Research on Cancer Viruses

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THERE IS NO DOUBT that virology now holds great promise in research efforts on the cause and prevention of cancer. Virology has but recently attained this high status. Only in the past few years has the accumulated evidence of a half century of investigation proved sufficient to convince the more skeptical scientists that viruses cause cancer in animals.

The French bacteriologist Amédée Borrel was the first to make the suggestion, in 1903, that cancer might be a viral disease. His countryman, the eminent virologist Charles Oberling, later pointed out in "The Riddle of Cancer" that Borrel reached this conclusion when he failed to find the "microbe of cancer." For years his idea was defensible "mainly because no other offered a satisfactory interpretation."

Then, in 1908, the Danish scientists V. Ellerman and O. Bang succeeded in transmitting leukemia from one chicken to another by injecting cell-free filtrates of blood and organ extracts. At that time, however, leukemia was not generally considered a neoplastic disease, so their work did not receive much attention.

Two years later, Dr. Peyton Rous, working at the Rockefeller Institute in New York, transferred certain spontaneous tumors of chickens by cell-free filtrates. One of these neoplasms was the source of the Rous sarcoma virus. This work was viewed with some skepticism because of the prevailing opposition to an infection theory, but subsequent work established beyond any doubt that these were true neoplasms and that there were no living tissue cells in the filtrate. Now we know that Rous' discovery marked an important stage in the history of experimental cancer research. The Rous sarcoma dramatically progresses in degree of malignancy through successive passages in the laboratory, either by cell transplants or tumor filtrates (fig. 1).

In the early thirties, a young man named Dr. Richard Shope, also at the Rockefeller Institute, was studying rabbit tumors—in particular, a papilloma occurring in certain wild cottontail rabbits. Using the same basic technique employed by Rous, Shope extracted and filtered the papilloma tissue and injected the filtrate into domestic rabbits. Figure 2 shows the results after various periods of time.

The Shope papilloma agent cannot be recovered from tumors in the domestic rabbit; the animal can be infected with a filtrate, but the papillomas that arise generally cannot be transmitted from one domestic rabbit to another. Nevertheless, the presence of a virus is signified by the appearance of antibodies in the blood as the tumor develops.

Several other virus studies bore fruit during the thirties. In 1934, Dr. Balduin Lucké at the University of Pennsylvania described the transmission by a cell-free extract of kidney tumors in the leopard frog.

At the Roscoe B. Jackson Memorial Laboratory, in Bar Harbor, Maine, a geneticist, Dr. John Bittner, and others were working with inbred mouse strains. He made reciprocal crosses between high and low mammary tumor strains of mice, expecting to find approximately the same incidence of tumors in the progeny of high-strain mother-low-strain father crosses and low mother-high father crosses. But only the progeny from mothers of the high-tumor strains had the same high incidence of cancer.

In studies to determine the nature of the

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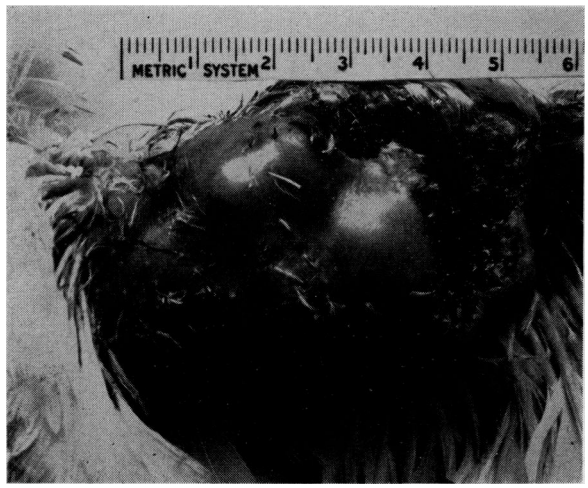

Figure 1. Sarcoma in the wing of a chicken 10 days after inoculation of the most potent Rous sarcoma virus preparation currently available

mother's influence, Bittner foster-nursed mice from high-tumor strains with mothers of low-tumor strains. The foster-nursed mice were relatively or entirely free of cancer, and so were their descendants for generations. In later studies, the reverse was also shown to be true. It was also found that a low-tumor strain mouse had to receive milk from a high-tumor strain foster nurse within about 10 days of birth, if it was to develop cancer. Thus was discovered the Bittner milk agent, now accepted as a virus.

Research in the Fifties

It was a long time before the studies conducted by the early pioneers of virus-cancer research were generally considered to be anything but isolated laboratory curiosities. Other paths of cancer research appeared vastly more promising than virology. In the last decade, however, a number of investigators have become interested in virus-cancer research, and have produced an impressive amount of information about animal tumor viruses and the fundamental nature of viruses and cell components.

The beginning of this new, active period is marked by a study by Dr. Ludwik Gross of the Bronx Veterans Administration Hospital who in 1951 successfully transmitted mouse leukemia with filtered extracts by injecting newborn mice of a susceptible strain. His best results were obtained when he inoculated mice no more than 16 hours old. Gross also reported an unexpected result—some of the inoculated mice developed tumors of the parotid, a salivary gland, and miscellaneous other types. Many of these tumors rarely, if ever, occur spontaneously in uninoculated mice of this strain.

At the National Cancer Institute, Dr. Sarah Stewart attempted to reproduce Gross' results. But, although she used the same method, none of the mice she inoculated got leukemia. They developed primarily parotid gland tumors instead.

Teaming with Dr. Bernice Eddy, also at the National Institutes of Health, Dr. Stewart increased the potency of the parotid tumor fil-

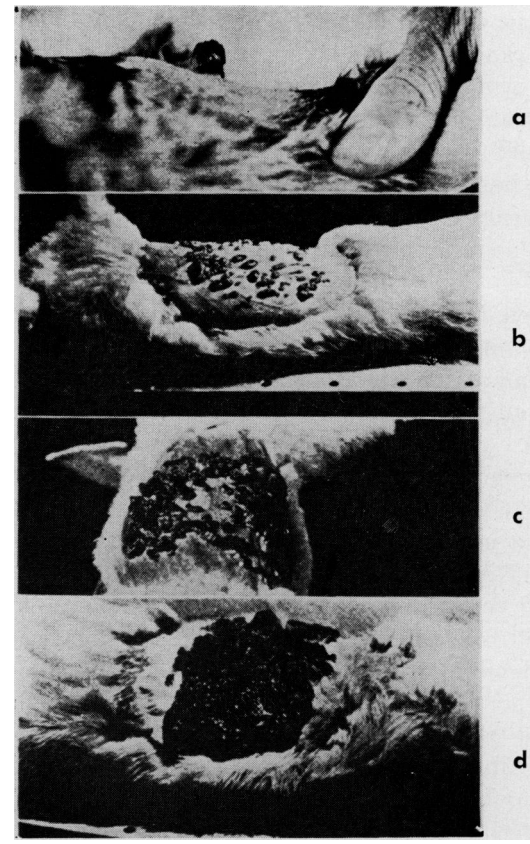

Figure 2. (a) Papilloma on the inner left thigh of a wild rabbit (under the microscope these growths resemble extremely keratinized warts), (b) result of inoculation of domestic rabbit with filtrate of the papilloma tissue after 23 days, (c) after 52 days, and (d) after 118 days

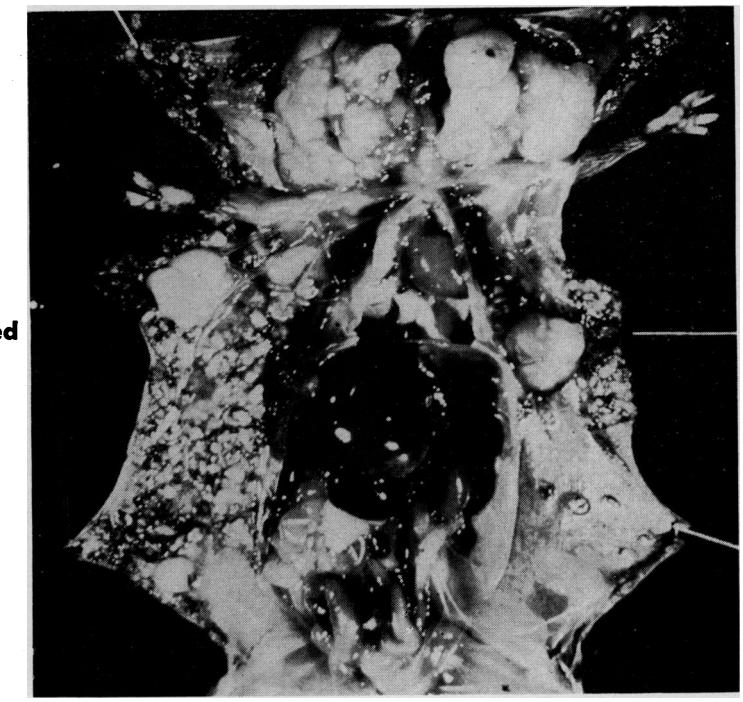

Figure 3. Autopsy of mouse inoculated when newborn with polyoma virus

trate by carrying it in tissue culture. When newborn mice were injected with this filtrate, the results were remarkable (fig. 3). Not only did the mice develop primary parotid tumors, but 22 other types of tumors as well, including tumors of the thymus, adrenal glands, and mammary glands. The agent was thus named "polyoma virus." Some of the mice developed tumors within 6 weeks. Again, none developed leukemia. The investigators obtained similar results when they inoculated mice with mouse leukemia extracts incubated in tissue culture.

It is now widely believed that Gross' original material contained two viruses, the leukemia virus and the polyoma virus.

Stewart and Eddy have also shown that the polyoma virus has the unusual ability to cross animal strain and species barriers, for, although a mouse virus, it produces tumors in hamsters and rats as well.

A study conducted at the Sloan-Kettering Institute for Cancer Research in New York City about 3 years ago shed new light on the virus-tumor relationship in animal leukemia. Dr. Charlotte Friend reported her discovery of a virus that induced a leukemia-like disease in adult as well as infant mice within 2 to 3 weeks after inoculation.

Dr. Joseph Beard, the eminent virologist at Duke University, has pointed out that the studies by Friend and Gross have "firmly established the principle of virus etiology of well-known examples of mammalian leukemia." And, he adds, "There now exists a considerable body of information which is not only compatible with the hypothesis of the viral etiology of human leukemia, but which provides a substantial and reasonable background for pursuing investigations in man" ("Nature of the Viruses of Avian Myeloblastosis and Erythroblastosis" in Proceedings of the Third National Cancer Conference).

Dr. Leon Dmochowski, who with the electron microscope photographed virus-like particles in a variety of mouse and chicken tumor tissues, has conducted some interesting studies on human leukemia. In a collaborative study at the University of Texas M. D. Anderson Hospital and Tumor Institute in Houston, Dr. Dmochowski reported seeing virus-like particles in a biopsy from an enlarged cervical lymph node of a patient with acute lymphatic leukemia. He also reported that cells in the lymph nodes had undergone a number of changes similar to cell changes in affected organs of mouse leukemia and chicken lymphoma-

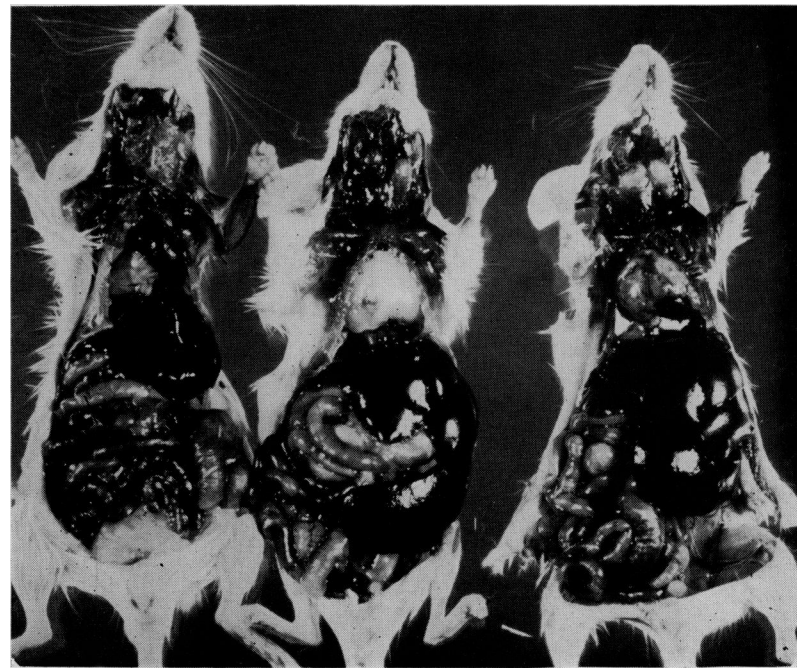

Figure 4. Mice inoculated with the Moloney virus *center* at 64 days of age, and *right* at 48 days. *Left*, a control C strain mouse

tosis. Visceral lymphomatosis is a common malignancy of chickens, known to be caused by a virus.

A year ago, one of our scientists at the National Cancer Institute reported a discovery that is, I believe, a real landmark in virus-cancer research. It is a fascinating story, beginning with Dr. John Moloney's studies of the properties of Sarcoma 37, an experimental mouse tumor. In the course of his investigation Dr. Moloney prepared a cell-free extract of the tumor and injected it into healthy mice. The result was quite unexpected. Within 8 months, the animals developed a type of leukemia that is indistinguishable from spontaneous leukemia in mice.

Following this lead, Moloney prepared extracts from leukemic tissue of the mice that first developed the disease, and injected these extracts into mice. By repeating this process several times, he obtained an extract so virulent that it caused leukemia within 10 weeks in 100 percent of the mice injected on the first day of life.

The leukemia agent is a virus, and the electron microscope has revealed particles that may be the virus. Unlike other mouse leukemia viruses, the Moloney virus causes the disease in several different strains. It is also active against adult as well as newborn animals. None of the mice inoculated with the virus has developed any form of cancer except leukemia (fig. 4).

Along with the numerous reports of new virus-caused animal tumors, there have been many discoveries, often seemingly unrelated, in research on virus and cell constituents, their modes of behavior, and other characteristics. Recently we have come to see that all these results are very likely pieces of the same large and intricate puzzle.

Avenues of Research

This realization has been greatly responsible for the acceleration and vitality of virus-cancer research today. At the same time, the present phase of research in this field presents a number of problems and obstacles that demand wise and careful attention. With this need in mind, 15 distinguished scientists met at the National Institutes of Health in September 1958 to explore new approaches in virology and other sciences that might lead to major advances in human cancer. The group made four proposals: (*a*) basic study of viruses and animals, using electron microscopy and available animal tumors as models; (*b*) greater emphasis on

training of biologists, zoologists, and chemists in the basic medical sciences related to virus-cancer research; (*c*) improvement of sources and distribution among laboratories of living host and viral materials; and (*d*) expanded financial support to include large-scale interdisciplinary explorations over long periods of time. These are excellent suggestions, and most of them have already been acted upon.

Additional conferences were held in November 1959 and in March 1960 to further explore the problems of research on viruses and human cancer. These meetings were attended by many of the Nation's leading virologists.

Establishing the role of viruses in human cancer might seem a simple matter of finding virus in malignant tissue and then demonstrating that it caused the disease. However, there is a fundamental difficulty here: at the present time we have no way to demonstrate the carcinogenic effect of viruses on humans. We must, therefore, develop laboratory techniques that will attack the problem indirectly.

A key tool in the development of such techniques will probably be tissue culture. The number of laboratories where human cells are being grown in tissue culture has greatly increased in recent years, thus facilitating the search for and study of viruses in human tissue. Research of this nature is making wider use of techniques such as treatment with X-ray or cortisone that permit human tissue to grow in experimental animals.

Other fundamental studies are equipping us with knowledge of the relationship between the host animal and the virus. Dr. Ray Bryan at the National Cancer Institute has conducted some revealing studies on the Rous sarcoma virus in chickens. He has shown that there is a quantitative relationship between the amount of virus inoculated and certain biological properties of the tumor such as size, length of time before the tumor develops, and length of time before it kills the animal. In other words, Bryan's work makes it possible to refute the old argument that a tumor cannot have been caused by a virus if the virus cannot be extracted.

One of the principal questions that must be answered is, how do viruses enter a cell and make it cancerous? For many scientists, studies on nucleic acids offer the most promise in this area. In cells the nucleic acid DNA is localized in the chromosomes, which carry the genetic information of cells and determine their form and function. Cells also contain another form of nucleic acid, RNA, most of which is in the cytoplasm.

Viruses are known to consist largely of nucleic acid, either RNA or DNA, and protein. But until fairly recently it was not known whether nucleic acid alone could be responsible for virus activity. Then, almost simultaneously, Dr. Heinz Fraenkel-Conrat at the Berkeley Virus Laboratory and Dr. A. Gierer and Dr. G. Schramm in Germany found that the RNA of the tobacco mosaic virus showed infectious activity.

This work pointed up the vital role of nucleic acid in virus activity, and of course, the possible role of nucleic acid in cancer.

In January 1960, scientists at Sloan-Kettering and the National Institutes of Health reported that DNA has been isolated from the polyoma virus discovered by Stewart and Eddy. Cancer was produced in laboratory animals by inoculating them with tissue culture fluids in which the isolated viral DNA was carried. This strongly indicates that DNA can enter a living cell and change the DNA of the cell to make it cancerous.

Such work has breathtaking implications, and it acts as a powerful stimulus to the scientific imagination. Studies on bacteria and bacterial viruses have shown that genetic material, and thereby hereditary traits, can be transferred by a virus from cell to cell, by a process known as transduction. This transfer might cause an abnormal, malignant change in the cell. Or, a viral nucleic acid might shed its protein coat and enter a cell, become incorporated into the genetic structure of the cell, and modify it so that the cell begins to reproduce abnormally. Bacteria studies also support the concept that latent viral nucleic acid in a cell might be activated by chemical or physical agents, and thus initiate malignant growth.

These possibilities and many others constitute a broad challenge to the scientific community as a whole. Intense, collaborative efforts in many disciplines, genetics, cellular biology, chemistry, immunology, to cite just a few, are needed. The National Cancer Institute is en-

couraging such activities through a greatly expanded program of grant support for virus-cancer research. Some of the investigators participating in this program are virus experts entering the cancer field for the first time. Emphasis is on the long-term support of the scientist himself, as opposed to support of a specific project, and support of some of our grantees in this field has been recommended for periods up to 10 years. I believe that these practices, which were recommended by our advisers, will help to insure the most productive work possible in virus-cancer research.

If viruses do cause cancer in man, and if these viruses are isolated, what then? How will we apply our knowledge to help save lives?

Prevention

Naturally, prevention is what we look to as an end result of all cancer research. There has been some success in developing vaccines against virus-caused cancer in animals. Stewart and Eddy have devised a procedure that immunizes hamsters against polyoma virus. Friend has developed a formalin-killed vaccine that protects mice challenged with live leukemia virus. And successful vaccines against visceral lymphomatosis in chickens have been developed by Dr. Ben Burmester of the Department of Agriculture's Poultry Research Laboratory in Michigan. On the other hand, attempts to detect antibodies against the Moloney virus have been unsuccessful, and have therefore hindered work on the development of a vaccine from this virus. This illustrates an important point: the isolation and identification of a cancer-producing virus may not lead to the speedy development of a vaccine.

Some day, it may be possible to produce a vaccine that will prevent cancer from developing in man. It might, of course, take years to determine its effectiveness, unless a vaccine for acute leukemia were developed. In that case, the effect of a vaccine given to babies would soon be obvious, since acute leukemia most often strikes young children.

If human cancer is a virus disease, another approach might be the use of drugs designed to destroy the virus either before it induced cancer or very early in the course of the disease. Laboratory studies of a virus that infects bacteria have shown that selective action by such drugs is possible. The virus studied induces the formation of a particular enzyme necessary for the reproduction of the virus within the cell. A powerful anticancer agent, 5-fluorouracil deoxyriboside, will seek out this enzyme, which is only in the bacteria infected by virus, combine with it, and thus block the reproduction process.

Some intriguing studies of cancer treatment in humans have shown that infection of cancer cells with certain viruses destroys some of the cells. The effect is temporary, since the patient soon develops antibodies against the virus. In further studies, attempts are being made to inhibit the host's production of antibodies against these viruses, to develop methods of reaching the cancer with sufficiently powerful doses before antibodies develop, and to produce tumor-destroying properties in other human viruses.

Virus-cancer research has come a long way in the past 50 years. And the efforts of dedicated scientists in countries all over the world assure us that our knowledge of this complex field will steadily increase. This is indeed an era in which we are continually having to reevaluate and readjust our concepts. It is difficult to imagine what new findings may be just beyond today's horizon. But I am sure it is no mere dream that research on viruses and cancer may eventually give us valuable new knowledge and skill that will help to prevent or arrest the development of many human cancers.

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