A New Approach to Cancer Research

by Renato Dulbecco

Since their discovery, half a century ago, viruses have been investigated with ever-increasing interest. They have yielded a wealth of information about the organization and the dynamics of life, yet they have not given away all their secrets. Viruses are to biology what atoms are to physics—the smallest units capable of autonomous and stable existence. Like atoms, viruses can be broken down further into elementary components, the study of which reveals the most intimate secrets of life.

Viruses can be obtained in pure state, and as such are kept in vials in refrigerators and freezers in laboratories all over the world. These pure viruses are made up of very tiny particles, which are as lifeless and inert as a pure substance on the shelf of the chemistry laboratory. However, when the virus particles come in contact with a living cell, an interaction takes place between the two, in which the virus becomes very much alive. In the living cell one of the elementary constituents of the virus, chemically known as nucleic acid, enters a frantic process of reproduction. In this process the nucleic acid of the virus undergoes the essential life processes that occur in all cells during their growth; thus the reproduction of a virus in the cell recapitulates the basic phenomena of life in their most simplified form.

Virus reproduction is an effective and fascinating model for an experimental study of life for several reasons—the relative simplicity of the processes, compared to those of the cells of the body; the possibility of causing a very large number of virus particles to develop their life cycle at the same time; and the possibility of using precise methods of investigation. For these reasons the study of viruses has attracted many scientists of different backgrounds, including chemists, physicists, geneticists, mathematicians, and physiologists. Each one of them looks at viruses from his own highly specialized angle, and from the various fragments of information that every specialist contributes comes an ever-increasing knowledge of life.

The penetration of a virus particle into a living cell, a process which we call infection, is never inconsequential. In many cases the virus multiplies at a terrific speed, increasing between a thousand to a millionfold in a short time, and eventually killing the cell. In other cases the cell is not killed but modified. This event is of great biological interest because it gives a tool for studying cellular modifications, which occur constantly in organisms and are the main factor in many normal and pathological processes.

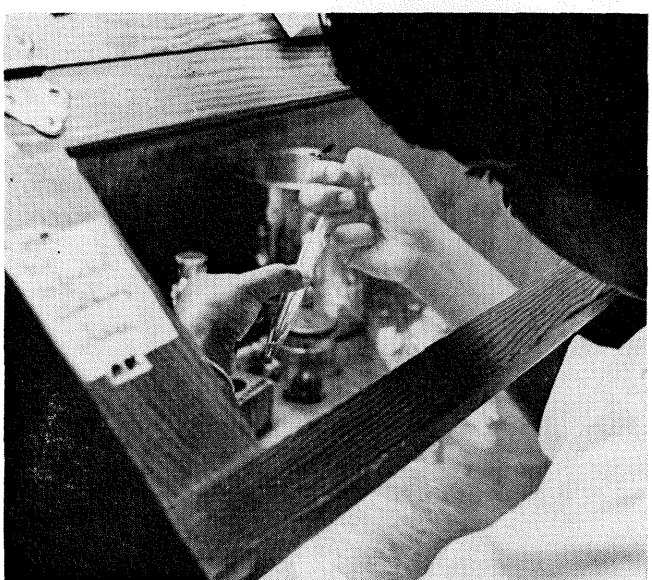
One possible modification of cells infected by virus is their transformation into cancer cells. We can visualize this process in the following way: A single particle of virus enters the body of an animal, interacts with one cell, and transforms it. The transformed cell now becomes released from the restraints that normally control the growth of the body cells and starts to grow and multiply relentlessly, giving rise to a large, ever-growing cell population, which is the cancer. Not all viruses do this, but only those of a special class, which are defined as tumor-producing or cancer-producing viruses.

At Caltech we have been interested for many years in the basic aspects of virus replication. In this framework, cancer-producing viruses have been studied for several years; however, only recently has an intensive effort been concentrated in this area. The interest in these viruses rose considerably two years ago, due to the discovery, at the National Institutes of Health, of a virus called polyoma, which is able to produce cancer in mice and hamsters. In examining the properties of this new virus we soon found that it was possible to develop a satisfactory system for following and measuring its multiplication. When this was found we set to work in an effort to learn how the virus causes cancerization. We had no practical question in mind when we started, but as the project developed, findings of practical significance were discovered — a not uncommon occurrence in science.

One main aspect of this work was that we did not take the traditional approach in cancer research and use animals; we used tissue cultures exclusively. These are cultures of cells derived from animals or embyros. The cells are grown in test tubes, bottles, or special plastic dishes, and they are maintained in properly outfitted incubators, where the cells find conditions very closely resembling those existing in the body.

This approach involves a specialized and delicate technology, but allows a study of the events following virus infection far more direct than would be possible with experimental animals. Furthermore, this approach extends the range of investigations beyond the usual limits of experimentation; for instance, it makes it possible to carry out experiments with human material.

This unconventional approach was made possible by one of the first results obtained in our work. We discovered that, by allowing the polyoma virus to interact with cells deriving from mouse or hamster embryos in plastic dishes, some cells became transformed into cancer cells, and gave rise to a growth which—in spite of the artificial conditions used—acquired all the characters of cancerous growth as we know it in the body. When cells from this growth were injected into an animal of the same strain as that



A research assistant transfers cultures of cells that have been made cancerous by interaction with polyoma virus.

from which the culture was obtained, the cells grew and gave rise to a conventional cancer; but cells of the same culture which had not been exposed to the virus never grew in the animal. This success provided an ideal experimental system which enabled us to carry out all the phases of our work with cell cultures in test tubes and plastic dishes.

Among the results of theoretical significance which were obtained by using this system we found that, although the virus interacts with most cells in a culture, it causes the transformation of only a small proportion of them—about one in a thousand. We also found that the transformation into a cancer cell, if it happens, occurs very shortly after the infection of the cell by the virus. These results indicate that a normal cell is transformed into a cancer cell by virus only when a very special type of virus-cell interaction occurs, and that this interaction causes the immediate and total transformation of the cell.

By using our system we were able to study the cancer cells very shortly after they were formed. The initial results of this study showed that the cancer cells always differ from the normal cells in certain characteristic properties of the cell surface. These surface changes were discovered by studying the spontaneous movement of the cells on a glass slide and by observing how strongly the cells adhere to the slide.

A most intriguing problem

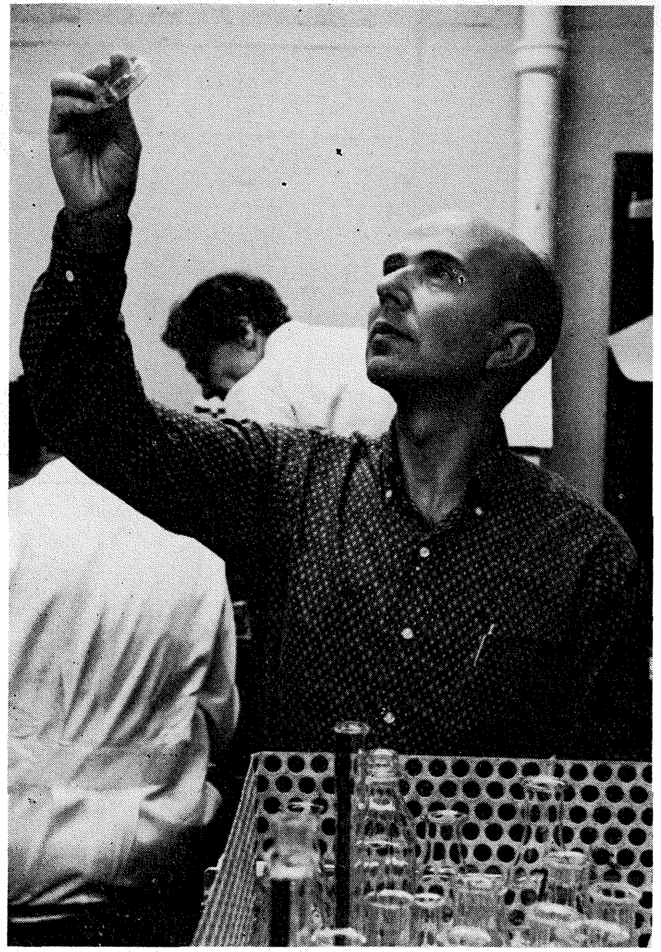
We do not yet know the chemical reasons for these changes, but we are in the process of investigating them. What meaning these surface changes may have for the cancerous transformation of cells is a most intriguing problem. Due to the great significance of the surface for the function of the cell, the surface changes may cause the abnormal behavior of the cancer cells in the body, and may therefore hold the secret of the main difference betwen a normal cell and a cancer cell. However, the surface changes may only be a consequence of some other key change. In any case, these changes are at the present moment of great interest to us as a marker of the action of the virus.

A very interesting finding emerged when we tried to find out what had happened to the particles of polyoma virus that had caused the cancerous transformation of the cells. We were expecting to find the virus growing in the cancer cells, but were surprised to discover that the cancer cells were free of the polyoma virus in its usual form. This showed that cells made cancerous by the virus continue to be cancerous even in the absence of the infectious form of the virus.

To try to understand the fate of the virus in the cancer cells, we made numerous attempts to find a number of the elementary components of the virus in the cells, but the results were consistently negative.

The fate of the virus in the cancer cells is still shrouded in mystery, and with it the basic mechanism of the transformation of the normal cells into cancer cells. The results obtained so far in the study of this problem have reduced to two the number of possibilities as to the fate of the virus. Either the virus-transformed cells contain a small fragment of the virus—for instance, a fragment of its nucleic acid—which would not be recognizable by the means now available, or they do not contain any trace of the virus. These alternate possibilities are being actively investigated, since solving these problems would greatly contribute to our understanding of what is cancer.

These findings have significant bearing on the possible role of viruses in human cancer. This is an important medical problem, because if human cancer is due to a small number of viruses, the prevention of the disease, if not its cure, might be feasible with our present means. Although this problem has been studied a great deal in the past, no clear answer has been given, and it is still unknown whether human cancer is caused by viruses. Our results suggest that two difficulties may be responsible for the inconclusiveness of the results, and they suggest new approaches to avoid these difficulties. One difficulty is the impossibility of making adequate experiments by following the conventional approach; the other is the previous lack of knowledge about certain properties of cancer-producing viruses.



Dr. Renato Dulbecco examines a culture of cells infected with polyoma virus.

Cancer experiments on man

Traditionally, to determine whether a cancer of an animal was caused by a virus, extracts of the cancer were tested by inoculation into animals of the same species, where the virus would elicit the formation of the cancer. In the case of human cancers, tests by inoculation into man are impossible. Extracts of human cancers have been inoculated into the usual experimental animals, such as mice and hamsters, and viruses have from time to time been isolated, but these viruses were characteristic of the experimental animal inoculated. There is every reason to believe that these were contaminant viruses present in a latent state in the animal itself, rather than viruses derived from the human cancer. It is most likely that, to reveal a human tumor-producing virus, experiments will have to be conducted by using man as the experimental animal. This seemingly insuperable difficulty may now be easily circumvented by using cultures of human cells. On the basis of the results obtained with polyoma virus, a test based on the production of cancerous growth in these cultures in plastic dishes would have the same significance as if the cancer had been produced in a human being.

The second difficulty derives from the unrecognized possibility that the virus causing the cancer may not be found in the cancer cells themselves. The traditional procedures of searching for a cancer virus in the cancer itself may therefore fail, even if experiments are carried out with human cells. The results obtained with polyoma virus suggest a different approach. The polyoma virus is found, as a seemingly harmless virus, in many healthy mice—most of which never develop cancer. When tested under proper conditions, however, the carcinogenic capacity of the virus can be easily revealed.

Considering the situation in man, we know that many viruses can be found in healthy individuals. Some of these viruses are not definitely associated with any disease. Among these a cancer virus may well be hidden; such a virus may cause cancer in a few of the individuals infected, and may disappear later from the cancer cells themselves. These considerations suggest that viruses commonly found in normal individuals should be studied for their possible carcinogenic activity in cultures of human cells. If any is found that can produce the cancerous transformation of the cells, its connection with known human cancers could be studied epidemiologically.

We hope that these ideas will contribute to the clarification of the relationship between virus and cancer, and that in this way research conducted to serve the interest of science will end up also serving the needs of man.