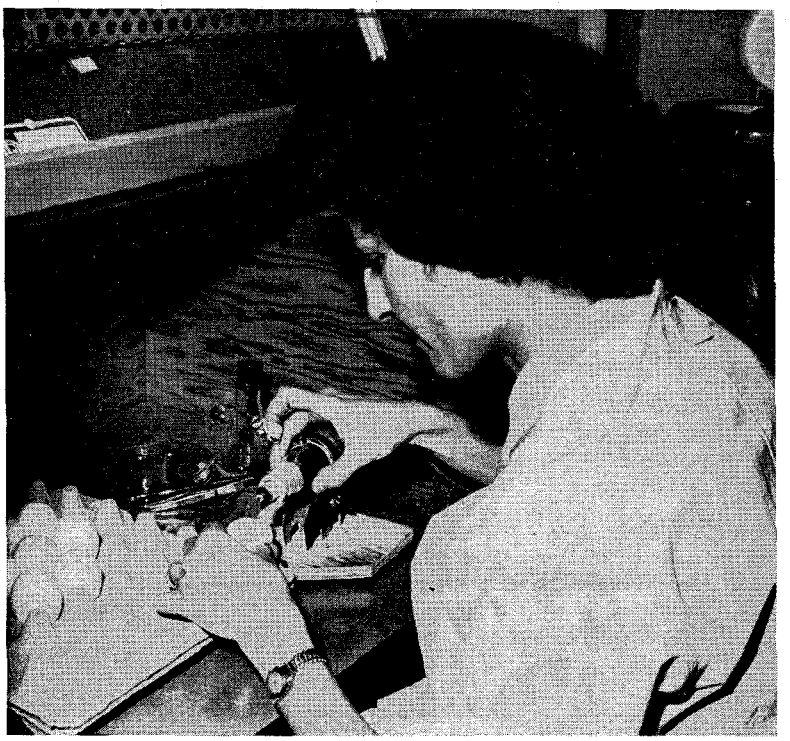
In Caltech's biology laboratories, workers infect fertile egg membranes with Rous sarcoma virus to induce tumors.



Cancer and Viruses

by Howard Temin

It is often said that we do not know the cause of cancer but this is not completely correct. We know many *causes* of cancer. What we do not know is how to *prevent* cancer.. To explain, we can first indicate some general causes of cancer in laboratory animals and then see how a better understanding of one of these may be of general importance.

The difficulty in identifying causes of cancer is especially well seen in the case of smoking and cancer. It seems that there is a strong correlation between the occurrence of one type of cancer – lung cancer – and smoking. "Correlation" means that when one event occurs a second is *likely* to occur. Some people have then concluded that this correlation alone is enough to indicate a causal relationship between smoking and cancer. A causal relation means that when A occurs, then *B* occurs. In this case, smoking causes the cancer. However, it is equally possible that some common factor *C* causes both *B* and *A*. Such a situation would give rise to the observation that whenever *A* occurs, *B* occurs – but here *A* would not be a cause of *B*; *C* would be a cause of both *A* and *B*. (It should be added that there is other evidence linking smoking and cancer.)

In working with animals, however, we are able to decide what are causal connections, as opposed to non-causal correlations. We can do this by setting up controlled experiments in which only one factor is changed. In the case of smoking and cancer in people, it may be that only a certain "group" of the population will smoke, and that people in this group are more likely to get cancer than other people. When working with mice or chickens or rabbits, we can either select the animals at random or we can see that they are as similar as possible. By use of these controlled experiments, we find that there are at least three types of causes of cancer: (1) chemical, (2) hormonal, and (3) viral.

Chemical cause of cancer

If we take two groups of mice that are identical genetically and are kept in identical pens and we paint the backs of one group every day with water and the other group with any one of a number of chemicals (for instance, methylcholanthrene), no tumors will develop on the backs of the mice in the first group, but tumors will develop on the backs of those

in the second group. In this case, we are able to say that the chemical is causing the cancer; this has been the only variable in the treatment of the two groups of animals.

Hormonal cause

Similarly, we can transplant the ovary of a mouse to its spleen, which means putting the ovary in a different part of the body with a different blood supply. In all cases, this ovary will then develop cancer. We think the cancer develops because of the disturbance of the hormonal relations of the ovary to the pituitary.

Viral cause

The third cause is the virus. If we inject a virus – for instance, the Rous sarcoma virus – into a chicken, the chicken will develop a cancer and usually die. If we take control chickens and inject water or killed viruses, they will not develop cancer. So, we can say in this case that the virus has caused the cancer.

These are laboratory experiments; in each one of these cases we can say that we know a cause of the cancer. However, these causes are not very relevant to naturally occurring cancer, because these particular circumstances do not exist, and still cancers *do* appear.

Knowing that we can cause cancer, we look to see if there can be some common factor. A cancer is a wild, unchecked growth of cells in an organism. This is merely a definition on a cellular level of what a cancer is.

Cancer cells were first normal cells, so we can say that a normal cell gave rise to a cancer cell. Further, we can say that when these cancer cells divide, they give rise to more cancer cells. We can say this because it is possible with transplantable laboratory cancers to take a single cell and start growth in another animal. We can then – speaking broadly – say that the change from a normal cell to a cancer cell is a genetic change; it is something which is inherited. We can then look at the various causes of cancer we have listed to see whether these could have some effect on the inherited part of a cell.

The concept of genetic change is one of the most important concepts in biology. There are several sites where genetic changes can occur. A cell consists of two parts, the cytoplasm and the nucleus. In the nucleus there are bodies called chromosomes. These chromosomes insure that the egg of a mouse gives rise to a mouse, and the egg of a frog to a frog. Genetic change would come about by a change in the number or size of chromosomes.

Chromosomes are divided into regions called "genes;" these genes can change without changing the appearance of the chromosome. This is another site for genetic change – a gene mutation. In addition, there may be other sites for genetic change in the cytoplasm, although less is known about these.

We can now look at the classes of causes for cancer and see how they might operate. Chemicals can cause mutations and they can derange cell division; thus they could alter the genetic sites in a cell in several ways. Or they could operate in what we call "selective" fashions; genetic changes could occur spontaneously (which means that they happen because of something we don't know about) and the chemicals could act as selective agents for the altered cells. Hormone unbalance could act in the same way. And, as far as we knew up to 10 years ago, viruses could only act in a selective fashion, too.

Viruses are small entities which grow in cells and kill them. The viruses we ordinarily think of are those which cause poliomyelitis or influenza. They enter a cell, reproduce in the cell, and produce many progeny viruses while killing the cell. Cancer is a disease in which there is too much growth of cells. A virus which kills cells would appear to have only a very indirect relationship to cancer.

However, our ideas about viruses have changed drastically in the last decade. This change was caused by work with viruses which infect bacteria. Recent work done here at Caltech has extended these ideas and indicated that cancer viruses are different from other animal viruses – that they do not kill cells, but by their presence cause a genetic change in the infected cells.

The Rous sarcoma virus

In 1910, at the Rockefeller Institute, a man named Peyton Rous isolated a virus from a chicken tumor – which means he took the tumor, ground it up, and passed it through a filter that held back cells. (This was in the early days of the work of discovering viruses. Only six viruses had been discovered before this one. The definition of a virus at this time was merely something transmissible that passes through a filter.) Rous took this filtrate and injected it into chickens and got new tumors, and from these new tumors, he could get new virus. For the last 50 years this Rous sarcoma virus, named after its discoverer, has been kept in laboratories.

In order to try to understand how this virus acts, we do not work with chickens, or even with eggs. They are too complicated. We work instead with cells isolated in glass dishes – what we used to call *tissue* culture and now call *cell* culture.

There are two major problems of cell culture. The first is keeping the cells happy and growing; the second is keeping bacteria and molds unhappy and not growing. Once cells are removed from an organism, the elaborate defensive mechanism of an organism is no longer available to them. We substitute a high concentration of antibiotics and work under sterile conditions so as to keep bacteria out. The cells

grow attached to the bottom of small glass dishes. The dishes are kept in incubators where the environment is carefully controlled; the cells are kept in a humid atmosphere, at a constant temperature and a constant pH. The cells are fed by a rich medium something like blood. They grow fairly well in such circumstances, but not quite as well as in a chicken. After three or four months in culture, they stop dividing. However, earlier than this, the cells appear to be fairly normal.

A chicken, or any organism, is a group of cells held together by a matrix. To get isolated cells it is necessary to dissolve the matrix. To do this we take pieces of chicken embryos, treat them with trypsin (one of the digestive enzymes) until the cells are separated from each other. The cells are then put in small dishes where we can study them. They form a sparse layer of fibroblastic cells.

We then add virus to these cells, allow the virus to enter the cells, and add an overlay of nutrient medium. At the end of a week, when we look at the culture again, we find areas or foci of altered cells. We know that these foci are caused by the virus, because, if no virus is added, no foci appear. Also, if the amount of virus is increased the number of foci increase proportionately. And, most important of all, the cells of the foci release lots of virus.

Cell changes

Under higher magnification, we find that the cells in the foci look different from the original cells. They are no longer fibroblastic, but are round and refractile. To see if these cells are alive we replate them in another dish. There they grow. If we plate some on cells killed by x-rays, they form small colonies which may descend from one cell and are then called clones.

So we have seen that a virus which causes cancer in chickens alters the appearance of cells in tissue culture, and that these altered cells grow and divide and release more virus which causes cancer in chickens, or alters more cells. At this point we can see that our original ideas about viruses must be modified. Not all viruses kill cells. The growth of some viruses is not incompatible with further division of the infected cells.

As our work has continued, we have discovered another fact about the Rous sarcoma virus. Not all of the foci produced by the virus are alike. Some, instead of being composed of round refractile cells, are composed of long fusiform cells. Virus from the long fusiform cells makes foci of long fusiform cells.

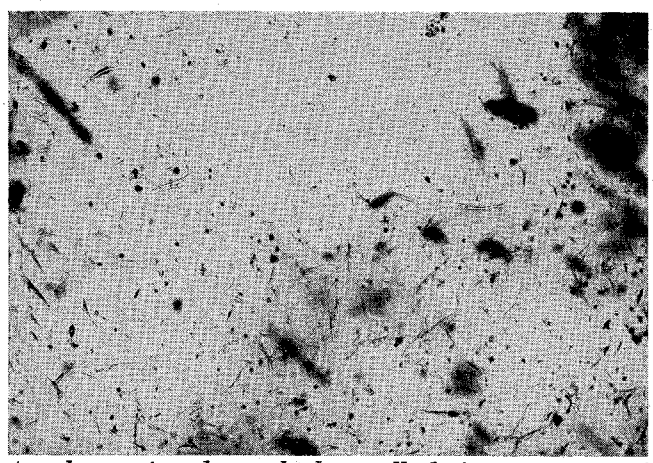
In order to evaluate this observation we must know more things. First, the virus producing foci of long cells is descended from the virus producing round cells – or, in other words, it is a mutant of the round virus.

Genes in viruses

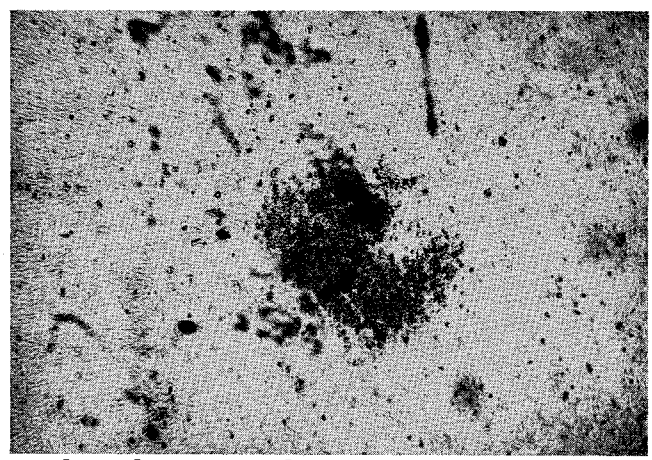
A short digression is perhaps in order. Biologists look upon viruses as organisms in the sense that they have life cycles with genetic continuity. Therefore, we speak of genes in viruses – though perhaps there are only a few – and then we can speak of changes or mutations in these genes.

Second, the virus controls whether an infected cell is round or fusiform. We establish this result by the following experiments. A clone of fibroblastic cells, which are presumed to be identical, can be infected with the two types of viruses and the two types of foci are produced. More directly, if we look at the progeny of an infected cell we find that about one in a thousand or so of these cells has changed spontaneously into another type of cell. This change seems to happen because the virus carried in that cell has mutated.

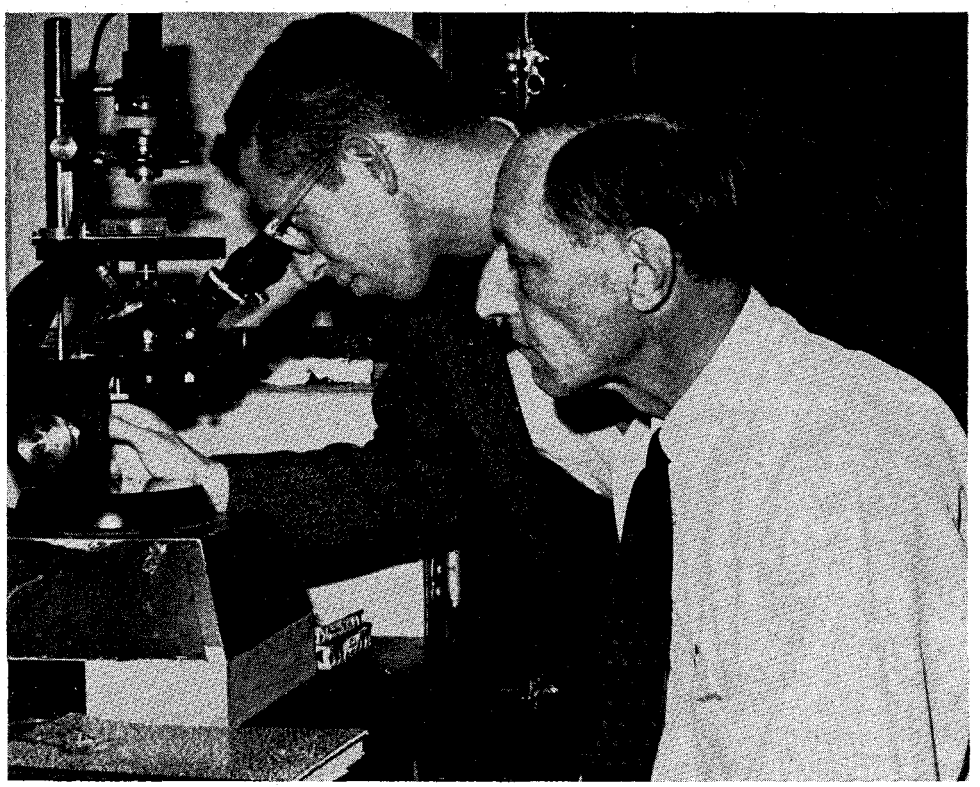
Genes do not operate in a vacuum. A gene will depend for its expression on what other genes are in the cell, the conditions under which the cell is kept, and so on. Another similarity of the Rous sarcoma virus to a cellular gene is that its expression



A culture of embryo chicken cells before injection of the Rous sarcoma virus.



Similar culture seven days after infection with virus. A single virus particle initiates the change.



Howard Temin examines infected cells from the iris of a chicken's eye, grown by Boris Ephrussi (seated), visiting professor of biology from the Sorbonne.

is affected by the genome or past history of the rest of the cell. For example, if we take a virus which causes foci of round cells on chicken cells, and place this virus on duck cells, we get foci composed of, not round, but fusiform cells. We can conclude that in a functional sense the virus becomes equivalent to part of the genome of the cell.

There is still one more thing we infer about the virus. When a virus mutates inside the cell, it changes the appearance of that cell and its descendants. Since this change is a rare event, a study of the cells in which the virus has changed enables us to say how many genetic copies of the virus there are in the cell. The answer comes out to be *less than two* on the average. Other experiments show that the inheritance of the virus in a cell is regular, indicating that the previous answer is not due to intracellular selection. The existence of such a small number of genetic units of the virus in the cell, and the regular inheritance of these units, shows that the virus, in some structural sense, as well as the functional sense discussed before, becomes a part of the genome of the cell. Probably it does not attach to a chromosome, and may not even be in the nucleus, but becomes part of the general apparatus of the cell which controls what a cell is.

In discussing the causes of cancer then, we can see that, from a functional point of view, there is little difference between chromosomal or gene mutation and infection by the Rous sarcoma virus. Both sets of events cause genetic changes in the cell. There is one difference however. A gene mutation requires a change in some pre-existing structure. The viral infection, as far as we know, introduces a new genetic structure. A gene mutation is a change in something that is inside a cell; a gene that controls formation of one enzyme mutates to something else – it doesn't *form* this enzyme. In the case of the viral infection, the change in the cell is an *addition* of something – not a change in something that is already there. What meaning this difference has is not yet clear. It may be that the virus by its presence affects some pre-existing structure.

Once we know that the virus acts to cause a genetic change in the infected cell, we can ask how this genetic change is related to the production of a tumor. Such studies are now going on in our laboratory.

This work I have been describing has been done with one cancer virus. There are other viruses which cause cancer – but we do not know whether they act in a similar fashion to the Rous sarcoma virus or not.

It could be said further that this work we are doing is all very well, but what all of us are really interested in is people – not chickens. I can only repeat the story of the English gentleman leaving the opera one night who passed a man under a lamppost, looking for something in the gutter. On being informed that the searcher had lost his watch, the first man got down to help, and looked and looked. Finally he asked the searcher if he had dropped his watch right here under the lamppost. "No," the man said, "I *really* dropped it around the corner – but I'm looking for it here because there's so much more light."

We are in the position of the man under the lamppost. What we are primarily interested in is not chicken cancer, but since we have so much light there, we look. Maybe we will find something better than a watch.