Viral Carcinogenesis: Role of DNA Viruses

Scientists have grappled with the idea that viruses could cause human cancer for at least 70 years, but only within the last 10 years have they accorded it widespread respectability. The idea was suspect for so long because cancer in humans simply did not behave like other diseases of viral origin. It did not appear to be infectious, and there were no confirmable isolations of a causative virus. During that time, however, a comparative handful of pioneers accumulated evidence that proved that viruses did in fact cause cancer in animals. So the recurring question was: If viruses can cause cancer in animals, then why not in humans?

The concept that viruses can cause cancer is attractive for two reasons. Introduction of viral DNA or RNA—and thus of new genetic information—into cells can account for their permanent transformation to the malignant state. Second, identification of a viral cause for a cancer may permit the development of a vaccine to prevent and ultimately eradicate the disease just as vaccination has virtually eliminated smallpox in the United States (see box). Given the dread with which people view cancer—an attitude that may have contributed to earlier reluctance to consider that cancer had a viral etiology and was possibly contagious—the prospect of a vaccine is indeed a heady one.

Because the disease called cancer is actually a number of different diseases, demonstration of viral involvement in the etiology of one type of cancer, or of several types, does not mean that the problems of cancer etiology will be solved. According to some estimates, the majority of cancers may be chemically induced. Alternatively, the virus may be a necessary but not sufficient contributor to development of cancer. Other factors such as genetic disposition, immunological deficiency, or exposure to chemicals or radiation may also be needed. A requirement for two or more causes, acting together in the proper sequence, may help to explain why—if viruses are involved—there is little evidence that cancer is contagious.

Interest in the DNA viruses as human cancer virus candidates at first centered on the adenoviruses and papovaviruses. Adenoviruses, which cause respiratory infections in humans, and papovaviruses, such as SV40 and polyoma virus, produce tumors in experimental animals and transform animal cells in culture. But despite the fact that they are widespread, there are few indications that they are implicated in human cancers. Adenoviruses and papovaviruses have served as valuable tools for studying malignant transformation in the laboratory.

The Herpesviruses

During the middle 1960's, however, the herpesviruses, a family of complex DNA viruses, attracted the attention of a number of investigators who were seeking to establish a link between cancer and viruses. The lines of evidence for such a connection now stretch from several laboratories. They point to at least three herpesviruses that may be involved in human cancers and also to several that cause cancer in animals and may serve as models for studying human disease.

Proving that a virus causes human cancer has proved so elusive because Koch's postulates—which have served for almost 100 years as the criteria for establishing that a disease was caused by a given infectious agent—cannot be fulfilled. One postulate requires the isolation of the agent from all infected organisms. But viruses cannot be seen in fresh human tumor cells, nor can infectious particles be recovered from them. Although this complicates proof of a causal relationship, it is not surprising because when DNA viruses such as herpesviruses reproduce in cells they kill them—a consequence that is necessarily incompatible with development of cancer. Only after "tricks" have been perpetrated on tumor cells—such as culturing them, sometimes with other kinds of cells—can infectious virus be demonstrated. This raises the possibility that the virus is a contaminant. Another postulate requires an experiment that cannot be performed on humans with a suspected oncogenic virus: induction of the disease in a suitable animal by a pure preparation of the agent.

Thus investigators must rely on indirect or circumstantial evidence to prove their case against herpesviruses. Their strategies include epidemiological studies, now usually done in conjunction with immunological studies to determine whether the virus has left traces of its presence in the form of antibodies against it in the patient's blood; study of tumor cells to detect the presence of viral DNA or RNA or of virus-associated antigens; comparison with virus-induced animal tumors; and study of the oncogenic potential of the virus in both cultured cells and in living animals, especially nonhuman primates.

Since 1964 when M. A. Epstein and Y. M. Barr discovered a virus in cultured Burkitt's lymphoma cells, this herpesvirus—now called the Epstein-Barr virus (EBV)—has been a prime candidate as a human cancer virus. Burkitt's lymphoma is a cancer of the lymphoid system that afflicts children in certain areas of Africa. Among the investigators who have studied EBV, frequently in collaboration, are George Klein of the Karolinska Institute, Stockholm; Werner Henle and Gertrude Henle of the University of Pennsylvania Medical School, Philadelphia; George Niederman of the Yale University School of Medicine, New Haven, Connecticut; Paul Gerber and Gary Pearson, both at the National Cancer Institute (NCI), Bethesda, Maryland; and Dharam Ablashi of the NCI Frederick Cancer Research Center, Fort Detrick, Frederick, Maryland.

Like the other herpesviruses, EBV is widely disseminated in the human population. In studies conducted in areas of Africa where Burkitt's lymphoma is endemic, up to 90 percent of the children had antibodies to EBV before the age of 2 years. But the virus is not restricted to Africa. In the U.S., approximately 75 percent of the population acquires antibodies before adolescence. Early infection usually produces no characteristic illness. Epstein-Barr virus has been closely associated with Burkitt's lymphoma, a cancer of the lymphoid system, and with nasopharyngeal carcinoma. The same virus, or one so closely related

to it that they cannot be distinguished by current techniques, causes infectious mononucleosis, a disease whose symptoms may mimic those of leukemia except that mononucleosis is self-limiting and relatively mild.

Most of the evidence now linking EBV to human cancer relates to Burkitt's lymphoma. Investigators have detected a number of distinct, EBV-related antigen-antibody systems that can be used as indicators of viral involvement in Burkitt's lymphoma and that may reflect the progress of the disease. For example, patients with Burkitt's lymphoma have eight to ten times as much antibody against viral capsid antigen (the capsid is the protein layer surrounding the DNA core of the virus) as do normal controls. Although EBV is common, Burkitt's lymphoma is rare. According to Werner Henle, a prospective study now under way should determine whether the children who develop Burkitt's lymphoma are among those few who escape early infection (a situation analogous to that thought to pertain to infectious mononucleosis) or whether the disease occurs only rarely, for unknown reasons, among those who were infected in infancy.

Antibodies to another EBV-related antigen complex, called early antigens because when cultured cells are infected with EBV they appear early in the virus replication cycle, are rarely seen in healthy people but attain high concentrations in patients with Burkitt's lymphoma. These antibodies could be used to predict the probable course of the disease. Patients in whom the antibodies disappeared or declined after chemotherapy became longterm survivors—living 5 to 10 years without a

Cancer Vaccine Prospects: Not Soon

Identification of a viral role in the etiology of cancer could open the door to developing a vaccine to prevent the cancer. Carcinomas—the type of cancer associated with herpes simplex viruses—are by far the most common, accounting for some 85 percent of human cancer. Albert Sabin estimates that carcinomas he has linked to HSV I and HSV II constitute 30 percent of the total. An effective vaccine would thus be a major contribution to human welfare. The way to achieving this goal, however, is beset with even more perils and difficulties than are usually encountered in vaccine development.

A typical antiviral vaccine consists of a virus preparation that will elicit an immune response and enable the host to fight off subsequent invasion by the virus. The virus used for vaccination must either be inactivated or attenuated so that it cannot produce serious disease. Alternatively, it can be a relative of the pathogen which resembles it sufficiently to provoke an immune response but not enough to cause a severe infection. The cowpox virus used for smallpox vaccine is a good example of the latter. A current example—and one relating directly to the cancer problem—is the vaccine developed for Marek's disease by B. R. Burmester, H. Graham Purchase, and their colleagues at the United States Department of Agriculture's Regional Poultry Research Laboratory in East Lansing, Michigan.

Marek's disease is a malignant lymphoma of chickens that, until recently, was the most important cause of economic loss to the poultry industry. It is also an infectious disease caused by a herpesvirus, Marek's disease virus (MDV). For their vaccine, the East Lansing group used a herpesvirus of turkeys that is related immunologically to MDV but apparently not pathogenic to either species. Immunization with the turkey virus prevents Marek's disease but does not prevent reproduction of MDV (which occurs in the feather follicles) or virus spread.

Use of an analogous technique to produce a cancer vaccine for humans would be fraught with hazard, to say the least; it would be necessary to prove—somehow—that a relative of a human cancer virus candidate had no oncogenic potential in humans. Administration of an inactivated candidate virus does not appear to solve the problem. Fred Rapp has shown that HSV I and HSV II, following photodynamic inactivation and loss of infectivity, still transformed hamster cells, as did HSV inactivated by ultraviolet light. Heat-inactivated viruses can also retain their oncogenic capacity, according to Dharam Ablashi and his colleagues. Herpesvirus saimiri, after heating to 56°C, could not infect and kill cultured cells, but it induced malignant lymphomas when injected into owl monkeys. In view of the theories that cancer is caused by defective DNA viruses, inactivation may actually increase the hazards.

Investigators are exploring approaches to vaccine development that do not require the injection of viral DNA —presumably carrying information necessary for transformation—into humans. Among them are the use of proteins or glycoproteins of the viral envelope or membrane or of virus-associated antigens from transformed cells to elicit an immune response in the host. Although these strategies avoid administration of genetic information to humans, they could be ineffective if the antigens are only weak stimulants of the immune system, or if the immune system is itself defective and cannot make an adequate response—a possibility considered by some to contribute to cancer initiation.

A final problem is the inadequacy of current techniques for assessing vaccine effectiveness. Onset of clinical symptoms cannot serve as a useful criterion. Most herpesvirus infections are not accompanied by detectable symptoms, and cancer itself has a long latent period between infection and disease development. Nor would the presence of circulating antibodies be indicative of vaccine effectiveness; recurrent infections can occur in individuals who have such antibodies. Additional information about the response of the immune system to herpesvirus infection will be required before suitable criteria for vaccine effectiveness can be selected.

Researchers are hopeful that they can develop a vaccine against human cancer, but these problems, plus the need for thorough testing for safety and effectiveness in animals before human studies can be initiated, all militate against an early solution.—J.L.M.

relapse. If the antibody concentration did not decline, the prognosis for the patients was generally poor. Investigators think it unlikely that antibodies to a passenger virus (one that just happened to be present but was not causative) would be of prognostic value.

Although EBV particles are not found in Burkitt's lymphoma cells removed by biopsy, other traces of the virus can be detected. These include certain EBV-associated antigens and also viral DNA. Cellular DNA hybridizes either with viral DNA or with RNA transcribed from viral DNA. The results of the hybridization experiments indicated that each lymphoma cell contained multiple copies of EBV DNA. No one knows why the viral DNA is not completely expressed in tumor cells. It may be defective or partially repressed. A small number of cells in a few cultured cell lines derived from biopsied tumor cells did produce infectious viral particles or incomplete noninfectious particles. All of the cells, however, produced an EBV-associated nuclear antigen that may be analogous to the T (tumor) antigens produced in cells infected by oncogenic animal viruses such as SV40.

Another indication of the oncogenic potential of EBV is its capacity to transform lymphoid cells in culture. Normal lymphoid cells do not proliferate in culture and die out after a short time. Transformed cells, which harbor the viral genome, do grow in culture, and they have the characteristics usually associated with transformation, including loss of density-dependent inhibition of growth.

Production of cancer in laboratory animals with EBV would provide additional evidence that it causes related disease in humans, and would also provide a model system for studying therapeutic techniques. George Miller and his colleagues at Yale University induced lymphomas in a small number of marmosets by inoculating them with materials containing EBV. Although Miller has not conclusively proved that EBV itself caused the tumors, similarities between the human disease and that in marmosets make this the most likely explanation.

Herpesvirus saimiri, an oncogenic virus of nonhuman primates, may provide another model for investigating human lymphomas. This virus causes no overt disease in its natural host, the squirrel monkey, but induces lymphomas or occasionally lymphocytic leukemia in marmosets and owl monkeys. The behavior of the virus resembles that of EBV, both in its effects in living animals and in cultured cells. For example, neither EBV nor herpesvirus saimiri particles can be demonstrated in biopsied tumor cells but they are occasionally produced in cultured cells. Moreover, the antigen-antibody patterns associated with the latter virus resemble those of EBV.

Evidence implicating the herpes simplex viruses I and II (HSV I and HSV II) in the etiology of human cancer is similar to that outlined for EBV. Investigators have elucidated a series of correlations between certain types of cancer and past infections with the viruses, the presence of antibodies to virus-associated antigens, the presence of virus-associated antigens in tumor cells, and, in one case, the presence of viral DNA in tumor cells. The herpes simplex viruses also transform cells in culture.

These viruses are again familiar human pathogens. Herpes simplex virus I primarily infects regions around the lips (where it causes the common "cold sores"), the oral cavity, and the eyes. Herpes simplex virus II usually infects

What Is Cancer? What Forms Does

It is not yet clear whether cancer is many diseases exhibiting a common pattern of general symptoms or one disease that is manifested in many forms depending, primarily, upon the organ from which it evolves. In any case, more than 100 clinically distinct types of cancer are recognized, each having a unique set of specific symptoms and requiring a specific course of therapy. These types can, however, be grouped into four major categories:

► Leukemias are diseases in which abnormal numbers of leukocytes (white blood cells) are produced by the bone marrow. This enhanced production resembles the body's normal response to a massive infection, but in leukemias most of the leukocytes do not mature into functional cells. Leukemias are one of the most common malignancies of childhood, but they strike people of all ages. Preliminary results from the National Cancer Institute's Third National Cancer Survey of 1969 suggest that leukemias will account for about 3.4 percent of the 655,000 cases of cancer that will be diagnosed this year.

► Lymphomas are diseases in which abnormal numbers of lymphocytes (a type of leukocyte) are produced by the spleen and lymph nodes. The disease is thus quite similar to leukemia, but in some lymphomas the immature lymphocytes aggregate in the lymphoid tissues. Hodgkin's disease is the best-known form of lymphoma. The cancer survey indicates that lymphomas account for about 5.4 percent of diagnosed malignancies.

► Sarcomas are solid tumors growing from derivatives of embryonal mesoderm, such as connective tissues, cartilage, bone, muscle, and fat. Leukemias and lymphomas could be considered subgroups of sarcomas, since bone marrow and lymphoid tissues are derived from mesoderm. Sarcomas, leukemias, and lymphomas are the predominant forms of malignancy observed in laboratory animals and in cell cultures, but sarcomas themselves, according to the survey, account for only 1.9 percent of human malignancies.

► Carcinomas, solid tumors derived from epithelial tissues, are thus the major form of cancer. Epithelial tissues are the internal and external body surface coverings and their derivatives, and thus include skin, glands, nerves, breasts, and the linings of the respiratory, gastrointestinal, urinary, and genital systems. Carcinomas account for about 85.3 percent of malignancies. (The remaining 4.0 percent of malignancies include tumors derived from mixed tissues, such as ovaries and testes, and all others that are not readily classifiable.)

All of the more than 100 types share three major

genital areas and is transmitted venereally. Both may persist in the host for long periods of time and produce recurrent infections.

Beginning in the middle 1960's, epidemiological studies such as those conducted in the laboratories of André Nahmias at the Emory University School of Medicine, Atlanta, Georgia, Laure Aurelian at Johns Hopkins University School of Medicine, Baltimore, Maryland, and Joseph Melnick at Baylor College of Medicine, Houston, Texas, implied an association between HVS II, which Nahmias had identified as the cause of up to 95 percent of genital herpes infections, and cancer of the uterine cervix. Both conditions were correlated with increased numbers of sexual partners for the women surveyed. Women with cervical cancer had higher frequencies of antibodies to HSV II than did controls.

The problem with interpreting these results is that women likely to contract one venereal disease, genital herpes, might also be likely to acquire the other, cervical cancer. In other words, the two conditions could be independent consequences of a high degree of sexual activity. The solution to the problem requires close matching of women with cervical cancer and control women with regard to age, race, socioeconomic status, and all the variables indicative of sexual activity.

In one such study with carefully matched women, William Rawls, in Melnick's laboratory, found that women with antibodies to HSV II had a risk of acquiring cervical cancer more than twice as great as that of women without antibodies. There was no greater incidence of breast cancer in women with antibodies than in women without. Melnick concluded that the risk of developing cervical cancer appeared to be more closely related to HSV II infection than to attributes associated with number of sexual partners.

Virtually all epidemiological studies conducted to date have been retrospective ones in which the information about herpes infection was gathered after the onset of cancer. Nahmias and his associates are currently conducting a prospective study, begun in 1963, to determine whether women who have had genital herpes infection or who have antibodies to HSV II develop more cervical cancers than women who do not have the antibodies. The long latent period of cancer and the need to include large numbers of carefully matched women compound the difficulty of such studies. Nahmias' preliminary results indicate that women with antibodies to HSV II do have a higher incidence of cervical cancer than do women without antibodies. Women who were pregnant when genital herpes was diagnosed had an even higher incidence. Nahmias is now investigating whether changes in the female genital tract during pregnancy may predispose to initiation of cancer by HSV II.

Malignant transformation of cells by viruses probably depends on expression of some viral gene or genes. For adenoviruses and papovaviruses, substances called nonviron antigens are viral gene products thought to be associated with transformation. Nonvirion antigens are coded by the viral DNA but are not themselves part of the viral particles. Since they are foreign substances, the host species will make antibodies against nonvirion antigens that can be detected in blood serums.

Discovery of HSV nonvirion antigens in human tumor cells or of their antibodies in patients' blood serums would provide strong evidence for a causal role of these viruses in human cancer. Both antigens and antibodies have been detected. Albert Sabin, now

It Take? How Does It Kill?

characteristics that define cancer: hyperplasia, anaplasia, and metastasis. Hyperplasia is the uncontrolled proliferation of cells. Contrary to popular belief, however, hyperplasia does not imply an enhanced rate of proliferation: malignant cells display the same variations of rates as healthy cells. The malignant cells simply do not respond to the host organism's (as yet unknown) signals to halt division, and thus produce a localized accumulation of tissue.

Anaplasia is a structural abnormality in which the cells resemble more primitive or embryonic cells and in which adult functions are absent or diminished. A malignant lymphocyte, for example, would not retain the capacity to fight infections. Anaplastic cells also lack orientation with respect to the parent tissue; instead of an orderly spatial arrangement, their distribution is often jumbled.

Metastasis is the ability of a malignant cell to detach itself from a tumor and establish a new tumor at a remote site within the host. This ability reflects both the lessened cohesiveness of cells within a tumor and the capacity of malignant cells to sustain themselves while floating freely in the blood stream or lymph ducts. Unmetastasized tumors can frequently be removed by surgery or irradiation, but there is much less prospect of treating metastasized malignancies.

Cancers are generally not, in themselves, fatal; that is, with rare exceptions, they do not produce toxins or otherwise kill the host directly. Rather, they seem to have a curious priority on nutrients within the host, nourishing themselves at the expense of other tissues. This malnutrition produces a phenomenon called cachexia, the generalized emaciation and ill-health of the host. Cachexia has frequently been considered to be the ultimate cause of death in a majority of cancer patients, but a recent study suggests that many other factors are involved in such cases.

Julian L. Ambrus and his associates at the Roswell Park Memorial Institute, Buffalo, New York, have studied more than 500 cancer deaths at that institution and found that the chief cause of death was infection, which accounted for 36 percent of the deaths and was a contributory factor in an additional 13 percent. Most of these infections were caused by antibiotic-resistant bacteria. The second most important factors were hemorrhaging and blood clots, which together accounted for 18 percent of deaths and were contributory factors in another 43 percent. Other factors included organ failures caused by invasion by cancer cells, 10 percent, respiratory failure, 19 percent, and cardiovascular insufficiency, 7 percent.—T.H.M.

at NCI, and Giulio Tarro of the University of Naples found that serum from patients with advanced cancer of the lip, mouth, oropharynx, nasopharynx, kidney, urinary bladder, prostate, uterine cervix, or vulva did contain antibodies to nonvirion antigens of HSV I, HSV II, or more frequently to both. Patients with cancer of the lip, mouth, or oropharynx had antibodies only against the nonvirion antigen of HSV I which primarily affects head and mouth regions. Sabin and Tarro did not find antibodies in normal adults, patients with recurrent herpes infections, or a large number of patients with cancers other than those listed. Thus the antibodies appear to be specifically associated with cancers—all of them carcinomas—of the urogenital and oral regions. The antibodies are present only in patients with advanced metastatic cancer.

Ariel Hollinshead of the George Washington University Medical School, Washington, D.C., and Tarro found that soluble cell membrane antigens extracted from carcinomas of the lip and of the uterine cervix would react with antibody to HSV nonvirion antigens. Soluble antigens from an intestinal tumor or from normal vaginal tissue did not react with the antibody in the immunological assay. Thus, it appeared that lip and cervical carcinomas contained HSV nonvirion antigens not found in normal tissue or an unrelated tumor.

Researchers hope that they can monitor the effectiveness of cancer treatment by measuring the concentration of antibodies against virus-associated antigens. Hollinshead and her colleagues found that 70 to 90 percent of the patients with carcinomas of the head, neck, or uterine cervix had antibodies to HSV nonvirion antigens. Removal of the tumors might be expected to remove the antigen source with subsequent disappearance of the antibodies against it. But Hollinshead and Tarro, in a collaborative study with Rawls and Paul Chretien of NCI, found that individuals who had no clinical signs of carcinoma after successful treatment still retained antibodies to HSV antigens.

Hollinshead does not know why the antibodies did not disappear. She points out that the results parallel those of Chretien and his colleagues. They found that individuals cured of certain carcinomas still had deficient cellular immune responses, while individuals cured of other types of cancer did not have impaired cellular immunity. Hollinshead hypothesizes that herpes simplex viruses may themselves suppress the activity of the immune system. Alternatively, the immune defect may be a preexisting condition that contributes to cancer development.

Antibodies to other antigens (apparently not the same as the nonvirion antigens studied by Hollinshead and Sabin) associated with HSV II do appear to correlate with the clinical state of patients with cervical cancer. Aurelian and her colleagues found that cultured human epidermoid carcinoma cells, infected with HSV II, produce an early antigen (or antigens) that reacts in an immunological assay with antibody in serums from patients with cervical cancer. There was no reaction with serums from normal controls or from patients with other cancers, including carcinomas. The presence of the antibody correlated well with the extent of the disease. Ninety-one percent of patients with invasive cancer had antibody to the antigen but only 35 percent of patients with very early cancerous changes had it.

Antibody of Prognostic Value

Moreover, the antibody may be of prognostic value. Aurelian and her colleagues did not detect it in 22 patients who had had invasive cancer but who were free of the disease after treatment. They also studied four patients in whom antibody had been detected prior to treatment; in the 2 years after radiation therapy, antibody was found in the individuals whose cancer recurred but not in the two who remained free of clinical symptoms.

Finally, Gilbert Chiang, in Nahmias' laboratory, has recently detected herpesvirus nuclear antigens in biopsies of invasive cervical cancers. Thus, there is evidence that at least three groups of HSV-associated antigens—non-virion, early, and nuclear—are found in cervical cancers.

Virus-associated antigens are not the only traces of herpesviruses detectable in cancer cells. Bernard Roizman and his colleagues at the University of Chicago detected fragments of HSV II DNA in one specimen of human cervical cancer tissue. In contrast to Burkitt lymphoma cells that contain multiple copies of the EBV genome, the tumor cells contained about 40 percent of the HSV II genome. According to Roizman, only 5 percent of the HSV genome was transcribed into RNA in the tumor cells while approximately 50 percent is transcribed when the virus reproduces in and kills cells. These findings support the hypothesis that transformation is effected by defective viruses, possibly when they are integrated into the cell genome. Roizman and his colleagues are using hybridization techniques to determine whether different cervical tumors contain common DNA sequences whose expression is required for transformation.

Roizman is now attempting to pin down the role of the various HSV-associated antigens in transformed cells. He had previously identified a large number of proteins—approximately 50—specified by the HSV I genome in infected cells. Many of them did not appear to be viral structural proteins. In one phase of the work, Roizman is collaborating with Sabin. Using materials supplied by Sabin, he hopes to demonstrate that one (or more) of these nonstructural proteins is identical to the nonvirion antigen (or antigens). Such a result would directly confirm that the antigens are in fact nonvirion (present evidence is indirect), and markedly strengthen the case for herpesviruses as carcinogens, especially if the nonvirion antigens are shown to be the product of DNA fragments common to all cervical tumor cells. In this case, they might well prove to be the substances causing transformation.

It appears that inactivated or defective herpesviruses can transform cultured cells. Infectious particles reproduce and thus kill the cells by lysing them. Fred Rapp at the Milton S. Hershey Medical Center of Pennsylvania State University, Hershey, found that HSV I and HSV II transformed hamster embryo cells after the viruses had been inactivated photodynamically. Photodynamic inactivation involves treating the virus first with a dye, then with light. When the dye forms a complex with viral DNA, subsequent absorption of light energy causes a reaction that damages the DNA and results in loss of infectivity. Rapp and others have shown that HSV I and II, inactivated by ultraviolet light, also transform hamster cells.

Because of his results, Rapp has expressed concern about the use of photodynamic inactivation for treating recurrent genital herpes infections. Such treatment is frequently successful, but Rapp suggests that it is potentially hazardous if inactivated HSV is indeed carcinogenic. He thinks that

(Continued on page 1112)

(Continued from page 1070)

use of the treatment should be suspended until its oncogenicity can be determined in animals.

A large percentage of the human population has been exposed to one or more of the herpesviruses, yet relatively few get cancer. Most investigators think that other factors—in addition to DNA viruses—must contribute to initiation of the disease. Of prime interest is the role of the immune system (which will be discussed more fully in a future article). The immune system is generally thought to prevent tumor development by detecting tumor cells—because of their tumor- or virus-associated antigens—and destroying them. A deficiency in the immune system, whether the result of a genetic defect, infection, or immunosuppression (as in transplant patients who suffer an increased cancer incidence), could therefore contribute to cancer development.

Another possibility is that two or more viruses may cooperate in initiating transformation. For example, Sol Spiegelman and his colleagues at Columbia University, New York, found particles resembling RNA tumor viruses in Burkitt's lymphoma cells. These findings raised the possibility of an interaction between EBV and an oncogenic RNA virus in Burkitt's tumors. Spiegelman and his colleagues used an animal model to test this hypothesis. From experiments on chickens, in which they studied the interaction of Marek's disease virus (an oncogenic herpesvirus of chicken) and an RNA tumor virus, Spiegelman concluded that both could contribute to tumor growth under their experimental conditions.

Although evidence implicating DNA viruses in the etiology of human cancer is accumulating, numerous questions remain unanswered: What viral genes are necessary for transformation? Where and how is the virus maintained in the human body during the long latent period before cancer develops? How is viral DNA incorporated into cellular DNA? What controls the expression of viral DNA and triggers transformation? What is the role in cancer initiation of other human cancer virus candidates? of chemicals? and of the immune system? The cancer problem sometimes seems to have as many questions as Hydra has heads—and when one is lopped off, two grow back.—JEAN L. MARX