other stages. This sequence of events has been most clearly demonstrated in the case of mouse skin tumorigenesis. In this model, appropriate doses of carcingoens can initiate in an irreversible manner the formation of altered cells with the potential capacity for the proliferation to tumors. However, tumors do not usually develop until another type of chemical, called a promoter, is applied subsequent to the initiating dose. The promoting agent appears to facilitate the proliferation of the altered cells and, in the early states, its activity appears to be at least partially reversible. Knowledge in this area is highly relevant to the testing of new chemicals for their carcinogenicity.

Tumors induced by chemical carcinogens, even those induced by the same compound in the same animal, have surface structures that differ in their immunological properties from those of all other tumors. The basis of this tremendous diversity among chemically induced tumors is not understood at present. (See: Cancer Immunology).

THE FUTURE

Much further research is needed before complete understanding is obtained of the activation of chemical carcinogens, their cellular targets, the nature of the reactions of the carcinogens with those targets, and the consequences of the carcinogen-cell interactions that are relevant to the induction of malignancy and the proliferation of the altered cells. Much of the ground work for these studies has been laid in the areas of chemical and viral carcinogenesis and in the broader field of molecular biology.

By providing the information and the tools needed to protect the public from environmental carcinogenic hazards, studies of chemical carcinogenesis can make enormous contributions to public health. Because it is now possible to study the process of cancer formation with new insights, new techniques, and new precision, research in this area is likely to provide new means to prevent, control, or even reverse the cancerous process.

AREAS OF PROMISE IN CHEMICAL CARCINOGENESIS

In order to achieve the answers to the fundamental and practical problems yet unsolved, it will be necessary to expand our national program in chemical carcinogenesis. Given much increased support, the following studies in chemical carcinogenesis offer great promise:

1. Development of test systems for identifications of chemical carcinogens, with a review of requirements for test systems, with recommendations for minimal conditions, and development of special systems, including use of additional animal species and *in vitro* systems.
2. Selection of materials to be tested, these must include those which are highly suspect based on epidemiological and epizoological data, structural features, and mutagenic, teratogenic, and growth-promoting actions as well as those to which man is extensively exposed, including natural hazards, pharmaceuticals, and compounds associated with occupational hazards.
3. Studies on man, including special population groups, based on geographic pathology, occupational risks, and population "laboratories."
4. Effects on chemical carcinogens on immunological process.
5. Interaction between chemical carcinogens and factors that regulate growth and differentiation.
6. Role of hormones in chemical carcinogenesis.
7. Interactions of chemical carcinogens with each other and with other extrinsic factors, such as viruses and radiation, and intrinisc factors.
8. Identification of target cells in tissues undergoing carcinogenesis.
9. Distribution, metabolism, and excretion of carcinogens in susceptible and resistant animals.
10. Nature of interactions between chemical carcinogens and tissue components at the molecular level.
11. Early event in carcinogenesis, especially in macromolecular synthesis and ultrastructural changes.
12. Comparison of the biochemical properties of chemically induced tumors with those of the parent normal cells, and the significance of these properties to the carcinogenic process.
13. Relationship between carcinogenicity, mutagenicity, teratogenicity, and immunosuppressive activities in various species and strains.
14. Observations on cell population dynamics in relation to chemical carcinogenesis.

Radiation Carcinogenesis

Studies in radiation carcinogenesis are directed toward 1) determining the carcinogenic hazards to humans of both natural and man-made radiation and 2) determining the mechanisms by which radiation produces cancer.

The realization that ionizing radiation is carcinogenic for man came remarkably soon after the discovery, just before the turn of the century, of x-rays by Roentgen and the purification of radium by the Curies and Henri Becquerel. Within a few years, the first skin cancers were beginning to appear on the exposed fingers, hands, and faces of the early workers in the field. By 1908, the injurious effects of ionizing radiation on the bone marrow and peripheral blood were known, and by 1911, the first cases of leukemia began to be reported in individuals previously exposed to significant radiation doses. However, the carcinogenic hazards of internally deposited natural and artificial radioactive substance were not appreciated until the now-classic report of Martland in 1931 on the development of sarcomas of the bones in radium watch-dial painters.

With isolated exceptions, experimental studies of these processes languished until World War II, when the development of the atomic bomb gave a powerful impetus and urgency to such investigations. The first systematic studies of the effects of radiation dose and dose rate on longevity and incidence of leukemia in mice were reported in 1947. Since that time, the leukemias and lymphomas have continued to be the most intensively studied of the cancers induced by ionizing radiation in mice and other experimental animals.

However, a broad spectrum of other types of cancers has also been induced under appropriate conditions, including tumors of the skin, subcutaneous connective tissues, bone, lung, intestine, ovary, thyroid, pituitary, and liver. Moreover, ionizing radiations have proven to be a most useful tool for the elucidation of the fundamental mechanisms of carcinogenesis.

MECHANISMS OF ACTION

Ionizing radiations may induce cancers by at least three different mechanisms: somatic mutation, the activation of latent oncogenic viruses, and the disturbance of growth regulatory mechanisms. The latter two concepts were quite unsuspected 25 years ago, yet today they are well documented. On the other hand, somatic mutation, which was the only plausible hypothesis in existence a generation ago, has remained so far unproven. It has long been known that ionizing radiation is powerfully mutagenic, but the frequency of transformations produced by x-rays among cells in culture seems too high to be attributed to randomly occurring mutations. The recent successful development of techniques for the induction of radiogenic cell transformations *in vitro* as well as the new opportunities for the genetic analysis of somatic cells offer great promise for the unequivocal establishment of whether radiation induces cancer through mutations. Moreover, since both the dose and dose rate can be calculated with precision and the time of the initial events can be known with certainty, radiation-induced carcinogenesis may well prove to be an important model system for the elucidation of the earliest steps in carcinogenesis, which are inherently more difficult to time with chemical carcinogens or viruses.

RADIATION AS AN INDUCER OF A MOUSE LEUKEMIA VIRUS

The leukemias and thymic lymphosarcomas induced in mice by whole-body x-ray exposure were also presumed initially to be the result of somatic mutation. However, in a series of experimental

studies, it has been demonstrated conclusively that the results seen in this induction system are not consistent with what would be expected from the induction of mutations. For example, although these tumors arose locally in the thymus, local irradiation over the thymic region alone was entirely ineffective in inducing them and shielding of the bone marrow was able to confer a striking degree of protection. Finally, and most convincingly incompatible with the somatic mutation hypothesis lymphoma development, abolished in irradiated mice by prior thymectomy, could be restored by the later grafting into them of histocompatible thymus glands from unirradiated donors. The tumors arose in the thymic grafts and could be shown, by their transplantation behavior and karyotype (chromosome complement), to be derived from the nonirradiated cells of the graft and thus induced by a completely indirect mechanism. Were carcinogenesis a result of mutation, it should occur in the irradiated cells themselves.

Meanwhile, the key to this paradox had been provided by the discovery of Gross that viruses with leukemogenic potentialities could be extracted from the "spontaneous" leukemias and lymphomas of certain highly susceptible strains of mice. Soon thereafter, a similar subcellular, presumably viral, leukemogenic agent was successfully extracted from lymphomas that had been induced by radiation in two low-leukemia strains. Since that time, this agent has been purified and proven to be a virus which is closely related to the original Gross mouse leukemia virus with respect to its morphology, its immunogenic characteristics, its vertical transmission from one generation to the next through the embryo, and its ability to act as a "helper" for the defective murine sarcoma virus. (See: Viruses and Cancer.) However, this agent differs from the Gross virus in its pattern of host strain susceptibility and, most importantly, in its requirement of "activation" or "switching on" from a latent, oncogenically inert state, by exposure to ionizing radiation or to a variety of chemical agents. As a consequence, radiation-induced leukemias in mice are now interpreted as being caused by a latent leukemia virus "induced" in bone marrow cells by radiation. This virus, once released, is capable of producing cancers in thymic cells, whether irradiated or not.

This phenomenon is of great theoretical interest, since, in many ways, it is remarkably analogous to the extensively studied process of lysogeny and induction of temperate bacterial viruses in their specific bacterial hosts, suggesting that many of the molecular mechansisms of the latter process may have direct applicability at the mammalian level in this "temperate" leukemia virus system. The recent development of quantitative *in vitro* assays for the mouse leukemia viruses should give great impetus to this field of investigation, which may well have important etiologic implications for the human leukemias as well.

It has been reported that there is a small but seemingly real increase in leukemia incidence among children born of mothers whose abdomens were exposed in the course of diagnostic radiographic procedures during pregnancy. Those procedures involve very much smaller doses than those which have been estimated to be leukemogenic for adults, which suggests that the human fetus may be much more sensitive. Indeed, it may well be that these childhood leukemias are the human counterparts of the latent viral activation mechanism described above. Certainly, the extremely low doses of radiation which are known to

induce vegetative viral replication in lysogenic bacteria are entirely consistent with such a possibility. These considerations suggest that that the specific problem of radiation leukemogenesis warrants much further study.

DISTURBANCE OF GROWTH REGULATION

The best, though not the sole, examples of tumors arising by the sustained disturbance of a growth regulatory feedback of equilibrium are those induced by ionizing radiation in the endocrine glands, especially in the ovaries, the thyroids, and the pituitary glands of mice and rats.

In the mouse ovary, granulosa cell tumors and luteomas develop in high yield after doses as low as 50 r. That this induction mechanism is also an indirect one has been demonstrated by experiments that showed that shielding of one ovary, grafting of an unirradiated ovary, or the injection of either female or male sex hormones could prevent the development of such tumors in the other, irradiated ovary. Later work confirmed the interpretation that tumors arose in such irradiated ovaries as a consequence of a reduced ovarian output of estrogen, leading to the production of an increased level of gonadotrophic hormone by the pituitary. When this increase in gonadotrophin was prevented by any of the measures listed above, tumors failed to develop, despite the fact that the ovary had been exposed to the same x-ray dose. Additional confirmation is provided by the induction of identical tumors in an unirradiated ovary transplanted to the spleen or pancreas, from which its estrogen output is carried via the portal vein to the liver and inactivated. The pituitary is again stimulated by the low systemic blood estrogen level to secrete an increased amount of gonadotrophin, which will ultimately produce the same tumors in the grafted ovary unless the systemic estrogen level is restored to normal either by leaving the other ovary *in situ* or injecting exogenous estrogen. It has been shown that analogous mechanisms are responsible for the development of pituitary tumors in mice subjected to thyroid ablation with radioactive iodine. In the rat, the same treatment leads to the development of thyroid rather than pituitary neoplasms, but the mechanisms are fundamentally similar.

It is thus clear that by inducing an injury which leads to the sustained disturbance of regulatory mechanisms, ionizing radiation can initiate a chain of events leading indirectly to oncogenesis. The carcinogenic agent in this circumstance is abnormal hormonal stimulation. The role of radiation is disruption of the "feed back" regulatory functions of irradiated endocrine glands.

These studies are of importance to the understanding of how hormonal factors may influence the appearance of tumors. (See: Cancer Biology, and Interaction between Etiologic Agents.) They are also likely to be directly relevant to the well-documented evidence that incidence of thyroid neoplasms is significantly increased in individuals exposed during childhood to low therapeutic doses of x-rays for enlargement of the tonsils, thymus, or other lymphatic tissue, a practice now thoroughly discredited.

Viruses and Cancer

Research in the production of cancer by viruses has several objectives:

(1) Determining whether or not viruses cause human cancers,

(2) Studying the means by which known cancer-causing viruses change normal into cancer cells. Should it be shown that viruses cause cancers in man, ways might thus be found to prevent, interrupt, or reverse this process.

(3) Determining, in the different types of cell-virus interactions, the precise role of the viral genes and their products. Such studies should increase the understanding of the genesis of all kinds of cancers, including those induced by chemicals and radiation.

(4) Analyzing the mechanisms that regulate the expression of viral genes. Knowledge of how genes in general are expressed, regulated, and inhibited in living systems can ultimately be exploited for prevention and therapy.

HISTORICAL BACKGROUND

The concept that an infectious agent might be involved etiologically in the cancer process was advanced in 1908 by Ellerman and Bang, who observed that the mode of transmission of a malignant leukosis in fowl was similar to that of an infectious and contagious disease. In 1911, Peyton Rous demonstrated that the infectious agent involved could go through a porcelain filter which would not permit the passage of bacteria. He thus attributed the disease to a "filterable virus." Both these pertinent observations attracted little attention at that time, because the cancerous nature of fowl leukosis was not as yet clearly recognized. A few years later, however, Rous also demonstrated that the same filterable agent could induce a solid tumor, a sarcoma, when administered intramuscularly.

An initial controversy over whether most cancers might be due to viruses soon died out, because Rous's discovery remained an isolated finding for many years to come. In 1932, however, Shope described a new agent in wild cottontail rabbits that could transmit a wartlike growth not only to cottontails but also to domestic rabbits. Although most warts in cottontail rabbits remained benign or regressed, those of the domestic rabbit sometimes changed into highly malignant, invasive carcinomas. Moreover, although the warts of the cottontails readily released an infectious filterable agent, it appeared impossible to extract such an agent either from the benign or malignant tumors of the domestic rabbits. Something of a viral nature persisted in the rabbits' growths, however, because extracts from them could be used to immunize other healthy animals, both wild and domestic, against the original virus.

In 1938, Peyton Rous, in a further pioneering observation, noted that the application of a chemical carcinogen to the skin of an infected animal increased its susceptibility to this infection and greatly accelerated the conversion of benign tumors to cancers. Thus the history

of viral carcinogenesis began, with a series of odd findings which to this day, defy the most fertile scientific imaginations.

The "milk factor" of mice, a virus isolated from mammary gland tumors, was described by Bittner in 1938. The milk factor is passed from mother to offspring through suckling. This agent proved difficult to experiment with, and its etiologic role difficult to evaluate because the virus is only one factor in cancer production, others being hormonal status, genetic constitution, temperature, diet, and even overcrowding.

Ludwig Gross, in 1951, discovered a virus that induces leukemia in mice. The demonstration that viruses can cause leukemia in mammals, as well as in birds, elicited an upsurge of interest and research activities, which were intensified by the remarkable similarity between the leukemias of mice and man. Efforts to develop "animal models" applicable to human leukemia research resulted in the isolation of a large number of new mouse leukemia viruses, all containing RNA as their genetic material and all related serologically, although distinct in a number of other biological properties. Polyoma virus, the first DNA virus capable of inducing a wide variety of cancers in several species of mammals, was isolated in 1957, further raising hopes of an early solution to the human cancer problem. This solution was envisaged most generally in the form of a vaccine against "*the* human cancer virus."

By now, some 100 additional viruses have been discovered, which cause virtually all kinds of cancers in every major group of animals, including subhuman primates. However, there is still no unequivocal evidence of a human cancer virus, and the situation now appears far more complex than could have been envisaged 20, or even 10 years ago.

PROBLEMS OF IDENTIFYING HUMAN CANCER VIRUSES

Viruses were accepted as causes of cancers in animals when they were shown to satisfy the following postulates:

(1) The agent could be isolated from cancer tissue;

(2) When injected into a healthy animal, it reproducibly induced a similar cancer;

(3) The same agent could be re-isolated from cancers so induced.

To apply these same postulates in the case of cancer viruses in man is probably impossible. First of all, despite the plethora of viruses which can be shown to cause cancers in animals other than man, no virus can be demonstrated at all in the great majority of cancers—in addition to those of man. Second, to inject into a human being a virus capable of causing cancer is ethically and morally inconceivable. Third, such an experiment probably would yield negative results under any circumstances. From among those viruses that have been isolated from animal cancers, only a few, including that of Mareks disease, a lymphoma of chickens, are known to induce leukemias or tumors in their natural wild host. In other species, viruses cause noncancerous disease or asymptomatic infections and may, only under special conditions, produce cancer. This is the case with polyoma and SV40, as leukemia and sarcoma viruses.

Even in the case of virus-caused warts, which occur in many different species of animals as well as in man, the growths remain limited and usually regress spontaneously.

The actively oncogenic viruses used in the laboratory are predominantly strains whose virulence has been greatly increased by selection and further exaggerated by the choice of highly susceptible inbred animal hosts, at least for the first transfers. Thus the experimental system represents a highly simplified situation and is not at all the natural relationship that any one of these viruses has with its natural host. For example, it commonly observed that when a cancer-causing virus is first isolated from its natural host, it tends to be very restricted with regard to what animals it will infect, showing very exacting requirements for hosts with a particular genetic constitution. For instance, at first, the Gross virus could be grown only in newborn mice and furthermore only in newborns of the very special strain from which the virus had been isolated originally.

Similarly, polyoma virus grew initially only in the same or genetically closely related mice and in newborn hamsters, animals which are unusually susceptible to cancer induction. All laboratory Syrian hamsters are descendants of a single pair and are thus genetically very much alike. It is only upon repeated passage that the exacting host specificity was gradually lost by these viruses.

The past success in isolating animal cancer viruses is due largely to the existence of inbred strain of animals which genetic differences have been eliminated by inbreeding. There are no comparable inbred strains in humans, and even if there were, passage of virus from individual to individual would not be permissible. Clearly, because of the impossibility of carrying out the critical experiments previously mentioned, any human cancer virus that might have been present in human cancer extracts would thus in all likelihood, remain unrecognized by the application of previously available procedures. Wholly new methods were called for and these now have been devised.

VIRUSES SUSPECTED OF CAUSING HUMAN CANCER

Despite the fact that a causative virus has not been isolated from any human cancer so far, sufficient circumstantial evidence has been acquired to compel the assumption that one or more viruses are indeed involved in the causation of human cancer. It is, therefore, imperative that all the necessary steps are taken now to plan for large surveys both among cancer patients and the normal population, in order to study the distribution and natural history of agents that may cause human cancer.

At the moment, several viruses are under particularly intensive study.

Herpes-type viruses

In the past "herpes-like particles" were observed with the electron microscope in various human cancer tissue or preparations from the sera of leukemia patients. The infrequency and lack of constancy with which they were seen in cancer tissues made it appear at that time that they were no more than "passenger viruses," with no particular significance either for the cause or the progress of the disease. In any event, repeated attempts at isolating them and inoculating them into experimental animals failed. However, following a report of their presence in cells of the Burkitt tumor (whose peculiar distribution was in itself and indication of its possible infectious origin), new attempts were

made and as a consequence, a variant of a herpes virus was successfully grown out of cultured white blood cells taken from patients with Burkitt's tumor. This variant is now known as the *EB virus*, for Epstein and Barr who isolated it. The EB virus, as well as other herpes-like particles seen in tumors, is similar in size and shape, but not identical, to other herpes viruses of man (which include, common DNA-containing viruses, such as the one that causes fever blisters). The association of a virus with a tumor does not, in itself, permit assigning the virus an etiologic role. Nevertheless, the EB virus has been the object of great interest both because of its particular origin and because it has afforded a first opportunity to study a tumor-associated herpes-like virus in vitro. Moreover, its behavior can now be studied in large groups of people, since sufficient viral antigen is available for seroepidemiological studies. Studies, which are now underway, reveal that 100 percent of the African children with Burkitt's tumor have high titers of antibody against this virus, as compared to less than 50 percent of the control children. Similar surveys made in this country among cancer patients and healthy controls, reveal that patients affected by any one of a variety of 30 different types of cancer did not differ from the control population (in which the virus is fairly common) in terms of their antibody titers against EV virus. However, high titers were commonly found in patients with nasopharyngeal carcinoma. In addition, two apparently totally unrelated conditions, infectious mononucleosis and sarcoidosis, also were found to be strongly associated with EB virus infection, with the highest titers reported for the latter. EB virus has not yet been shown to have a clear causative role in three of these conditions; only in the case of mononucleosis did the serological study convincingly show a typical pattern of antibody production related to the disease. Students who lacked antibody when first tested were found to develop a high titer of antibody against EB virus at the same time as they developed clinical signs of infectious mononucleosis, clearly linking the virus with the disease. The result is of great interest; although infectious mononucleosis is a self-limited, nonmalignant disease, it involves the same type of cells as are involved in leukemia, and these cells go through a phase of very rapid and, for a time, uncontrolled growth. Recently, and independently, epidemiologic studies have reported that people who have had overt infectious mononucleosis have a higher risk of developing lymphoma later in life. The very intriguing associations uncovered so far between EB virus and several diseases, two of which are cancers deserve much more rigorous studies.

Other herpes-type viruses have meanwhile been isolated from human cancers and successfully grown in human cells in culture. Out of some 180 tumor cell lines established from patients with leukemia and lymphoma, virus has been successfully isolated in 80 instances. The human cells that are the source of these viruses can be grown on a large scale *in vitro*. Thus it should be possible to recover, purify, and concentrate these virus in sufficient quantities so that their definitive characterization can be attempted.

Vaccine against EB virus

In view of the possibility that EB virus might be found to be the cause of Burkitt's tumor and of nasopharyngeal carcinoma, the possibility of developing a vaccine against EB virus is being explored.

Of great importance in the development of a vaccine is the need for reliable systems for safety testing; it is essential to have an "animal model," i.e., an animal susceptible to the virus in the same manner and in no lesser degree than the human. Subhuman primates develop antibodies against EB virus, indicating that they are susceptible to infection, although none has developed tumors so far. Monkeys may provide a suitable model system for a vaccine testing, a role not unlike the one they played during the development of the polio vaccine. If such tests become necessary on a larger scale, however, they will be expensive, requiring large numbers of virus-free monkeys, costly materials, and highly skilled workers.

Although, as discussed in the following pages, vaccines will probably not be appropriate as preventive measures against all cancer-causing viruses, there is good reason to think that in the case of a herpes-type viruses, a vaccine might accomplish effective prevention. A herpes-type virus causes a highly malignant and contagious lymphomatosis in chicken (Marek's disease). Its devastating spread through chicken flocks has caused great economic loss to the country. The disease is now completely preventable by vaccination of young chicks with a nononcogenic but closely related strain of virus. It is of great interest that this unusual vaccine, while it prevents the appearance of lymphomatosis, does not seem to limit either the multiplication or spread of the virus of Marek's disease. Indeed it seems to be a common attribute of many herpes viruses that they can multiply extensively in an individual who already has a high titer of antibody to the virus. Antibody in this case seems unable to prevent *infection* although, as the example of Marek's disease shows, it does seem able to prevent the appearance of cancer.

Herpes simplex type II has been reported consistently associated with pre-invasive cervical carcinoma. Here again, a causal relationship has not been established (see: Epidemiology), but further studies on this problem need to be urgently pursued.

Most of the questions raised by the association of certain herpes-type virsuses with several types of cancer still remain to be answered. Furthermore, there are already indications that many more such associations exist, involving herpeslike viruses and other forms of solid tumors and leukemia. All await concerted research efforts for findings that can either eliminate them as causes of cancer or else make clear the need for the urgent development of preventive measures.

Viruses and Breast Cancer

The question of whether or not a virus is involved in human breast cancer has been brought closer to a solution by recent exciting findings. Cell lines from human breast cancers have been successfully established in tissue culture. In several instances, RNA-containing virsuses, which resemble the mouse mammary tumor viruses, have been found that produce no changes in cultured cells but can be detected by electron microscopy and biochemical tests. They are able to infect other human cell lines in which they continue to multiply.

Since the mouse virus is present in, and is indeed transmitted through, the milk, human milk specimens have been examined with the electron microscope. Similar particles have been found more frequently in the milk from women belonging to "high breast-cancer

families" than from others in the general population. This correlation is particular marked among the Parsis, who are a highly inbred group.

The significance of these findings is increased by the recent isolation of similar viruses from cultured mammary cancer cells from two additional animal species, the rat and the monkey. In all cases, *in vitro* production of virus on a large scale is being attempted to provide material for systematic characterization studies, and some very encouraging results have been obtained with the virus isolated from monkey mammary cancer. The latter represents a particularly exciting finding because it is the first mammary virus that can, thus far, be produced in sufficient quantity to sustain exhaustive and systematic investigations.

In view of the successful isolation of these agents, there is now an urgent need to develop the knowledge and technology for large scale production of the other breast cancer viruses in tissue culture, as well as assay systems to test their oncogenic potential in cultured cells and in animals.

Once large scale production of viruses or viral antigen is achieved, an immediate serological survey should be carried out to ascertain—through sero-epidemiological studies—the distribution of the virus of human origin in the population and its relationship to human breast cancer.

C-Type viruses

Virus particles with a distinctive "C-type" morphology have been detected by the electron microscope in specimens of both human leukemia and lymphoma, as well as of leukemias of cats, dogs, and cattle. These C-type particles are similar in appearance to the virus causing well-known leukemias in the chicken and mouse.

C-type particles from cats, chicken, and mice have been successfully grown in the laboratory. They have been conclusively shown to be the cause of leukemias in all three species. In all other species, including man, attempts to propagate the particles have met with seemingly insurmountable difficulties. Recently, however, extremely ingenious methods have been developed for their indirect detection.

NEW DETECTION TECHNIQUES

C-type viruses in chickens include the leukemia-inducing virus and the Rous sarcoma virus, mentioned previously. A very valuable characteristic of the Rous virus stimulated a successful search for corresponding sarcoma viruses in mice and cats. The Rous sarcoma virus of the chicken and also the sarcoma viruses from mice and cats, as it was later found, induce transformation in cultured cells of the species from which they originate. Foci of transformed cells appear as discrete, round thickened areas of cell growth, each caused by one "transforming unit" of virus—i.e., one virus particle. Counting the number of transformed foci permits a quantitative assessment of the transforming activity of the sarcoma virus present in the culture. Leukemia viruses, on the other hand, usually do not cause foci nor other changes that can be used for their detection.

Extremely interesting detection systems have been worked out for viruses of species from which both a sarcoma-causing and a leukemia-

causing virus have been isolated. For example, it has been observed that certain sarcoma viruses are "defective" in the sense that they cannot form foci in cell cultures, or tumors in animals of certain genetic types, without the presence of an accompanying leukemia virus which acts as a "helper." The "helper" aids the sarcoma virus to set up an infection. In this way a "defective" sarcoma virus can be revealed by a "helper" leukemia virus, since its entrance into a cell is then followed by the formation of a transformed focus.

The existence of this type of "helping activity" indicates that two types of viruses might be needed simultaneously, in a dual infection, for some types of virus-caused cancers to occur. Since the existence of such phenomena has been established in three species of animals, it might well exist in humans too.

These new findings are making it possible to reveal otherwise undetectable leukemia viruses in tissue culture systems. If one can find a "defective" sarcoma virus that infects the same species as the leukemia virus, the "defective" virus can reveal the existence of leukemia virus in the culture because the "helper" action of the latter will reveal its presence through enabling the "defective" sarcoma virus to induce a visible, transformed focus. Thus, an active search has been undertaken for a virus associated with human sarcomas, that would help develop a similar system for the detection of a human leukemia virus.

In case a human sarcoma virus is not detected soon, an alternative approach already exists. It has been observed that when different viruses are tightly packed together under high centrifugal force, they form aggregates that may behave as a single virus. Tightly packed together in this way, a defective mouse sarcoma virus and its cat leukemia virus "helper" can infect cat cells in culture, which the mouse sarcoma virus alone cannot do. A progeny of hybrid virus is produced that can go on growing continuously in cat cells. However, the hybrid is "defective" and requires the continuous presence of cat leukemia virus for producing foci of transformation.

Thus, in this case, it is not a defective cat sarcoma virus which is used, but an artificially changed mouse sarcoma virus, which has acquired cat infective properties and can now be used in cat cell cultures as a substitute to the cat sarcoma virus to detect the cat leukemia virus. Such a system could be created for the detection of a human leukemia virus if a minimum amount of human C-particles can be extracted and purified to create the first aggregate virus.

These examples show how pertinent extensive studies on chicken, mice, and cat viruses are to the human situation. The systems developed in other species provide ways to bypass the inability to propagate the human C-type virus in cell cultures, or to use it to induce leukemia in animals. Its capacity to help a defective virus, is now all the proof needed to establish its existence. In all likelihood that proof will now not be long in coming.

C-type virus particles have by now been isolated from and transmitted in several species and observed in many others. They all seem serologically related and whenever their oncogenic potential could be tested, they have all produced leukemias and sarcomas.

The C-type viruses of mice, in particular, have been well studied by biophysical and immunological procedures. A new antigen was thus discovered in such viruses to which the name of "group specific" antigen was given since it is common to this whole group of mammalian leukemia and sarcoma viruses. Detection of this antigen in a cell reveals the presence of viral genetic material (viral genome), since such an antigen is a product of a viral genome's activity. Such group antigens have been repeatedly demonstrated in murine leukemia and sarcoma cells, which was to be expected since such cells contain an active virus genome. Surprisingly, however, they have also been recently demonstrated in normal embryonic, but not adult tissues, of *all* mouse strains, including those believed to be completely free of either the leukemia or sarcoma viruses. This finding has led to the development of the challenging theory that C-type viruses, such as the agents of mouse leukemia and sarcomas, are transmitted vertically from generation to generation, probably as part of the cell's genetic material. Moreover, since they seem, in mice at least, to exist in *all* individuals, their occurrence can no longer be considered abnormal. In fact, it is speculated that they may have some unsuspected role to play in the process of embryonic development. According to this theory, all adult animals carry a dormant, unexpressed viral genome. The difference between those who develop cancer and those who do not, the hypothesis continues, resides in the fact that the viral gene, or genes, are expressed in persons with cancer, whereas they remain repressed and therefore undetectable in the tissues of the normal adult. This hypothesis is supported by much circumstantial evidence which indicates that the development of cancer has to do with gene expression. Several human cancers are also characterized by the presence of fetal antigens, a type of protein that is normally present in human embryos but is found in the adult *only* in tumor cells. (See: Cancer Immunology). At the moment, much of this hypothesis is pure speculation, but it has the merit of offering the first unifying theory for cancer etiology. It views cancer as a biological event determined by the "switching on," or derepression, of universally prevalent specific "oncogenes." Such a theory encompasses the "naturally occurring" cancers, as well as with those induced by radiation, chemicals (see: Etiologic Interactions), and other viral agents (because of the evidence of the need for a "helper" virus, in some cases, to elicit oncogenic activity in defective viruses). Other endogenous factors, such as aging and immunological and hormonal status, are viewed as additional potent determinants of gene derepression at the level of the whole animal. With the present competence in virology and molecular biology, such a theory lends itself to experimental verification. It is obvious that, should it become so verified, it will have a radical influence on the thinking and overall approach to the prevention and treatment of cancer. Two research approaches toward control of cancer would under these circumstances, acquire considerable importance: a search for an understanding of the mechanisms of gene regulation (see: Cancer Biology) and a search for ways to strengthen the normal protective immunological mechanisms, so that they can successfully reject cells carrying abnormal antigens. (See: Cancer Immunology).

HOW DO VIRUSES CAUSE CANCER?

The way in which viruses cause cancer is a crucial question. Even in the unlikely event that viruses are proved to cause cancers only in animals other than man this question remains important. If the mechanism by which one kind of agent can transform normal cells into cancer cells becomes known, it may open the way to understanding how other agents do so. Because viruses can transform cells *in vitro*, they are extremely useful laboratory tools. Even a single viral particle can effect transformation. Used in conjunction with modern, quantitative methods of cell culture and biochemistry, viruses make it possible to study carcinogenesis in more rigorous and quantitative ways than is possible in animals.

Even compared to bacteria, virus particles are simple from a biochemical point of view. In general, they consist of a core of nucleic acid which is a single molecule of either DNA or RNA, and an envelope composed of a small number of protein molecules. They have the added advantage that both their components, nucleic acid and proteins, are sufficiently different from cell constituents that they can be identified within the infected cell. Many ingenious methods have been devised to exploit these differences between viral and cell components. Viruses are all obligate parasites of living cells which can multiply only if they use the cell's synthetic machinery. Thus, their study cannot be dissociated from a study of the host cell. It is the *interaction* between virus and cell which determines the outcome of viral infection, rather than viral properties alone.

Virus-cell interactions

Virus-cell interactions show great variety and complexity. The most intimate form of association between a virus and a cell takes place whenever the outcome of viral infection is neoplastic transformation. (See: Cancer Biology). If transformation occurs, infection may not be followed by the rapid production of a large number of new virus particles (or "virions"), but rather by the slow production of a few particles, or by no virion production at all. The virus particles may in fact be impossible to detect in the cell with the only consequence of infection being the appearance of new cellular characteristics. Chief among these characteristics is a new autonomy, both in regard to multiplication and behaviour towards neighboring cells and tissues—i.e., the acquisition, by the cell, of neoplastic properties. Biochemical studies of such a cell reveal that—as is also true after treatment with chemical carcinogens and "spontaneous" transformation—a host of metabolic changes accompany cellular transformation. Again, the problem is: which change is critical? And again, the answer now seems to be very close.

Those oncogenic interactions between cells and the small DNA viruses that have been probed in sufficient detail, reveal that, among a wealth of different effects, a small number of events seem to be of paramount importance to the fate of the cell. (1) The viral genetic material (genome) replicates only a few times—perhaps only once; (2) The virus induces cellular DNA synthesis; (3) The virus is thereafter associated with the cellular genome; (4) A new antigen (T-antigen) is produced which is the same in all cells transformed by the same kind of virus; and (5) A new cell surface antigen, the "transplantation antigen," appears.

The T-antigen has given the first clue that at least some of the viral genome must persist in the cell. Because the T-antigen is characteristic of the virus rather than of the host cell or the animal, it seems likely that it is specified by viral genes. The recent demonstration, in several cell-virus systems that a hidden virus can be "unmasked" in some transformed cell and can then yield complete virions able to infect susceptible cells, proves that the *whole* viral genome can persist in the transformed cells, although in a largely "repressed" state.

Moreover, it is now possible, in some cases, to get rid of the viral genome. When this occurs, transformation is reversed. Thus, the viral genome is not only required to bring about transformation but also to maintain it. The viral genome in the transformed cell must obey the latter's regulatory mechanism for DNA replication, because each transformed cell transmits the viral genes to its daughter cells as a part of its own genetic complement.

Unlike the T-antigen, which is characteristic of the virus, the new cellular "transplantation" antigen is characteristic of the species of origin of the transformed cell. The appearance of a new protein induced by the virus—but for which the information may reside in the cellular genome—suggests that the virus is capable of modifying the cell's own pattern of protein synthesis. Biochemical studies reveal that, indeed, the cellular "translation" mechanisms can be affected by oncogenic virus infection.

Some important questions that still have to be answered concern the exact role played by the virus. In other words, what are the functions on the viral genome? What does it code for directly? What changes in the mechanism of protein synthesis are really coded for by the cellular genome but "induced," i.e., "de-repressed," by the virus?

It is of practical importance to define the part played by the virus. If transformation is due to a viral function alone—such as, for example, by its specification of a molecule that would be the equivalent of a powerful "proximate carcinogen"—preventive and therapeutic measures would have to be directed primarily at the virus itself. However, if the critical effect of the virus is to activate a pre-existing cellular mechanism, knowledge of the mechanisms for gene regulation will be necessary to stop the development of cancer.

It is noteworthy that such conclusions are not unlike those reached through studies in chemical carcinogenesis.

Viral genome integration

Viral DNA is closely associated with or integrated into the cellular DNA of the genome and the viral information can be transmitted along with the cellular genes. However, many of the cancer-causing viruses are RNA viruses. Therefore, one of the major conceptual problems in virology has been to develop a theory that would take account of these viruses as well.

In 1964, it was hypothesized that a phase of DNA synthesis must follow infection by an oncogenic RNA virus. During this phase viral genetic information would be transcribed from RNA into DNA. In 1970, this hypothesis received solid support when an enzyme was discovered which transcribes DNA on an RNA template (in contrast to the usual mode of transcription in which RNA is transcribed from DNA.) This enzyme, which seems to be part of the virus particle, is associated with all oncogenic viruses so far tested and with only two

of the many viruses so far tested that have not been shown to be oncogenic. This "RNA-dependent DNA-polymerase," as the enzyme is called, can function *in vitro* synthesizing a DNA that is complementary to a viral RNA model, or "template." These findings make it easier to conceive how, after being copied into DNA, the viral genetic information can become so intimately associated with the cellular DNA as to be transmitted, together with cellular genes, during the process of cell division. The discovery of this particular enzyme provides a tool for exploring possible genetic similarities between oncogenic RNA viruses and also a radically new and extremely sensitive way of detecting cryptic viruses in human cancers.

DNA, including the DNA found in the human chromosome, is formed of two molecular strands which are coiled around each other in a double helix and held together by chemical bonds between obligatory pairs of the four base units. Such bonds can form spontaneously, and the molecular configuration of the bases is such that each kind of base can only bind to a single other one. If two complementary strands have been separated *in vitro*, they tend to rejoin, each base fitting to its complementary one, to make a precise reconstitution of the original double-stranded molecule. This rejoining cannot happen if the two single strands are not complementary. In a mixture of different kinds of single strands, complementary strands "recognize" each other and bind to each other to form new double strands.

The newly isolated RNA-dependent DNA-polymerase can transcribe the base sequence of a viral RNA onto a DNA strand, *in vitro*, so it is now possible to synthesize relatively large quantities of such virus-specified DNA. If a virus is the cause of a particular cancer, its genetic material may be present in the cancer cells in the form of DNA, even though it may not reveal its presence. It is possible to extract cellular DNA from tumors, purify it, and separate its strands. This DNA can be mixed with synthetic single-stranded DNA, carrying the base sequence of the RNA virus. Should a complementary viral genome (i.e., DNA specified by the same virus) exist in the tumor extract, the two should bind together, reconstituting double-stranded molecules. The latter can be recognized. Thus, the identification of such double-stranded "hybrid" molecules would, in itself, prove the existence of the same viral genome in the tumor, and on the basis of present evidence, strongly indicate the latter's viral etiology.

Many human cancers could now be tested in this way for association with any one of a number of RNA viruses. The availability of such methods underlines again how urgently larger amounts of human C-type particles are needed in order to make such tests possible and to be able to understand their role.

PREVENTION OF VIRUS-CAUSED CANCERS

Vaccines

If viruses are shown to cause human cancers, how can infections by such viruses be prevented?

The first consideration would be a vaccine. Work is already underway to develop a vaccine against certain types of viruses, particularly those of the herpes group, and specifically against the EB virus. Enough has been learned of the natural history of these viruses to know that they fulfill one necessary condition that might make a

vaccine effective: they are transmitted "horizontally"—that is, people become infected *after* they are born and following vaccination, their immunologic system can produce antibodies against the fully infective agent.

However, there are agents, of which the mouse leukemia virus is a prototype, that are transmitted "vertically"—that is, through the egg, the sperm, or the embryo. Mouse leukemia, moreover, is due to one of the ubiquitous C-type particles now reported to exist in a cryptic state in many species, including man. Since animals are born already infected, they are "tolerant" to their virus and unable to produce antibodies against. (See: Cancer Immunology). Use of a conventional vaccine against such agents is, therefore, impossible.

Though there is no clear evidence yet that the same situation exists in humans, it remains a distinct possibility. Therefore, it is critically important to pursue and develop approaches to prevention other than vaccination.

COMPETING DEFECTIVE VIRAL GENOMES

One new approach has already been proposed, suggested by model systems first developed by molecular biologists using a simpler organism, QB phage, a bacterial virus. This approach involves the deliverate synthesis of mutant viral genomes which are "defective." In the case of the bacteriophage, for example, mutants are synthesized *in vitro*, that lack the genetic information necessary to complete their life cycle. Such defective mutants are then used as antagonists, or competitors, to the original virus. They are capable of entering cells and occupying all the replicating sites, so that production of the virulent virus, when it is present, is blocked. One such antagonist particle per cell is sufficient. However, if such particles enter an uninfected, healthy cell, they are not able to mulitiply by themselves, since they lack the necessary genetic information for the synthesis of a "replicase" enzyme. The replicase molecules only exist within cells infected by the virulent, nondefective agent. Defecitve mutants are completely specific in their effect. They are a biological "magic bullet," uniquely designed to interfere with the synthesis of the *one* virus from which they themselves originally were derived.

Such a method can also be applied in principle to mammalian cells. The required methodology, however, does not exist. It calls for the immediate development of appropriate methods for finding, isolating, and selecting viral mutants in somatic cells. The merit of the proposal is that it gives a direction to future research and efforts should be made to make the proposed new scheme applicable to cells of higher organisms and, particularly, to the control of cancer-causing viruses.

TREATMENT OF VIRUS-CAUSED CANCERS: INTERFERON

Because infections by most viruses are usually self-limiting in the organism, it has long been suspected that a special defense mechanism existed against viruses. In 1957, it was observed that, immediately following viral infection, animals cells released a substance capable of protecting neighboring uninfected cells from infection not only by the same virus but also by other viruses as well. The active substance was called "interferon."

Interestingly, each animal species produces its own particular kind of interferon. Thus, the interferon produced by mouse cells protects

mouse cells against virus infection—but not rat cells or human cells. Interferons are small protein molecules, elaborated and released by living cells in response to the entry into the cells of a virus or of any natural or synthetic molecule called interferogen, capable of mimicking certain properties of viral nucleic acids. Interferon is not a defense against foreign antigens (see: Cancer Immunology) but against foreign genetic material, such as a viral nucleic acid. The first property of interferon tested was its ability to limit the spread of virulent viruses in lytic virus-cell interactions, i.e., infections resulting in the production of large numbers of particle and cell death. Recently, however, it has been reported that interferons are also capable of inhibiting oncogenic transformation.

Unfortunately these interesting substances are not easy to work with. Interferon preparations are usually difficult to concentrate and purify. Moreover, interferon production decreases sharply within a few days following its induction, either in the animal or in culture. For practical reasons, cells in culture can only be used for one cycle of interferon production. No effective way of inducing the production of larger amounts in interferon over longer periods of time has been found, although it is now possible somewhat to "stabilize" interferon preparations.

New techniques have recently been developed for growing cells in culture on a large scale, thus making larger amounts of interferon available. As a consequence, it has been possible to show that interferon has a much broader area of competence than was suspected at first. Very recently, its importance has assumed a totally new dimension; it has been shown that interferon is able to act also as an *oncolytic* agent—that is, as an agent capable of effective and specific destruction of cells that are *already* transformed.

Interferon was shown:

(1) To prevent transformation by two "horizontally" transmitted leukemia viruses;

(2) To delay the progression both of leukemia and transplanted solid tumors;

(3) To delay and/or possibly prevent the appearance of "spontaneous" leukemia in mice infected with a "vertically" transmitted leukemia virus;

(4) To arrest the progression and, in some cases, induce the regression of vertically transmitted leukemia, as well as transplanted mammary tumors.

In mice with either moderate and advanced leukemia, or with transplanted, solid mammary tumors, regressions were obtained with large and repeated doses of interferon given daily over several weeks. In mice with advanced leukemia, administration of interferon resulted in reduction of the size of the lymph nodes and spleen. However, this regression was accompanied by toxic symptoms, and, despite the fact that treated mice survived longer, no "cures" were achieved. In mice with early to moderate leukemia treated similarly, the early reduction in the size of lymph nodes and spleen was followed in some cases by slow regression of the disease and a return to apparent good health. One-third of the animals thus treated have survived for several months—a good portion of their natural life span—and are still alive without symptoms of disease. Similarly, slow regression of cancer nodules and prolonged survival were observed in mice with mammary

cancer. Microscopic examination of tissues formerly harboring active cancer cells showed that interferon had apparently sought out and destroyed most of the cancer cells—those which carry viral genetic material—without having any apparent effect on normal, noninfected cells.

How interferon acts

The important implications of the results just described warrant a short discussion concerning the mode of action of interferon and of what prospects exist for a similar therapeutic use of human interferon. Early observations suggested that interferon exerts its effect extracellularly. More recent evidence, however, indicates that interferon goes through cell membranes into cells, where it is capable of effectively blocking the mechanisms of both "lytic" and "transforming" interactions, redirecting them towards an "abortive interaction" whose result is cell death without production of infectious viral material.

Thus the spread of a viral infection is controlled through the failure of the infected cells to produce new virus, and cancer is prevented by the death of cells that have undergone transformation. Additional supporting evidence for this interpretation of the mode of action of interferon can be found even among the results of the experiments just described; namely, the toxic symptoms followed by death which were observed in treated animals with advanced disease. In advanced leukemia, leukemic cells invade most organs and represent a sizable proportion of their total mass. Sudden destruction of all the cancer cells might be expected to produce toxicity and, indeed, to be capable of causing death. Interferon itself is not toxic and is tolerated at very high doses, and toxicity was not seen in less severely affected animals treated similarly. Thus, the death of those animals that had advanced leukemia was probably due to sudden and massive destruction of cancer cells. On the basis of present knowledge of the way interferon acts, it is clear that it must be used in high doses to accomplish tumor regression; in an animal, any dose of interferon is immediately diluted, becoming distributed to all tissues where it enters each cell, infected or not. Inside virus-induced cancer cells, interferon might encounter more than one viral genome; it has, in fact, been demonstrated that an average of 20 genomes may be carried by each cell transformed by polyoma virus.

Interferon production

At present, only small amounts of interferon can be obtained. All the cells of a mouse can produce only 30,000 units, and this only once. Yet 100,000 to 250,000 units have to be administered daily for several weeks to each mouse, in order to control its leukemia. Proportionately larger amounts of human interferon would be needed to control human cancer. An obvious limitation to therapeutic experiments with human interferon is that of production. There are, however, several possible ways to overcome the present difficulties. Very large scale human cell cultures have to be established as a source of human interferon. Extensive work on ways to concentrate, purify, and stabilize it need to be undertaken. When this has been accomplished, therapuetic trials will be feasible. In addition, its precise molecular structure (amino acid sequence) has to be worked out so that eventual *in vitro* synthesis can be attempted.

NEED FOR A BROADER PROGRAM IN VIRAL CARCINOGENESIS

The creation of a Special Virus Leukemia Program in 1964 acknowledged that research in viral oncology is a field of great promise. The program was enlarged in 1969, to become the Special Virus Cancer Program including virus research on all types of cancer. Leads susceptible of exploitation, either at the basic or the clinical level, have thus been assured of support. However, much still remains to be done, not only to use fully what is already known but to gain new knowledge.

For instance, almost all the cancer-causing viruses studied in animals are associated with leukemias and sarcomas, which are cancers of blood-forming organs or muscle, bone, and connective tissues. Yet, over 90 percent of the cancers in man are carcinomas, originating from epithelial cells such as those that line the digestive and respiratory systems as well as from the skin. With the exception of mammary tumors, carcinomas have yet to be clearly associated with viruses either in mammals or man. On the other hand, as mentioned previously, while oncogenic animal viruses produce cancers regularly under laboratory conditions, their role in wild animals, under natural conditions, remains very poorly understood. To understand the human situation better, it is necessary to learn more about the natural history of viruses in wild, outbred animals. Simplified laboratory models are invaluable, because they permit an approach to very specific and detailed aspects of the virus life cycle, but they are less useful in assessing how such viruses behave in their broad, natural environment.

Observations with laboratory models have to be re-evaluated in the light of studies made with "field" strains of oncogenic virus, such as those of chicken and mice. Animals infected with field strains of such viruses, although they may be releasing C-type virus particle from most of the cells of the body, are often indistinguishable from uninfected animals. The only discernible difference is that a greater proportion of infected animals—but still a minority—eventually develop leukemia. (Disconcertingly, animals of the only flock of chickens in this country ascertained to be "virus free" also have a fairly high incidence of leukemia late in life.) It is also often observed that, although laboratory strains of DNA viruses can transform cells *in vitro*, the transformed cells may not be capable of fully malignant growth when transplanted into animals. In addition, uninfected cells cultured *in vitro* over long periods of time, can progressively acquire characteristics usually attributed to cells transformed by viruses or carcinogens and become capable of growing into genuine cancers when transplanted into animals. So infection with a typical "cancer virus" does not always induce cancer, and the same type of cancer can appear, although less frequently, without it. Obviously factors other than the known viruses must also play a role. These factors could be genetic, including changes in the genetic material, or environmental, such as chemicals, radiation or even other viruses, whose effect might be either to enhance or to inhibit the oncogenic process. Several situations have been described which lend support to one or the other of these possibilities.

Viral oncology thus still has to answer many more questions, and is essential that not only its present momentum should be maintained through strong support, but that its scope should be considerably broadened.

AREAS OF PROMISE

The following areas of work offer great promise if research in viral oncology is given adequate and sustained support:

(1) The search for an unequivocal demonstration that viruses are responsible for human cancers. This will include studies on several viruses suspected of being implicated in human cancer and development of methods to make possible their large-scale production.

(2) Large and well controlled sero-epidemiological investigations of the pattern of antibody production against EB virus and other herpes-like viruses in man and primates.

(3) Studies on methods to propagate mouse and human mammary cancer viruses in cultured cells, and intensive experimental work on the mammary cancer virus isolated from monkeys.

(4) Studies on methods to propagate C-type virus particles in cultured cells.

(5) The search for a human sarcoma virus and development of systems for the indirect detection of cryptic "leukemia viruses" in man.

(6) Further development and use of methods, such as nucleic acid hybridization, for the detection and identification of viral genomes in cancer cells.

(7) Epidemiological, immunological, and biological research to clarify the possible role of animal viruses in human disease.

(8) Research on interferon, including its mode of action, methods for stabilizing and purifying it in order to establish molecular structure; the large-scale production of human cultured cells for interferon production and a search for more active interferogens.

(9) Development of methods for the isolation and study of virus mutants in order to clarify the respective functions of viral and cellular genes in all types of cell-virus interactions.

(10) Studies on the antigens of transformed and tumor cells, including their role in the process of transformation and in the maintenance of the neoplastic state.

(11) Research on structural, genetic, and serological relationships between viruses.

(12) Studies on cancer cell antigens, their relationships to each other and to the antigens of virions, their possible dependence on intracellular viral genomes and their relationship to the cancer cell's properties of invasiveness and unregulated growth.

(13) Research on the natural history of viruses both in animals and man, particularly those viruses that have given rise to oncogenic strains in the laboratory.

(14) Experimental tests of the validity of the "oncogene" theory for animals other than mice.

(15) Research in general on the repression and derepression of cellular genes, and in particular on the mechanisms by which viruses may affect these processes, or themselves be repressed by cellular factors.

(16) Studies of the mechanisms by which viruses induce DNA synthesis in cells, and of the significance of intracellular viral genomes in relation to the lack of control of multiplication in cancer cells.

Interactions Between Etiologic Factors

INTRINSIC FACTORS

Research in cancer etiology has clearly incriminated three kinds of environmental factors as causes of human cancer, chemicals, viruses, and radiation. However, 1) not all individuals subjected to anyone of them develop cancer, 2) different organs vary widely in susceptibility to different agents, and 3) it is common for cells in culture to undergo "spontaneous" transformations without known contact with any of these factors. It thus appears that intrinsic factors may also play an important role

GENETIC FACTORS

Many findings now link leukemia with birth defect and abnormalities, among which mongolism, mentioned previously, is but one example. There are even more striking associations of cancer with other gross genetic anomalies, particularly some rear abnormalities that are accompanied by extensive chromosome breakage, such as Bloom's syndrome and Fanconi's anemia. More subtle genetic factors apparently also operate as evidenced by small and relatively rare "clusters," in which some types of cancer appear repeatedly within families. In such cases, a particular genetic constitution seems to predispose the individual to a specific type of cancer.

For example, polyps of the colon (benign multiple growths on the interior lining of the lower intestine) develop after a certain age in otherwise normal members of certain families. This characteristic is inherited as a dominant trait. The polyps frequently become cancerous, and consequently carcinoma of the colon occurs much more frequently among those carrying the genetic determinant for this type of polyposis than in the general population.

Possibly the most striking examples of genetically determined appearance of cancer is provided by retinoblastoma, a rare hereditary cancer of the eye due to a single gene mutation. Retinoblastoma is inherited as a recessive trait (one which occurs only when a child receives the mutant gene from both parents) and it invariably appears in children who have inherited two such recessive genes.

Predisposition to cancer can be demonstrated even at the cellular level. SV40 virus added to cultured cells taken from members of a family with a high incidence of leukemia, induced a higher than usual rate of cell transformation not only in the cells of leukemics but also in those of some of their healthy relatives.

THE SOMATIC MUTATION THEORY OF CANCER

Genes, the functional units of heredity, undergo, on rare occasions, spontaneous sudden changes called "mutations." If such changes do-

not bring about the cell's death, they remain henceforth an integral part of the cell's genetic information and are handed down to daughter cells with each cell division. If such mutations occur in the germ cells—the cells giving rise to eggs or sperm—they affect future generations. If they occur in somatic ("body") cells, they affect only other somatic cells arising from those carrying the mutation.

The obvious role played by certain genetic factors in carcinogenesis, added to the fact that the properties of cancer cells are transmitted to their descendents like genetic traits, has lent great credence to the "somatic mutation theory" of carcinogenesis. This theory proposes that the cancer cell results from a very specific change of the cellular genetic material itself, a mutation.

Radiation and chemicals which cause cancers in animals induce mutations in microorganisms. Radiation can also cause mutations in animal cells, and similar evidence regarding chemical carcinogens has now been obtained using cultured cells. There is ample evidence that both radiation and chemical carcinogens affect the genetic material; for example, the wave-lengths of ultra-violet radiation that are effective in inducing neoplasia are also those that are "absorbed" preferentially by nucleic acids and that damage their molecules. Chemical carcinogens react chemically with nucleic acids. Irradiated cells, whether transformed or not, may have abnormal chromosomes, as do many cells transformed by chemical carcinogens or originating from "spontaneous" tumors.

All these observations, seem to favor a theory that implicates genetic change in the neoplastic transformation. They do not prove direct causality, however, nor do they suggest any specific mechanism, and therefore the "somatic mutation" theory of cancer has remained unsubstantiated and controversial.

AGING

There is a strikingly overall increase in the incidence of cancer with age. With the exception of a few types, the risk of most common types of cancer increases about 2.7—fold for each 10-year interval between the ages of 20 and 60. Thus the probability of developing cancer increases from 15 per year per 100,000 of the population at the age of 20, to about 1,000 per year per 100,000 at the age of 60. The reasons for this increase are largely unknown.

The so-called degenerative diseases, including coronary thromboses and other cardiovascular accidents, show a similar association with age. Thus, cancer appears in some ways to be a systemic as much as a cellular disease, the result of a deterioration of intercellular, hormonal, and immunological integration as much as of the transformation of one or more cells. The disorganization that accompanies aging may result from the many experiences and insults accumulated through a lifetime, including effects of diet, smoking, and pollution. However, the pattern of distribution with age varies considerably from one type of cancer to another and there seems to be no clear difference between the susceptibility of people of different ages to cancer induction by a given agent. Thus the overall increasing incidence of cancer

with age also apparently reflects the cumulative effects of multiple, continuous, and prolonged exposure to environmental carcinogenic factors.

HORMONAL FACTORS

Hormones play an important role in the appearance and progression of certain tumors. These tumors result from excessive and continuous hormonal stimulation of "target" organs or tissues. The latter include either primary endocrine glands overstimulated by pituitary hormones, the pituitary itself, or secondary sex organs, such as breast and prostate, which are target organs of sex hormones. Interesting exceptions are experimental induction of leukemia and kidney tumors with estrogens.

The importance of estrogens in the appearance of human breast cancer has recently been illustrated by the finding that women who become pregnant before the age of 20 are only half as likely to develop breast cancer later in life as the rest of the female population.

Tumors arising in organs that are targets of hormone action often are "hormone dependent," in that they need continuous hormone stimulation for their growth, at least during their early stages. They progress in the presence of hormone stimulation and they stop growing or even regress without it. This observation has prompted the effective use of oophorectomy (removal of the ovaries) in the treatment of disseminated breast cancer and orchidectomy (removal of the testes) in the treatment of cancer of the prostate, so as to deprive tumor cells of hormonal stimulation.

Hormonal carcinogensis is a good example of cancer induction by a disturbance of the body's regulatory mechanisms. Upon removal of the hormonal stimulus, the phenomenon of tumor progression may stop and the cells revert to less malignant states or even to latency. Thus, hormones seem to be "promoters" rather than "initiators" in the carcinogenic process (see: Chemical Carcinogenesis.)

Although an oncogenic virus plays a major role in the induction of the mammary tumor of mice, it is a hormone-dependent tumor as well. Thus, in this case, both an initiating and a promoting factor seem to have been identified. Virus-like particles have been seen in human breast tumors and viruses have recently been isolated from rat and monkey mammary cancers as well. (See: Viruses and Cancer.)

It is interesting to speculate on the possible relationships and relative role of virus and hormone stimulus in mammary tumors. At least for some hormones, the end result of their action seems to be the induction or repression of genes in their target cells, expressed as changed enzymatic activities. Should a viral genome be present in those cells, hormones might thus affect the regulation of expression of viral genes, with results of critical importance in view of the cancer causing potential of the virus.

Despite their hormone-dependent induction and early progression, these tumors are capable of progression through unknown mechanisms, to the point of being completely autonomous and impervious to control through removal of the hormonal stimulant.

ENZYME FACTORS

The importance of intracellular host factors at the level of the enzymatic machinery, has been demonstrated by the discovery of enzymatic activations and inactivations of chemical carcinogens. The capacity of tissues and individuals to activate or inactivate particular chemicals will obviously affect their susceptibility to cancer induction by carcinogens. It will thus be important to know for each tissue and each carcinogen what those enzymes are and to learn how to enhance a "protective" metabolic activity and/or repress an undesirable one. Encouraging beginnings are being made. It is now possible to stimulate some "protective" enzymes—such as that which destroys polynuclear hydrocarbon carcinogens in the lung of mice.

IMMUNE DEFENSES

There is growing evidence that cancers may appear as a result of the failure of a defense mchanism which normally kills cell variants, such as cancer cells. Increased cancer incidence in cases of immunologic deficiency as well as serological studies in animals and man bearing active cancers, provide evidence for the critical role of immune reactions in cancer etiology. It has been found empirically that newborn rodents (in which the antibody forming system has not yet begun to function) are more susceptible to cancer induction. That immune reactions against cancer cells do occur—with varying degrees of success—will be discussed under the topic of Cancer Immunology.

EXTRINSIC FACTORS

Cancer as it occurs in man, might result from an even more complex interplay of factors than it would appear from the foregoing account. Since the first report by Rous in 1934, it has become clear that the activity of a given carcinogen (whether a chemical, a virus, or radiation) can be strongly affected by others.

CHEMICAL CARCINOGENS: CO–CARCINOGENESIS

In studies of chemical co-carcinogenesis (see: Chemical Carcinogenesis), it has been found that some chemical carcinogens are well effective as "initiators" and others only as "promoters." As a consequence, at a given dosage, administration of both, in a particular order, is essential for the appearance of cancer. Thus, one such chemical by itself will be ineffective and apparently innocuous.

Striking examples were recently reported of the existence of co-carcinogenic factors in the human environment. The combination of asbestos dust inhalation and smoking greatly increases the incidence of lung carcinoma in man above that associated with smoking alone. Other different sorts of dust, common in the atmosphere, are suspected of the same effect. In an experimental system, rats at birth are given a carcinogenic nitrosamine (see: Chemical Carcinogenesis) in a dose insufficient to produce lung tumors by itself. However, if these rats subsequently inhale ferric dusts, which by themselves are innocuous, a large proportion develop bronchial and pulmonary cancers. "Non-

carcinogenic" sulfur dioxide (a prominent constituent of industrial smoke) given to rats in inhalation chambers in combination with benzo (a) pyrene (a carcinogen) causes bronchogenic carcinomas in rats, whereas none appear in animals treated with the same dose of one or the other alone. Further experiments with combinations of chemicals at low doses are obviously of great importance to our understanding of the causes of lung cancer in man.

RADIATION AND LEUKEMIA VIRUS

Not only can two chemical agents reinforce each other, but so can also two agents of different kinds. "Virus-free" mice, treated with x-rays to induce leukemia, develop a typical "virus-induced" leukemia, whose cells shed virus particles in the usual large numbers. In this case the radiation is a co-carcinogen to a viral initiator. (See: Radiation Carcinogenesis.)

CHEMICAL CARCINOGENS AND VIRUSES

It has been observed repeatedly that chemical carcinogens when applied to animals infected with a cancer-causing virus accentuate the effects of the virus. Recently, for example, rat and mouse cells in culture were treated with nontransforming C-type leukemia viruses as well as with different chemicals at doses far below that which would be expected to cause transformation. In all cases, cell transformations were obtained. Moreover, ozonized gasoline, noncarcinogenic alone, can apparently become carcinogenic when administered in combination even with common, nononcogenic viruses, such as influenza.

As yet, there is no general interpretation for all observations like those described above. They lead, however, to the suspicion that some human cancers may appear to be "spontaneous" only because they result from the combined effect of elusive factors, both intrinsic and environmental, each of them too weak to act alone. It is therefore important that future research determine *what* combinations of agents are effective in producing cancer in addition to *how* they do so.

A theory of carcinogenesis known as the oncogene theory, proposes that hidden viral genomes exist in many cells as "oncogenes": oncogenes would be the equivalent of an "initiating" carcinogenic factor, while chemical carcinogens or radiation would be reduced, in this scheme, to the role of "co-carcinogens" whose triggering action would remain, however, of critical importance. According to this theory, the co-carcinogen would counteract a cellular repressor that keeps the potentially oncogenic viral genome under control. The theory would not, in any way, reduce the importance of chemical carcinogens or radiation in the human environment, since it predicts that without them, potentially oncogenic, cryptic viruses would remain harmless. However, it would exclude a complete carcinogenic role for chemicals and radiation, an hypothesis which still remains to be proven. According to this theory, systemic host factors would generally act through favoring derepression. They might play a part in the appearance, survival, and growth of cancerous cells, through the disorganization or weakening of cellular, tissue, or systemic control mechanisms.

"Spontaneous transformation" of cultured cells would also be tentatively explained by the absence of tissue and systemic controls in a situation where a strong selective pressure exists towards increased metabolic autonomy. Repressor mechanisms that keep cellular genes conferring such autonomy in check would tend to be lost.

This novel theory of carcinogenesis would account for the involvement of both cellular genetic and epigenetic mechanisms. The nature of the genetic change would be an *addition* of viral genes, which is very much unlike the change postulated by the "somatic mutation" theory but would still implicate a modified cellular genome. On the other hand, the observed epigenetic changes of cellular translation mechanisms, might be among those that effect de-repression of critical genes and both changes are assumed to be required for the appearance of cancer.

Thus the history of the etiology of cancer went first from observations on man to those on organs and their cancers, and from there to isolated cells and sub-cellular structures. The complex and multiple interactions just reviewed show that even as genes and molecules are being probed, it becomes increasingly clear that cancer still must be thought about in terms not only of the whole organism but also beyond it, in terms of the whole physical, chemical, and biological environment.

AREAS OF PROMISE

Very little systematic research has been done on the interaction between different etiologic factors. The common practice in a complicated situation is to study simple systems before going to complex ones. Such work, however, should now be developed since it is both relevant to the way in which humans are exposed to factors causing cancer and likely to lead to a comprehensive understanding of carcinogenesis. Great encouragement and commensurate support should be given, for example, to studies on:

(1) Genetic features that determine enhanced susceptibility to physical, chemical, and biological carcinogenic agents.

(2) The nature of tissue and organ differences as to their different susceptibilities to carcinogenic agents.

(3) The aging process and what aspects of it affect susceptibility to cancer.

(4) Other intrinsic factors of possible importance in susceptibility to cancer, including emotional and psychological factors; stress; nutritional habits and caloric intake, etc.

(5) Hormone action and feed-back mechanisms.

(6) Interactions between hormonal status, gene regulation, and virus in mammary tumors.

(7) The immune-response mechanism in relation to susceptibility to physical, chemical, and biological carcinogens.

(8) Co-carcinogenesis between chemical carcinogens known to occur in the human environment, not only in different combinations with one another, but also in association with inert particulate matter, viruses, and radiation.

(9) Effect of radiation and chemical carcinogens on cryptic virus particles.

(10) Mechanism of co-carcinogenesis at the cellular and subcellular level, including initiation, promotion, and reversion.
(11) Analyses of "spontaneous transformation" in culture.

Molecular Biology

Molecular biology seeks to account for the basic phenomena of life, such as growth and reproduction, in molecular terms.

During the past 15 years intensive study of simpler forms of life, such as bacteria and viruses, has spawned the new science of molecular biology and has resulted in a series of brilliant discoveries.

THE UNIVERSALITY OF THE GENETIC CODE

The genetic code—the form in which the genetic information of a cell is stored—has been deciphered and has been found to be the same for all organisms. The means by which the genetic material replicates, how it "mutates," and so evolves, as well as the manner in which it is read and expressed—all of these seem to be essentially the same for all living things.

Therefore, much of what has come out of such studies already has universal meaning. This is a fact of supreme importance. It implies, for instance, that the results of research on the interactions of mutagens and carcinogens with the genetic material of simpler organisms like bacteria and viruses, may help to understand the process of tumor induction in man.

Genetic information is carried by giant, linear nucleic acid molecules, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). These are composed of subunits, each of which contains usually one of four kinds of bases. The language in which the genetic code is written consists of the order of the component bases. The "words" in the genetic language consist of groups of three bases each, "triplets," called *codons*. The genetic code is said to be universal because its codons have the same meaning in all forms of life.

The form in which genetic information is finally expressed is also universal—structural proteins or enzymes—and it is the nature of these proteins that gives every cell its individuality, directly reflecting each individual's genetic originality.

BASIC SYNTHETIC ACTIVITIES

The genetic material has two functions: 1) to replicate itself with great precision and 2) to direct all other biosynthetic activities. The latter are initiated by the copying of the order of the bases in DNA into a new nucleic acid, "messenger RNA"; this copying of the DNA is called *transcription*.

Messenger RNAs carry the information to ribosomes, the structures on which proteins are made.

Once on the ribosomes, messenger RNAs interact with other, "transfer" RNAs. These are particular small RNA molecules to which amino acids, the building blocks out of which proteins are made, become attached by special enzymes. One particular transfer RNA can

couple to only one sort of amino acid. A transfer RNA molecule can also recognize a specific "triplet" on the messenger RNA, so that it can then guide its attached amino acid to a specific place along the messenger RNA molecule. In this way, each of the many kinds of transfer RNA aligns one amino acid molecule, in an order dictated by the RNA message, to form, when the amino acids join together, a new protein chain. The nature and functions of a protein are entirely determined by the order of the amino acids it contains, which in turn, faithfully translates the genetic message; this part of the process is termed the *translation* of messenger RNA into protein. Only about 20 amino acids constitute the building blocks of proteins, and the transfer RNAs that recognize amino acids seem to be the same wherever they are found in bacteria, plants, or animals. Even the codons that constitute special signals, such as those for the beginning or the end of a protein, seem to be the same for all organisms investigated.

REGULATION OF GENE EXPRESSION

Although molecular biology suggests that the same general scheme for expressing genetic information is used throughout nature, there is no clear answer, as yet, to the question of whether or not all cells have the same way of deciding which genes shall be expressed at a particular time. In bacteria, the switching on or off of protein manufacture seems to be controlled mainly at the transcription level—that is, by turning on or off the synthesis of messenger RNA. A number of different repressor substances involved in the regulation of gene expression in bacteria have been discovered. It is not yet known whether a similar system exists in cells of higher organisms.

In bacteria, the genetic material of the cell is usually contained in a single, DNA molecule. Apparently, all this genetic material seems to be ready for use all the time. In cells from higher organisms, however, most of the genetic information is contained in highly complex structures, the chromosomes.

The DNA of the chromosome is associated with a class of proteins, called histones, plus nonhistone proteins and RNA. When found to the other components of chromatin, DNA is apparently less capable of playing its critical role in the synthesis of RNA and, ultimately, proteins. A major portion of nuclear DNA seems to be in a state of continuous repression in all cells of higher organisms.

Investigations of the precise molecular nature of this type of repression are being carried out at present, using purified components of chromatin, whose interaction and activities can now be studied in the test tube.

CARCINOGENESIS AND GENE EXPRESSION

Carcinogenesis seems to involve a disturbance of the mechanisms that regulate gene expression in cells of higher organisms. Carcinogenic agents, such as viruses, chemicals, and radiation, apparently act in higher cells by freeing from restraint many genes that are normally kept repressed within differentiated tissue. Evidence for such derepression is found in the stimulated or reduced synthesis of enzymes and the appearance of fetal and transplantation antigens specified by transformed cells following the carcinogenic change. It is imperative

to learn 1) how, in cells of higher organisms genes escape from repression, 2) which, of the many thousands of possible derepressions, can lead to cancer, and 3) a means to repress—to reimpose a ban on—the expression of potentially dangerous genes.

So, first of all, much more knowledge is needed about the molecular mechanisms by which genes are regulated in normal cells, in the process of becoming specialized—i.e., during normal development.

For example, there is a strong possibility that in differentiated cells, control over which genes are expressed can be exerted not only at the transcriptional level, but also at the level of translation or perhaps even the one at which amino acids are coupled to transfer RNAs. These are only two of the points at which interference by cancer-inducting agents could produce alterations in cellular characteristics, lasting over many generations, without actually changing the genetic material itself.

It is also still conceivable that the carcinogenic stimulas could result in a mutation of a "master gene" controlling mitosis, for example, which could lead, indirectly, to effects on overall gene expression.

However, there is already evidence to indicate that malignant transformation of cells by chemical carcinogens or viruses is accompanied by changes in the specificity of transfer RNAs.

There are also indications that hormones, drugs, and other substances that uniquely affect the function of mammalian organisms act by controlling the translation of specific messenger RNAs.

Simplified *in vitro* methods, like those used in the study of micro-organisms, have already been adapted to higher cells in order to test whether such controls of gene regulation apply with respect to cancer. In some cases, it appears as if "oncogenic information" can be integrated in the cellular genome although its expression may still be determined by host cell factors.

Much more is known about how to "derepress" certain genes in plant cells (i.e., how to endow the latter with metabolic and growth autonomy) and also "repress" them (as during tumor reversal). For these reasons, studies of plant tumor cells seem admirably suited for a first characterization, at the molecular level, of those mechanisms that may underlie not only their own, but all cancerous states.

This work is just beginning. It is using as a model the methods and understanding of molecular mechanisms that have come almost entirely from studies done with bacteria and bacterial viruses, owing to their comparative simplicity and the ease with which they can be manipulated. Present work with more cumbersome systems, such as animal cells, has fortunately shown that the basic findings already made with micro-organisms are also valid for their mammalian counterparts. Thus the decision to study simpler systems to start with has not been misleading.

LYSOGENY

There are reasons to believe that viral genetic information, responsible for the maintenance of the malignant state in experimental animals, is "integrated" with the host cell chromosomes. An analogous

situation has been encountered with a small virus of a species of bacterium. Certain strains of this bacterium carry the virus permanently in what is called a "lysogenic" state. In this condition, the virus does not multiply and destroy its host but is replicated and passed to the daughter bacteria at each cell division as part of the bacterium's own genetic material. However, the presence of this virus can be demonstrated, because methods to "induce" it have been found which result in its release from the bacterial chromosome followed by the destruction of the host cell and the release of a crop of virus particles.

Further research on the analogy between lysogeny in bacteria and of the mode of integration of potentially oncogenic genomes in chromosomes of mammalian cells may provide important leads to new control of virus-induced cancers.

THE USE OF MICROORGANISMS

More information is needed about the details of protein synthesis. Ribosomes are now known to consist of at least 50 distinct proteins, which indicates that their role in protein synthesis is likely to be much more complex than heretofore imagined. As previous work has shown, the details of such complex biochemical interactions are much easier to work out using bacteria. A check can then be made using human cells to decide whether there are any significant differences in the details of gene regulation and expression between bacteria and human cells. If there are no differences, even in detail, there is a strong likelihood that experiments using bacteria as model systems will give results applicable to human situations. But such a level of understanding can only be attained if research into the whole is actively continued.

METHODS IN MOLECULAR BIOLOGY

Molecular biology has been a spectacular success for two main reasons: 1) Following the lead of workers in the physical sciences, molecular biologists have tried to simplify experimental conditions to an extreme, and so have begun with the biology of the simpler forms of life or even of their isolated components in the test tube. Under these conditions, they were able to show that some of the seemingly mysterious events that occur in living cells can be duplicated under relatively simple and controlled conditions. 2) This approach allowed molecular biologists to formulate sophisticated hypotheses that could be tested by simple experiments under conditions that allowed a degree of rigor and control unattainable with more complex biologic systems. Even fundamental discoveries, such as the deciphering of the genetic code and the understanding of the processes involved in protein synthesis, were based on, and in many ways verified by, experiments carried out in the test-tube, using enzyme extracts, ribosome preparations, and simple precursors. Protein-like polymers can now be made using entirely artificial messenger RNAs. None of this knowledge would have been obtained, if molecular biologists had worked only with whole cells.

However, great as have been the successes of their methodology, the triumphs of the molecular biologists have come also from the precision of their thinking, the rigor of their criticisms, and the

coordination of their concerted efforts, a kind of approach that has now become possible and appropriate for cancer research.

The investigation in such simpler systems of the events relevant to carcinogenesis, applying the rigor and the coordination of effort traditional to molecular biology, is an important and timely undertaking.

THE PROMISE OF MOLECULAR ONCOLOGY

It has sometimes been stated that the past success of molecular biology has not cured a single person of anything yet. This is probably true because the knowledge gained concerns mechanisms so fundamental, well regulated, and therefore, intricately interwoven, that much additionl information is still needed before all their complexities can be understood and tools can be developed of the sensitivity and specificity needed to interfere uniquely with any one of them.

Cancer is not an ordinary disease. It might be a normal hazard, inherent in all cells of superior organisms which are constantly exposed to errors in the copying or expression of their genetic code, or to external factors (such as chemicals and radiation) or to the intrusion of foreign genetic information in the form of viruses capable of causing such errors.

It is not possible to estimate what degree of knowledge of fundamental molecular mechanisms will be required before such knowledge may be applicable to the control of cancer. Molecular oncology, in undertaking to study and to understand the most basic mechanisms whose dysfunction cause cancer, is likely to be slower than other areas of cancer research in providing answers susceptible to practical application in the clinic. However, the findings of molecular oncology are likely to be applicable to many types of cancer and to be radical in nature. They will also have an impact far beyond the problem of cancer itself.

AREAS OF PROMISE

It is not possible to designate areas with special promise in molecular biology because as yet the events that lead to cancer are not known.

Studies of the molecular biology of any type of cell should be greatly encouraged. Particular encouragement should be given, to attempts to adapt methods hitherto used exclusively for the study of microorganisms to similar investigations with higher cells.

Special encouragement should also be extended to investigations of mechanisms in the cells of higher organisms, such as those concerned in the control of gene expression, the integration of viral genomes within the cellular genome, and the processes that regulate the functions of cells within tissues, organs, and the body as a whole.

Immunology of Cancer

All vertebrates have a defense mechanism, the immune defense system, that protects them from disease-causing microorganisms. Its deliberate exploitation has conquered many infectious diseases and has been a major achievement of medical science in terms of preventing suffering and saving lives.

The progressive and inexorable nature of cancer has led physicians and laymen alike to believe that humans were incapable of defending themselves against it. There was a time, however, when many infectious diseases that are now easily eradicated or cured, seemed almost as inevitable in their course as cancer does today. Attempts to fight cancer immunologically go back to the early days of immunology. The modern scientific study of the relationship of immunity to cancer was undertaken in the early 1950s, with extensive work on experimental transplantable tumors and the phenomena causing the rejection of grafted normal and cancerous tissue in animals. A major conceptual advance in cancer research was achieved when it was established that cancers *do* arouse a specific immune response in the organism within which they appear.

Cancer cells, like bacteria and viruses, have their own characteristic antigens. An antigen is defined as a substance—usually a protein or polysaccharide—that the body recognizes as foreign and to whose presence it reacts by forming antibody. Antigenic differences represent the first known *qualitative* distinctions between cancer cells and their normal counterparts.

These qualitative differences between normal and cancer cells had escaped other methods of investigation but were revealed by immunological techniques—techniques that take advantage of the extraordinary power of discrimination of the immune defense mechanism itself. This mechanism is capable of distinguishing even minute differences between protein molecules, probably even one different amino acid in a chain of several thousands.

Cancer immunology may now be at the threshold of an era in the prevention and treatment of cancer not unlike that in which the conquest of some major infectious diseases was achieved.

Studies in the immunology of cancer have the following objectives:

(1) Determining the antigenic properties of cancer cells;

(2) Understanding the details of the mechanisms of immune reactions, particularly those evoked by cancer cells;

(3) Contributing to the knowledge of the etiology of cancer;

(4) Creating new and better diagnostic methods to increase the effectiveness of all forms of therapy;

(5) Developing new therapeutic methods based on an immunological approach.

"SILENT CANCER"

There is evidence that the immune response is an effective defense mechanism that may keep most cancers "silent" or may even destroy cancer cells in the organism. For example, persons with immunological deficiencies, whether the deficiency is inborn, as in agammaglobulinemia, or acquired, as by immunosuppression following organ transplantation, have an increased incidence of cancer. In animals, all procedures that interfere with the function of specific immunity result in increased susceptibility to both chemical and viral carcinogens. Also, in animals treated with carcinogens, the longer the latent period before cancer appears, the weaker its antigens, suggesting that more strongly antigenic cells may have appeared earlier and been eliminated.

Some cancer patients can live for long times in apparent equilibrium with their tumors, and a small number of well documented cases of "spontaneous" regression of advanced cancer exist. Both of these phenomena can probably be attributed to particularly effective immune responses. For example, neuroblastoma, a tumor of young children which originates in the adrenal glands, regresses spontaneously less rarely than other cancers. Recently, immunological tests on patients in remission have confirmed the existence of an immune reaction in many patients with neuroblastoma. It has also been reported, on the basis of studies of the adrenal glands from unselected necropsies on young children, that the incidence of nodules of neuroblastoma is 40 to 50 times greater than the overall incidence of clinical neuroblastoma.

Choriocarcinoma, another tumor which regresses more frequently than most other cancers, has two features of special interest: 1) it is highly susceptible to clinical cure by chemotherapy, and 2) it derives from embryonic tissue genetically and antigenically distinct from that of the host. A strong immunological response might be expected to exist against such a tumor, and such a response would facilitate the action of cytotoxic chemotherapeutic agent.

Cancers of the kidney, the prostate and the thyroid are detected histologically (in tissue samples) more frequently than they are seen clinically. In a series of 1,000 necropsies of routine thryoid material, about 50 percent of thyroids showed one or more nodules; of these, 21 percent showed histological signs suggestive of cancer. Such incidence is very much higher than the death rate for thyroid cancer which is approximately six per million per annum.

Thus, it is apparent that the incidence of "silent" cancers seems to be much higher than that of overt cancers.

Cancer, when first seen by a physician, represents a late stage of a process whose earlier stages have gone unnoticed. Newly arisen cancer cells are probably commonly eradicated immunologically and, in that event, go undetected. The immune defense system is therefore an efficient "surveillance mechanism," which in effect, may protect 75 percent of all people from developing the disease.

THE IMMUNE DEFENSE SYSTEM

The functions of the immune defense system are carried out mainly by two types of cells. They are both found in the lymphoid tissue, which is spread throughout the body. One kind, the plasma cell, produces antibodies, a family of proteins found in blood plasma. There are thousands of different kinds of antibodies, each designed to combine precisely with one antigen, the substance that stimulated its formation.

The other kind of cell, the lymphocyte, is dependent on the thymus, a gland that has been assigned important functions in the proper functioning of the immune defense system. "Thymus-dependent" lymphocytes circulate through the blood and other body fluids, patrolling the body for the presence of antigens. These lymphocytes are capable of becoming "sensitized" to antigen. The sensitized

cell is one which stores an immunological "memory" in a form that is still unclear; it is capable of "recognizing" an antigen and of reacting more readily to it following a second exposure. Moreover, the sensitized cell seems to be capable of transferring its information to other cells and thus of mobilizing an immune response on a larger scale should that become necessary.

Experimental evidence for these systems derives, for example, from the fact that animals from which the thymus has been removed neonatally are no longer capable of mounting an effective immune response; the grafting of a thymus or the injection of thymus cells fully restores their immune capacity. Also, in animals treated with very high doses of x-rays, which destroy all their lymphoid tissue and hence their ability to respond immunologically, immunological reactivity is restored when thymus and bone marrow are administered together.

Humoral and cell-mediated immune responses

The immune response takes thus two forms: 1) *a humoral response* mediated by antibody circulating freely in the bloodstream and other body fluids. 2) *a cell-mediated response*, carried out by sensitized lymphocytes. In the cell-mediated immune response, antibody remains an integral part of the cells that produced it; it is "cell-associated antibody." Lymphocytes carrying such antibody are carried in the lymph to tumor cells where their antibody combines with the antigen on the tumor cell's surface. The cell-bound antibody then becomes capable of rupturing and killing tumor cells. For free antibody to have the same cell-killing, or cytotoxic, effect, a third substance, in addition to antigen and antibody is required. This substance, called *complement*, is a factor present in normal serum. Without complement, free antibody can bind to antigenic cell surfaces but cannot kill cells. Complement is made of a set of many components, endowed with enzymatic activity. The nature and precise function of its various constituents are only just now beginning to be known.

This simplified account of the immune mechanisms does not begin to describe the great intricacy of the many cellular and biochemical systems involved. An even temporary deficiency of any link in the chain of events leading to effective immune response could lead to the survival of cancer cells.

FAILURE OF THE IMMUNE RESPONSE

If cancer cells are antigenic, why are they not destroyed by the immune response? Cancers that do develop may do so simply because their rate of growth exceeds the capacity of the immune response. From studies in experimental animals, however, more specific reasons are known for the failure of the immune response to prevent the emergence of cancer.

Immunosuppression

Many factors, such as aging, certain drugs (among which are chemical carcinogens) or neonatal thymectomy, may impair an individual's immune capacity and so favor the appearance of cancer.

Recipients of organ transplants undergoing immunosuppressive treatment have been reported to develop reticulum cell sarcomas.

The frequency with which this has occurred as well as the unusual sites of tumor occurrence suggest that development of this tumor has been facilitated by depressing immune reactions.

Immunological tolerance

Immunological tolerance, which represents a specific form of immunosuppression, exists when exposure to an antigen has taken place very early in life before the immune system has matured. The latter, thereafter, fails to recognize the antigen as "non-self" and so is incapable of forming antibodies against it. For example, rodents, and chickens that carry a "vertically" transmitted (from mothers to embryo) leukemia virus, or mice carrying the mammary cancer virus transmitted during suckling (See: Viruses and Cancer) are "tolerant" to those viruses. Even when heavily infected with large numbers of fully antigenic virus particles, they do not produce antibodies against them. Should some human cancers be similarly caused by viruses of this type, then man might be equally incapable of an immune defense against them.

Immune paralysis

Immune paralysis might occur if a large amount of antigen was released by large tumors which would thus overwhelm the host's immunological response. Such paralysis has been suggested to occur in animals with large tumors induced by viruses or chemicals. Following the removal of such a large tumor mass, the animals exhibit resistance to the reimplantations of their own tumor cells, suggesting that intrinsic failure of the immune response was not the cause of the original lack of immune reaction. Rather, the temporary paralysis observed was due to the fact that the system was unable to cope with too big a challenge.

Immunological enhancement

The important phenomenon of immunological enhancement was discovered in the course of transplantation experiments. Mice carrying grafts of a foreign cancer were given injections of serum containing antibody against the cancerous cells in the expectation that the antiserum would accelrerate rejection. However, it was found that cancer cells were better tolerated and rejection *delayed!*

Several important conclusions have been drawn from this apparent paradox of immunological enhancement:

(1) The antiserum, containing antibodies, instead of killing the cancer cells, appears to protect them. This result indicates that circulating antibody is not responsible for rejection of the transplanted tumor cells.

(2) The decisive component of the host's rejection reaction therefore, appears to be the cell-mediated immune response.

(3) The efficacy of the cell-mediated response may be diminished by circulating antibody. The latter is therefore assumed to "mask" or "block" antigen sites on the cancer cells, thus preventing recognition by killing lymphocytes.

(4) This "blocking" of antigenic sites may also result in reduced antigenic stimulation and thus diminish the immune response.

Thus, these experiments indicate that only part of the immune defense mechanism—the cell-mediated response—is effective in

eliminating cancer cells, whereas the other part, the humoral response, may interfere with successful tumor rejection.

These as well as many other corroborating findings clearly indicate that better immunological control of cancer will be possible, once ways can be found to selectively favor the cell-mediated immune response, or alternatively, to selectively depress the humoral one. Research on how to do this has become imperative.

Immunoselection

Cells within a tumor are presumed to be descendants of a single transformed cell. Thus, the bulk of the cells probably have the same types of antigens. However, as a result of cell variation occurring in the course of many cell generations (often accompanied by changes in the number of chromosomes), cells in the same cancer may end up with varying amounts of the same antigen. Those with the largest amounts will attract more cell-associated and free antibody and thus will have higher risk of being eliminated from the cell population, leaving cells with increasingly weaker antigenic properties as survivors. It is not clear yet whether such a selection process takes place or plays a significant role in human tumors, but it is likely to do so. Immunoselection may account for weak antigenicity of many cancers, and so help to explain the characteristic course of tumor progression—relatively slow growth at first often seemingly restrained, followed by progressive acquisition of more autonomy and greater invasive properties.

Antigenic modulation

As soon as antibody is present, cancer cells of certain animal leukemias cease synthesizing their leukemia antigens. This renders immunological reactions against these leukemias ineffective. Whether this phenomenon has general significance, or indeed if it exists in man at all, is not known.

The above phenomena represent problems standing in the way of effective elimination of cancer cells by immune reactions. They may play a role in man, individually and in combination with each other. The existence and critical role of "blocking" antibodies, for example, has been well demonstrated in cases of neuroblastoma. A highly reactive cell-mediated response was detected in instances of spontaneous regression, whereas the presence of "blocking" antibody could be detected in all cases of tumor progression studied.

The immunologic approach to prevention, diagnosis, and treatment of cancer is being greatly advanced by recently acquired knowledge of immune defense mechanisms and the conditions under which they fall.

Progress in cancer immunology also requires knowledge of the nature and the properties of those antigens that are characteristic of cancer cells.

ANTIGENS OF ANIMAL CANCER CELLS

In animal cancer cells, characteristic antigens are found either within the cell or on its surface.

Intracellular antigens can be detected both in cancers induced by DNA viruses (neo- or T-antigen), and those induced by RNA viruses (group-specific antigen). Being inside the cell, these antigens are inaccessible to immune reactions as long as the cancer cells are

alive. By contrast, the antigens of the cell surface render the cancer cell vulnerable to immunological attack. They have been named "transplantation antigens" because the usual technique for their demonstration involves transplantation of cancer cells from one animal to another. The very interesting fact has been established that transplantation antigens are distinct for each individual cancer induced by a chemical carcinogen; but that they are shared by all cancers induced by the same virus. The identification of a particular antigen in or on a tumor cell may thus reveal what virus has caused it.

Certain cancer antigens, which may be released by the cells which produce them, are found in the body fluids. "Fetal" antigens, to be described below, belong to this category.

ANTIGENS OF HUMAN CANCER CELLS

The successful application of serological techniques and the development of techniques for measuring cell-associated immune reactions *in vitro*, have resulted over the past five years in the demonstration of five distinct antigenic systems in human cancer. There are already several additional candidates for inclusion. It may soon become possible to classify human cancers according to their antigenic properties. Whether this classification will ultimately supplement or supersede histological classification cannot be predicted at the moment, although an immunological classification may become of great importance to human cancer etiology, early diagnosis, prognosis, and therapy.

The antigenic systems now recognized in human cancer are:

(1) The antigens of the cells of Burkitt's tumor and nasopharyngeal carcinoma, which are found also in association with infectious mononucleosis. The several antigens making up this system appear to be produced by a virus (EB virus) belonging to the herpes group. Viruses of this group cause two types of naturally occurring cancers in animals, a kidney tumor in the frog, and Marek's disease in chickens. EB virus has therefore become the prime suspect as the cause of some human cancers (See: Viruses and Cancer).

(2) An antigen of malignant melanomas, an intracellular antigen, which is detected in cells from tumor biopsy material and cultured cells. No virus has been identified yet that could produce this antigen, but the presence of the same antigen in melanomas from different individuals might be a first indication of a common viral etiology. Furthermore it has been found, very recently, that each melanoma may also have a unique cell surface antigen in addition to the common intracellular antigen. Again, there is precedent in experimental work for the co-existence of shared and unshared tumor antigens on the same cell. Two types of antigens are also found in tumors induced by viruses. In all these cases, the presence of the individual antigen may be masked by the common antigen.

(3) The antigens found in human sarcomas, osteosarcomas, liposarcomas and chondrosarcomas, which are common to all those tumors. There is thus mounting evidence that these sar-

comas may also have a common viral etiology. This would be consistent with the fact that sarcomas in chickens, mice, and cats are induced by RNA viruses. (See: Viruses and Cancer).

(4) The antigens of neuroblastomas. These antigens are demonstrated by a novel technique in which the capacity of tumor cells to form colonies in culture is measured. In the presence of specifically sensitized immune cells or cytoxic antibody, there is a reduction in the number of colonies formed, which can be quantitatively determined. By means of this technique, it has been shown that neuroblastomas possess common antigens and that specific immune reactions to these antigens are demonstrable in children with this tumor, as well as in their mothers, but not in other individuals.

(5) Three kinds of "fetal" antigens have now been identified in human tumors. They are called "fetal" because they are normally present in embryonic or fetal tissues. Such antigens must, therefore, be specified by genes that are expressed during fetal life but are later, normally repressed. Neoplastic transformation brings about derepression of such genes with the result that their products, the fetal antigens, are found in cancer cells. Three different examples of fetal antigens are now known—

(*a*) The "carcinoembryonic antigen," or CEA, which is present in the blood of patients with cancer of the colon and disappears after removal of the cancer. It is detectable in embryonic and fetal gut, pancreas, and liver, during the first two trimesters of gestation, but not in adult normal tissues. CEA is found, however, in all cases of adenocarcinomas arising in cells derived from a particular embryonic tissue, the entodermal epithelium, from which part of the inside lining of the intestine derives.

(*b*) The "alpha-feto protein" appearing in the blood of patients with hepatomas and embryonal carcinomas. This antigen is also a characteristically fetal product which is synthesized by cancer cells. Approximately 75 percent of patients with primary hepatoma have this antigen in their blood, whereas patients with bile duct carcinoma or metastatic disease to the liver do not.

(c) The third and most recently recognized fetal antigen is a gamma-protein, which is found in a wide variety of human neoplasms of diverse origin, both malignant and benign. The antigen is readily demonstrable in fetal serum but not in normal adult serum. Antibody to this antigen, however, is found only rarely and then only in patients with cancers. Because of the widespread occurrence of this antigen in human tumors, and the demonstration of either the antigen or its antibody in the patient's serum, this system may have importance in the development of immunologic diagnostic methods.

A variety of immunological techniques has been developed and applied to the search for human cancer antigens. These techniques need further refinement, and others must be created which have even greater sensitivity and reliability.

However, as a result of the discovery of the different human cancer antigens, 1) important insights have already been gained into human cancer causation, 2) diagnostic procedures have been developed of much greater sensitivity than could have been imagined only two years ago, and 3) leads have been disclosed to rational prevention or therapy.

So much has been accomplished in so short a time because not only—in this field as in others—do discoveries made in animals find application in humans, but also because much of immunological research can be carried out simultaneously in laboratory animals and in the clinic.

IMMUNOLOGY AND CANCER CAUSATION

In animals, the immunological analysis of cancer has revealed that the antigens fall into two classes:

(1) Antigens of solid tumors induced by chemical carcinogens, which are different in each particular tumor. Even two tumors induced in the same animal by the same carcinogen will generally possess different antigens.

(2) Antigens found in various tumors and leukemias induced by viruses, which are characteristic for each type of virus. A virus always induces the same antigens, whatever the organ affected or the type of tumor produced.

Human cancers, as noted previously, can also have antigens that are either unique to a single individual or common to different tumors, either of the same or of different histological types. Since, in animals, the tumors sharing antigens are those caused by viruses, the implications of shared cancer antigens in human cancers—a finding made only in the last two years—are obvious.

Immunological evidence, which may be revealing the "footprints" of hidden viruses is at the moment the most compelling reason to accept the concept that some human cancers are indeed caused by viruses. It is generally felt that confirmation of this prediction for several types of human cancers, in particular for Burkitt's tumor and sarcomas, is not far away.

THE IMMUNOLOGICAL DIAGNOSIS OF CANCER

As cancer antigens are identified and classified, the next step is to isolate, purify, and characterize them. In the case of *shared* cancer antigens, these steps will allow the development of batteries of both antigenic and antibody materials to be used in the clinic for diagnostic tests.

Human cancer antigens, like those of animal cancers, can be found either on the cancer cell surface, inside the cell, or, as is the case for the CEA and other fetal antigens, circulating in the blood. Tests for their presence are based either on direct or indirect detection of the antigen on cell surfaces or in the blood, or the finding in the blood of the corresponding antibody. Both methods are used in the laboratory.

Direct detection and characterization of cell-bound cancer antigens require a specimen of cancerous tissue, obtained through biopsy or surgery, and a pre-existing battery of antisera, some "tagged" with

either fluorescent or radioactive compounds to make it easier to identify positive reactions. Circulating antigens, which can be detected in samples of blood plasma, will react in the test tube with the corresponding antibody.

Detection of Colon Cancer

An ingenious method, involving a radioactive tracer, has been proposed for the detection of CEA in patients with suspected cancer of the colon. Since the amount of antigen seems to vary in the patient in proportion to the number of cancer cells he harbors, the same test can help, in addition, to monitor the patient's status and so permit the periodic evaluation of therapeutic methods. This test is now being applied experimentally in several large hospitals to patients with cancer of the lower intentine. Elsewhere, the test itself is undergoing further assessment for added refinements. If proven reliable, as well as simple enough and inexpensive, it could result in a method for routine screening of large numbers of people. Cancer of the colon is one of the most common types of human cancer and, at the stage at which it is often diagnosed, it is relatively difficult to cure. However, if it is detected very early, surgical intervention is frequently successful. Thus, a method that can be applied in routine screening of a population at risk is of great practical value. Thus, the new new immunological test or colon cancer, which may make possible a positive diagnosis far earlier than other forms of examination, would ensure patients of a much better chance for complete cure.

IMMUNOTHERAPY OF CANCER

Cancer immunotherapy, the treatment of cancers by immunological methods, is still only in its experimental phase. Knowlege acquired only very recently suggests many different ways by which immunotherapeutic methods can be developed. Some are already being tried clinically; others are as yet mere possiblities, which await either further evaluation in animal models or, in particular, unequivocal evidence of the involvement of specific viruses in human cancer.

Active Immunization against Oncogenic Viruses

Immunization is usually a preventive measure rather than a treatment. However, active immunization against DNA oncogenic viruses in animals (such as polyoma virus) is not preventive in the usual sense since, when it is given *after* infection with the virus, it still inhibits the appearance of tumors. It is completely protective if given early in the period elapsing between infection with the virus and the time tumors start developing. Later, or after tumors appear, it is ineffective. No virus resembling the polyoma virus is known or even suspected to play a role in human cancer. Vaccination of chickens against Marek's lymphomatosis, which fully prevents the disease, has been a much applauded scientific victory (See: Viruses and Cancer). The virus of the herpes group that causes Marek's disease is related to some of the viruses implicated in human cancer, which include the EB virus that was isolated from Burkitt's tumor. (See: Viruses and Cancer.) This encouraging evidence suggests that it may be possible to develop a vaccine, using an attenuated virus, against the EB virus at some time in the future.

Active immunization against cancer cells

Active immunization against cancer cells has been attempted. A variety of antigenic stimuli has been used, including preparations made from the patient's own tumor cells killed by physical or chemical means, similar preparations from tumors of the same histological type removed from other individuals, and cell extracts of different kinds.

The preparation of such materials requires previous knowledge of what types of cancers have individually distinct cancer antigens and what types have common antigens. In the case of cancers which are antigenically distinct, antigens for active immunization would have to be prepared from the patient's own tumor. If common antigens are involved, then all cancers of related antigenic type could serve as a source of antigen. With the rapid advances now being made in the analysis of human cancer antigens, the needed information should soon be available for choosing rationally, in each case, a source of tumor antigen for active immunization. Crude tumor extracts are probably the least likely to be effective as immunizing material because they contain an inadequate quantity of antigen, much of it being destroyed by enzymes released as a result of cell brakedown. The use of whole viable cells attenuated by irradiation or chemicals appears to be more promising. The small amounts of tissue received from the operating room are frequently a factor severely limiting the amount of material that can be prepared for immunization. If common antigens are involved, one can use tumor cells of similar antigenic type from other patients. Tumor cells can also be first propagated in tissue culture so as to increase the amount of source material, as well as to allow full expression of the tumor antigen in a non-immune environment.

Since the antigens capable of stimulating a cytotoxic immune response are part of the cell surface, the best source of immunizing material may ultimately be a fraction of the cell containing cell surface membrane, or just purified soluble antigen. Such materials are now being tested with tumors in animals.

Modification of antigenicity

Attempts are being made to increase the inherent capacity of tumor antigens to elicit an immune reaction.

Many cancer antigens may be so similar to normal cell constituents as to be relatively ineffective in eliciting immune reactions. Modifying the cancer cell surface by a variety of means, including the coupling of foreign proteins, polysaccharides, or chemicals, may serve to increase its immunogenicity. An effective antigen for immunotherapy may well be purified cancer cell surface components to which a strongly immunogenic substance would be coupled.

Another method for increasing antigenicity is being developed using hybrid cells (See: Cancer Biology) formed between normal cells of different species, which are strongly antigenic, and cancer cells, which are presumably weakly antigenic. Such hybrids, it is believed, will be less malignant than cancer cells, or completely nonmalignant, yet highly antigenic and so capable of stimulating tumor rejection. In laboratory animals, they have already been demonstrated capable of immunizing efficiently against tumor transplants; this approach is

relatively simple to carry out at present, as a result of new techniques (See: Cancer Biology), and so appears also to deserve further efforts.

PASSIVE IMMUNIZATION AGAINST CANCER

Passive immunization, i.e., the administration of pre-formed antibody, has been tried against leukemias induced by vertically transmitted viruses in mice. Since such viruses enjoy immunological tolerance, active immunization with virus or leukemic cells is not expected to be effective. Antiserum produced by nontolerant rats is completely protective if given to mice within two days of an inoculation of leukemic cells which—without such treatment—always produces leukemia. Given later, antiserum leads to increased survival time, but not to cure. Lack of sufficient amounts of complement, the factor required for the cell-killing action of antibody, may limit the use of passive immunization as a way of controlling cancers growing under conditions of immune tolerance. Whatever its merits, such a method could not yet be tried in humans, as highly specific anti-sera comparable to those used in the animals studies are not available.

Adoptive immunization

In strict usage, this term describes the colonization of a recipient by live and viable immunocompetent cells from an immune donor. It may be extended to include transmission of the immune state through cell fractions derived from immune cells. In animals, adoptive immunization has been found to protect against transplants of tumors induced by chemical carcinogens.

Of even greater interest has been the finding that a definite (although usually temporary) regression of primary tumors can be obtained in rats which have received sensitized cells from other unrelated rats, and even from sheep. The latter finding was surprising. It was not expected that sheep would be able to single out tumor antigens among the wealth of foreign antigens carried by rat cells, or even that any of the sheep's lymphoid cells would persist long enough in a rat to have any effect on its tumor. Further investigations revealed that not only whole cells from the immunized sheep, but also cell fractions, in the form of crude nucleic acid preparations, confer immune reactivity to the recipient rat. Thus, it may not be the immune activity of the foreign cells which exerts a beneficial effect in adoptive immunization of this kind, but rather their capacity to transfer sensitizing information.

It seems possible, therefore, that "adoptive" transfer of tumor immunity could be achieved in man with extracts prepared from lymphocytes of immune donors.

In man, a material of this type is already known in another context. This "transfer factor" can transfer cell-associated immunity to antigens of bacteria, from immune donors to non-immune recipients. The use of subcellular fractions rather than whole lymphocytes offers another advantage in that the danger of a graft versus host reaction—i.e., an attack by the foreign lymphocytes on the recipient's normal tissues—is eliminated. This danger is heightened in cancer patients who often have unexplained intrinisc defects of their immune response, particularly of the cell-mediated type. Moreover, present modes of

cancer treatment—surgery, radiation and chemotherapy—all in different degrees, further decrease a cancer patient's immune capacity. It is thus important to discover how to supply such patients with specific "pre-formed" resistance in the form of immune cells or subcellular fractions. This approach is now being explored in clinical trials.

Such methods, however, must be applied with great caution. The immune reactivity that is transferred cannot be precisely manipulated yet. It results, insofar as it is successful, in an increase in the humoral as well as the cell-mediated response. While the latter leads to the rejection of cancer cells, the first, as noted previously, can interfere with it. The apparent deficiencies of the cell-mediated defenses in tumor-bearing individuals might sometimes be due to an excess of free, "blocking" antibody. Were production of the latter to be stimulated further by immune transfer, an aggravated situation might result, with tumor protection and exacerbation of the disease.

TESTING THE IMMUNOLOGICAL STATES OF THE CANCER PATIENT

Passive immunization and adoptive immunization are essentially approaches that use another organism's immune defenses and that transfer either its antibodies or its sensitized cells or cell fractions to a tumor-bearing recipient.

Can the patient's *own* immune defense mechanism be stimulated and strengthened? Is he responding immunologically to his own cancer or is he immunologically unresponsive? The tumor immunologist can now provide some answers to these questions.

Innumerable investigations have been done on the general immune status of patients with cancer, assessing their capacity to form circulating antibodies to defined antigens, or to develop lymphocyte-mediated so-called delayed hypersensitivity reactions and to mobilize inflammatory cells. The general impression from these various studies has been that, in patients with cancer of all types, the humoral immune reactions are intact, but the *lymphocyte mediated immune responses are suppressed.* Recently, a striking correlation has been found between the ability bo develop contact sensitivity to the chemical sensitizer NCB (dinitrochorobenzene) and the prognosis following cancer surgery. The great majority of patients who could be sensitized with DNCB did not develop tumor recurrence or metastases, whereas those who failed to become sensitized showed early recurrence or metastases. This test is now being applied to large groups of patients in an effort to relate the type of cancer and its stage to the patient's capacity to develop delayed hypersensitivity reactions. One has to surmise that those patients who cannot develop new delayed hypersensitivity reactions may benefit poorly if at all from immunotherapy, whereas prospects are brighter with those who can.

STIMULATION OF IMMUNE RESPONSES

The immune capacity is not invariable; it is affected adversely by serious disease and it declines with age. Just as there are drugs that can depress it—among which are a number used in the treatment of

cancer—there are others which can augment it or make existing immune defenses more effective. This bolstering of immune responses can be accomplished even after a tumor has formed. It cannot yet be done, however, in a selective way; the stimulating action affects *both* the humoral and cell-mediated responses.

The agents most effective in enhancing the immune response in animals are products of bacteria and fungi, called "immuno-adjuvants."[7]

That infection interferes with tumor growth in animals has been repeatedly reported. Tuberculous chickens are less susceptible to Rous sarcoma virus, for example, and the appearance of chemically induced tumors is delayed in infected animals. In the clinic, also, intercurrent infection has been thought to have contributed to spontaneous remission in cancer patients. Streptococcal infections and also mixed bacterial toxins were widely used in cancer therapy during the early part of this century. Although the effects of such treatment were extremely variable, there were a few authenticated long-term remissions. In such cases, the infections or toxins may have raised the overall level of immune responsiveness.

Today, the activity of immuno-adjuvants can be measured in experimental systems. As a consequence, the search for, or the preparation of, sufficiently effective and nontaxic new adjuvants for use in cancer therapy becomes a valid research goal. Several of these are already being used to assist conventional types of immunization. They are now undergoing clinical trials for their effect on the immune response to cancer. BCG, an attenuated form of the bacillus of tuberculosis, is the one now being most widely tried, particularly in the treatment of acute lymphoblastic leukemia in children, and in these cases, a few very long remissions have recently been reported following its use. BCG seems to be most effective when used in conjunction with other forms of treatment. It might then stimulate immune reactions sufficiently for them to cope with the few leukemic cells remaining after the destruction of the bulk of cancerous cells by drugs or radiotherapy.

The mechanisms by which microbial products stimulate local or general immunological reactivity are as yet unknown and there is, at the moment, no chemically defined material which would achieve a state of heightened resistance without the side effects seen with the cruder materials now used. Several laboratories are now trying to isolate such pure substances. These would have value in the clinic, for not only might they be effective in increasing resistance to tumor antigens, but they might also counteract the immunosuppressive effects of the other forms of cancer therapy, which render patients susceptible to bacterial and viral infections.

One method of immunotherapy which has been remarkably successful was developed for the treatment of multiple intracutaneous carcinomas, such as basal cell carcinomas. Foci of cancer cells are destroyed as a consequence of delayed hypersensitivity reactions provoked by contact sensitization with allergenic chemicals such as dinitrochlorobenzene. The mechanism underlying the selective destruction of cancer cells is unknown and deserves close study. As yet efforts to control cancers in other sites by this method have been unsuccessful.

SELECTIVE IMMUNO-CHEMOTHERAPY

Among the many ways in which the property of specific recognition of the immune system could be used, selective immuno-chemotherapy is of special therapeutic interest. Most drugs effective in the treatment of cancer are also toxic to some normal cells, which greatly limits their use. However, such drugs could be attached to antibodies prepared against specific tumor antigens. The antibodies would seek out the cancer cells, attach themselves to them, and thus deliver the drug selectively where it is needed, greatly sparing normal cells. It is now technologically possible to experiment with this method.

THE FUTURE OF CANCER IMMUNOTHERAPY

Much more knowledge is required in the many areas of immunology that have promise. There is neither a rational, nor an empirical way, as yet, to augment the cell-mediated immune response, without, at the same time, enhancing the potentially harmful humoral response. Knowledge of the mechanisms of the cell-mediated immune response is still not complete. No one knows *how* a cell becomes "sensitized" or precisely *how* it "recognizes" its antigen. No one has a clear picture of *how* it kills a target cancer cell. Nor is it possible to influence specifically the different cells or mechanisms involved in the cell-mediated response.

The humoral response is also a complex phenomenon. It is becoming possible to modulate it—i.e., to stimulate the production of its different antibody components selectively. However, their respective functions with regard to the tumor rejection mechanism are not, as yet, understood. The mode of action of complement, the serum factor responsible for the killing activity of antibody, still remains largely unknown.

In addition to basic research, cancer immunology has a particularly urgent need for the development of testing methods. It is not sufficient to identify a cancer antigen. What is needed is an accurate assessment of the patient's *rejection potential.* Methods will thus be needed to measure the relevant components of the immune reaction, including cell-mediated and humoral defenses and their interactions with other physiological factors.

Such tests will have to be carried out serially on each patient so that his immune response to his tumor can be monitored in relation to 1) the stage of his disease and 2) the various forms of therapy he receives. Reliable immunological methods for the monitoring of cancer patients still remain to be developed. Present skin or serological tests, although very encouraging, are still only crude beginnings. However, new *in vitro* systems are particularly promising in this respect; not only do these systems allow the different cellular activities to be studied with great precision outside the body, but they will also make it possible to test proposed immunotherapeutic methods on isolated cells. Ways have been developed with which to elicit, in animals, the different forms of immune deficiencies found in humans, as well as most types of cancer found in man. As a consequence, good animal models now also exist with which it is possible to test therapeutic procedures.

The immunological procedures available today for cancer therapy have not yet found wide use because none has yet been shown unequivocally to alter the course of human tumor growth in a predictable fashion. Even in animals, they are only rarely effective in the treatment of established cancers and, even then, under conditions that are generally not reproducible in man.

Moreover, it is now recognized that increased antibody induced by immunization might, rather than benefit the patient, actually inhibit the killing action of sensitized lymphocytes. It is considerations of this sort that prevent most clinicians from using immunotherapy in its present state as an adjunct to the other current forms of therapy of cancer.

For active immunization, adoptive immunization, and adjuvant therapy to play a role in the future treatment of cancer, they first must be modified and refined as more is learned from laboratory studies about cancer antigens, their cellular location and chemical nature, and the sort of immune responses they elicit. It would be as unfair and as premature to be pessimistic about the prospect of cancer immunotherapy on the current evidence, as it would have been to judge the potential value of penicillin using only crude extracts of *Penicillium notatum*.

Answers to a number of critical questions must be obtained before an evaluation of the precise potential of immunotherapy is possible. In animals, immunologic mechanisms can prevent the appearance of tumors and significantly retard tumor progression. However, even a strong and maximally magnified tumor rejection mechanism cannot eradicate large, established tumors. On the basis of this evidence, future immunotherapy in man may be seen as an adjunct to other forms of therapy. Even in this role it may, nevertheless, be of critical importance to the chance of cure from cancer. It may be successful in counteracting the immunosuppressive effects of other treatments, in protecting from intercurrent infections, and, most importantly, in destroying residual nests of cancer cells that may escape the action of drugs and radiation and are the cause of the relapses of the disease. Similarly, following excision of the primary tumor, immunotherapy might prevent the appearance of metastases.

So, despite the caution necessarily exercised at present in its use, there is undeniably a wave of optimism regarding its future. Research in cancer immunology and immunotherapy needs therefore to be greatly expanded.

AREAS OF PROMISE

Following considerable expansion of the application of immunological knowledge and techniques to the problem of cancer, great promise exists in the following studies:

(1) Development of tests for the identification of specific immune deficiencies, the evaluation of immune reaction potential, and the monitoring of cancer patients.

(2) Immune factors associated with lack of progression and spontaneous regression of cancer.

(3) Naturally occurring immune deficiencies and their relationship to cancer.

(4) Immunosuppression associated with aging.
(5) Basic events in all forms of immune response, at the cellular and the molecular level.
(6) Cell surfaces, their different receptor and antigenic sites, and their structural, biochemical, and functional properties.
(7) Selective immunosuppression and strengthening of immune responses.
(8) The mechanism of immune enhancement and its control.
(9) Immune tolerance and paralysis.
(10) Antigenic modulation.
(11) Cancer antigens—their relatedness, structure, and function.
(12) Serological tests for the detection and identification of cell-associated and circulating antigens.
(13) Adaptation of serological diagnostic tests to screening of individuals at risk from cancer.
(14) Cell fusion and the use of hybrid cells for the active immunization of cancer patients.
(15) Radioactive labeling of antibodies against cancer antigens for the detection of metastases.
(16) Passive immunization against vertically transmitted viruses.
(17) Nature of the "transfer factor," its function and mechanisms of action.
(18) Development and use of nonspecific adjuvants n cancer treatment.
(19) Selective immunochemotherapy.
(20) Delayed hypersensitivity, its mechanisms and use in diagnostic and prognostic tests.
(21) Cell culture of lymphocytes, for the study of their function as well as the large-scale production of immunocompetent cells for therapy.
(22) Origin, nature, function, and chemical composition of complement.
(23) Combined used in the clinic of surgical, chemical, and radiological methods of treatment, with new immunotherapy approaches.

Clinical Investigation

Clinical investigation in cancer is essentially the culmination of the basic and preclinical studies in cancer research, and the development from these of techniques useful in preventing, diagnosing, or treating cancer in man. Clinical investigation can include such diverse investigations as the biochemical studies of purine metabolism in patients with leukemia; assays of enzyme differences between normal and neoplastic human cells; studies of cell kinetics in leukemias and solid tumors; the clinical pharmacology, absorption, excretion, and therapeutic effectiveness of new agents against cancer; clinical trials of radiation sensitizers, the use of immunological methods for detecting or treating human cancer, and immunological studies of organ transplants. Clinical investigations contributing directly to improvement in the main treatment modalities (surgery, radiotherapy,

chemotherapy, and emmunotherapy) have been detailed under those particular disciplines. Other areas of importance are mentioned here.

BIOCHEMICAL AND METABOLIC STUDIES

In groups of cancer patients with particular tumor types, studies are underway to determine if unique chemical, metabolic, or immunologic characteristics exist that will permit precise and early detection and evaluation of response to therapy.

The biochemical, physiologic and morphologic characteristics and the interrelationships of cancer and host tissues which may influence rate of cancer growth, patterns of metastasis, and response to therapy are being investigated. One example is the angiogenesis factor which is responsible for providing the blood supply to the tumor. Drugs which would antagonize this factor in man would be the utomost importance.

The comparison of the enzyme complements of neoplastic human cells and their normal counterparts has important implications for both etiology and therapy. For instance, the finding that an RNA-directed DNA-Polymerase is present in the leukemic cells of patients with acute lymphoblastic leukemia but is not present in normal lymphocytes suggests a viral etiology for the human disease and also a possible therapeutic role for inhibitors of this enzyme.

PREVENTION OF METASTASES

A particularly urgent problem in clinical research is the control or prevention of metastasis. Surgery and radiation therapy are already highly effective in controlling most primary cancers. It is the failure to prevent the dissemination of the cancer or to provide effective treatment after the disease has become widespread that accounts for the greatest number of deaths. Certain drugs are now under clinical investigation which in animals are much more effective in preventing metastases than in inhibiting growth of the primary tumor.

CARCINOGENESIS

Prospective studies are underway and should be increased in groups at high and low risk to various types of cancer (e.g., patients with mongolism, heavy smokers, uranium miners, etc.) to define the factors which may determine susceptibility or resistance to cancer, such as ease of cell transformation, genetic makeup, antigen and antibody titers, tissue levels of carcinogens, or alterations in body content or excretion of enzymes or hormones.

EPIDEMIOLOGIC STUDIES

The more extensive use of epidemiologic methods augmented with intensive laboratory and clinical investigation is suggested by the results achieved by Burkitt in Africa. This work established the remarkable coincidences between the geographic distribution of holoendemic malaria, a high incidence of Burkitt's tumor, and low incidences of abnormal hemoglobins in patients with this tumor. In some

studies, monitoring the environment for chemical components and correlating the data with epidemiologic, clinical, and other laboratory data should provide new insights into disease and form the basis for prevention.

The findings of such types of clinical investigation could be used as a basis for formulating and applying principles for broad application to large groups of people. The philosophies and techniques used in developing new therapeutic methods and disseminating their benefits to the population of cancer patients may also be employed in developing and applying new methods of prevention and detection to many potential cancer victims.

AREAS OF SPECIAL PROMISE INCLUDING THERAPEUTIC CLINICAL INVESTIGATION

Lines of research in clinical investigation which should be pursued intensively include:

(1) Clinical pharmacology of new drugs with particular emphasis on absorption, distribution, and ability to cross the blood-brain barrier.

(2) Search for enzyme differences between human cancer cells and the normal cells.

(3) Stage I evaluation of new drugs for activity against cancer in man.

(4) Studies of the cell kinetics of the leukemias and solid tumors. These, in turn, would suggest better dosage schedules and combinations.

(5) Stage II trials for the evaluation of combinations and different drug schedules.

(6) Stage II trials for the evaluation of new radiation sources which may circumvent the problems of anoxia in tumor tissues.

(7) Stage III evaluations of various types of carefully planned adjuvant therapy in an attempt to increase the cure rates from surgery or radiotherapy.

(8) Evaluation of the many options of immunotherapy both nonspecific and specific, active and adoptive.

(9) The development of more precise techniques for measuring the presence of small numbers of cancer cells which would be useful for detecting cancer and also for quantitating the response to various types of therapy.

(10) The development of immunological and surgical techniques for the successful long-term replacement by transplantation of vital organs, such as liver, lung, and bowel locally infiltrated with cancer.

Resources for Cancer Research

No discussion of the present status of cancer research in the United States would be complete if it dwelt on its achievements and potentialities to the exclusion of the serious problems that now confront it. Of paramount importance among these is the problem of manpower, both the specialized scientific and technical personnel required in areas of cancer research that are now developing their own particular methods

and concepts, and the highly specialized professional and technical experts required for the management of the cancer patient. Shortages in manpower resources could be one of the limiting factor to productivity in basic research, as well as in the general application of new findings in prevention, diagnosis, and treatment. Since, in the areas of treatment, the manpower needs have been specified already, the needs in research will be briefly outlined here.

The extremely rapid and revolutionary developments that have taken place in biological research over the last 20 years have meant that for biologists to remain abreast of their own fields, they have had to upgrade their knowledge continuously in other areas and to become familiar with complex physical and chemical methods as well as the new techniques of cell culture and virology. Young scientists now have to devote many years to acquiring knowledge and experience in the basic sciences as well as to achieve proficiency in the latest and complex laboratory technology. Most of them do recognize the intellectual challenge of modern biology and its great promise for the advancement of human welfare, and if they can be assured of economic and professional security, many more than at present will enter and remain in this field. Adequate and consistent support for their work is as essential as is the preservation of freedom of inquiry in a field where so much remains to be explored.

At the moment, many young people people feel reluctance to undertake long-term studies and to commit themselves to a research career in biology because of fear of economic insecurity. Should this situation last any longer, its ill effects will be felt for years to come and will have a maximum impact by the time those who should now be entering on research careers will be needed as leaders.

In addition to the purely financial aspect of manpower resource development in biological research, there are educational problems to be solved.

Modern cell biology and molecular oncology are new fields which have to develop their own manpower and experimental systems. Because of the novelty of the approaches involved, most universities do not have the graduate school facilities nor the personnel to train a new kind of biologists to study cells of higher organisms at the molecular level. However, this type of investigation is today an integral part of cancer research, and, as a consequence, the special training of a large number of young scientists is indispensable to its development. These scientists must be thoroughly prepared to think of biology in molecular terms, and familiary with special areas of chemistry, physics, mathematics, and physiology, in addition to having the more traditional skills of the biologist. Their professional training requires that the educational institutions should set up the proper curricula, and that strong incentives should be provided to attract some of our best young minds to the strenuous life of work in laboratory devoted to basic studies in molecular biology and oncology, and to keep them there.

The growing need for the use of the methods of molecular biology in the study of mammalian and human cells, however, should not divert biologists from fundamental studies using micro-organisms.

Immunology, much more than other fields of research, has lacked both the prestige of academic recognition, and the financial resources

to develop adequate manpower. Few academic centers have staff groups of immunologists who form working teams of the size and excellence necessary to ensure maximally effective activity.

Because immunology has not been given the general recognition it deserves as a discipline in its own right—through professorships and academic departments, it is taught separately only rarely in medical schools. As a consequence, immunology is often used as a research tool by investigators without adequate knowledge. On the other hand, sophisticated, competent research centers do exist where general immunological studies, of considerable potential aid to those specifically interested in cancer, are being carried out. However, not all the expertise of those centers has yet found its way into cancer research. The teaching of immunology in universities and medical schools, as well as the development of manpower highly qualified in this discipline, will therefore be a major requisite to assuring full application of the tools and methods of immunology to the solution of the cancer problem.

As has been true for radiology and pharmacology which, upon being applied to cancer have given rise to the very specialized disciplines of cancer radiotherapy and chemotherapy, the immunology of cancer is also becoming a specialized discipline with its own concepts and techniques, both in research and therapy. Thus the training of *cancer* immunologists is a particularly critical need, as well as, again, the creation of incentives to attract young people into the field and to retain them in it through career opportunities. Only thus will be created a body of professional cancer immunologists who will soon be needed if immunotherapy is to become a reality for the cancer patient.

Cancer research is beset by shortages other than financial and human ones. There will be ever increasing needs for large colonies of experimental animals for use in the screening of environmental carcinogens as well as to test viruses and viral vaccines of different kinds. Primates probably will have to be used because they reflect more closely human susceptibility to many viruses. Present primate resources are very inadequate and require rapid and major expansion.

Another kind of need is for the semi-industrial production of cells and biological products, which is easily possible with today's technology. Much research on important biochemical problems fails to be undertaken at present because biochemists often do not have the resources to prepare materials (such as cell fractions, viruses, etc.) in sufficient quantity to apply their techniques effectively to the biochemistry of the constituents of normal and cancer cells. It is important that facilities be created for the centralized production of such materials and that they be manned by personnel highly skilled both in cell and virus propagation on a large scale. The personnel must also be expert in the handling of dangerous materials, since large-scale production of oncogenic viruses with the potential of causing cancer in man will also ultimately be required. The centralization of such resources would allow stringent quality control and standardized procedures. To create facilities of this type now would not only provide for the present, but allow planning for future needs, and avoid an otherwise certain waste of effort and manpower through duplication and inefficiency.

The Panel agrees with the opinions of its consultants that a solution to the problem of manpower and of other shortages described is indispensable to the successful achievement of the conquest of cancer.

Afterword

The studies of the Panel have confirmed that the best of today's methods in prevention, diagnosis, and treatment of cancer, despite their remarkable effectiveness in certain cases, have nevertheless important inherent limitations. There is no doubt that the most effective ways to improve the control of cancer will be found through the acquisition of additional scientific and medical knowledge of the neoplastic diseases whose essential nature is, in particular, still a profound enigma.

The Panel further concludes that there is now a fresh intellectual ferment in the field, that a variety of fruitful approaches can be discerned that lend themselves to concerted efforts by many dedicated scientists, and that the present dynamic thrust is bound to result in progress of incalculable value to mankind if appropriately encouraged.

Much is known already for which a practical use can be foreseen in the goals of either preventing the disease or saving the lives of the people afflicted. However, from all fields of investigation on cancer emerges now the concept that neoplastic properties are determined by molecular disturbances at a level of cellular organization that is basic to life itself, the one that controls growth and the expression of gene function. The control of some forms of cancer, to perhaps a larger extent than this has been true of diseases that have been controlled in the past, may require more knowledge of the environment, of many basic biological phenomena in cellular metabolism, of viral infection, and of the mechanisms of immune reactions.

Many areas of research offer such leads and justify unprecedented expectations. Great developments both of concept and technique have resulted from research over the last two decades, and as a result, cancer research now finds itself at a critical transitional stage:

> More clearly than ever before, a large number of cancer hazards can be identified, so that ways to interfere with their action or to eliminate them altogether from the human environment can be devised.
>
> It is now possible to manipulate agents which cause the cancerous transformation in such a way as to prevent the induction of cancer in normal cells and sometimes even to reverse the cancerous process.
>
> The natural defense mechanisms which protect the integrity of the human body against cancer are now better understood, and ways are being developed to correct their failure and heighten their efficiency.
>
> For the first time, the very molecular fabric of the cell can be probed to analyze the events that endow it with the properties of uncontrolled growth and invasiveness that distinguish normal from cancer cells.

Because of these new possibilities, a number of different specific approaches are becoming recognized that make cancer control conceivable.

The variety of these promising approaches affords confidence that at least some of them will prove successful.

Present research cannot promise a single mircaculous breakthrough. It is more likely to lead to progressive improvements over a number of years. Effective control will be achieved for increasing numbers of particular forms of cancer—as indeed it has already been for a few of them—before it will become a reality for all. Future progress will be facilitated by the development of an additional large body of new scientific methodology, knowledge, and technology. Concerted, large, broadly-based research efforts are required.

It is the Panel's opinion that a large and essential component of any future effort must be the continued accumulation of fundamental information, in order to provide the rational basis on which to build better methods of prevention, diagnosis and treatment. The Panel also feels that exploratory investigations at the frontiers of present knowledge need considerable amplication and a measure of planning by the very scientists engaged in each particular field, so that concerted research programs can be organized and implemented.

Thus, the Panel is of the unanimous opinion that the Nation can decide, through the nature of its commitment to the task and the resources it is willing to allocate, how rapidly the effort towards the eventual conquest of cancer can proceed.

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