Viruses of Mice, Mosquitoes, and Men

A Primer of Virology

by James H. Strauss

A Caltech biologist reviews the structure, assembly, and replication of viruses – and the nature of research into ways to control them

All of us have been exposed to viruses at some time in the past, usually with unpleasant consequences. However, there is also a less personal view of viruses, the science of virology, which is the study of what viruses look like, how they assemble, and how they replicate. Virologists hope that an understanding of viruses will eventually lead at least to an amelioration and perhaps to eradication of viral disease.

First of all, viruses are very small, simple creatures that can infect either animals or plants or bacteria — and in some cases more than one of these groups of living things. They possess a single species of nucleic acid (either RNA or DNA but not both) containing the genes of the virus, the units of heredity that direct its replication. Wrapped around the nucleic acid is a protein shell that protects the virus and its genes as it moves through the universe looking for a person or a cell to infect. Having found a susceptible host, the protein shell carries out its second function, getting the nucleic acid inside a cell. Once inside, the nucleic acid goes about the business of a virus, that is, replication. The nucleic acid takes over the metabolic machinery of the host cell, an act that leads to the production of more virus particles rather than more cells.

Because a virus can only replicate within a living cell, the question has often been asked whether or not the virus itself is alive. The answer is semantic. If "alive" describes an organism that can replicate itself and has an independent evolutionary history, then clearly viruses are alive. If, in addition, "alive" implies metabolism and the production of energy, then viruses are not alive. A virus replicates but does not metabolize.

The very simplest (and smallest) viruses can code for only three genes, but with these three genes (and, of course, the entire host cell) they can manufacture replicas of themselves. The largest and most complex viruses contain as many as 200 genes, which is still very few compared to other living creatures. For example, a bacterium, a single microscopic cell, contains a few thousand genes. A mosquito has 100,000 genes, and a man carries several million. It is interesting to compare the size of a bacterium to that of a virus that might infect it. Bacteria, which are visible in the light microscope, are on the order of 2 or 3

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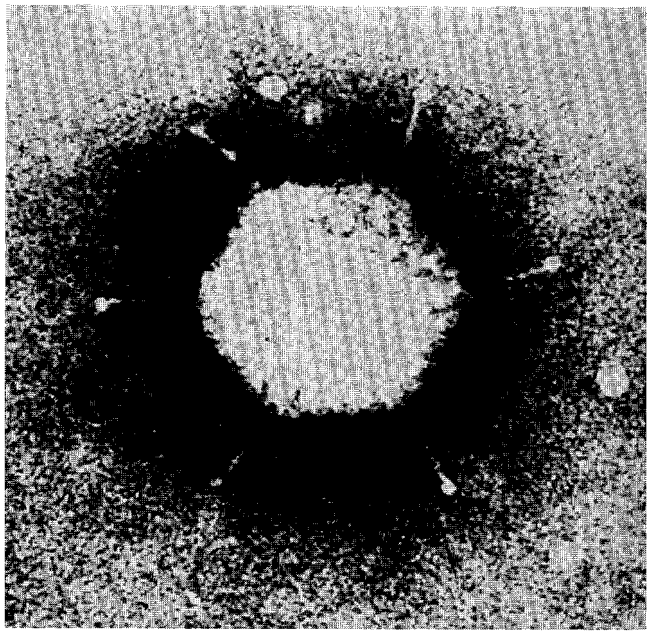
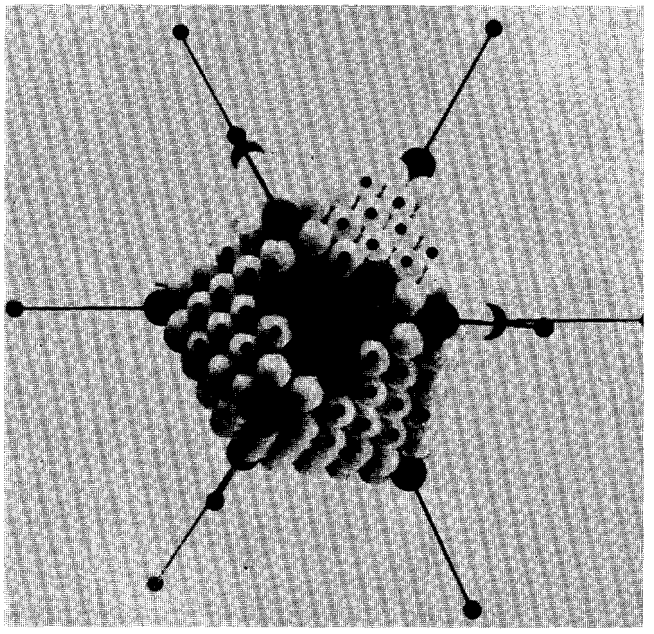
From Valentine and Pereira, *Journal of Molecular Biology* 13 (1965): 13. Courtesy Dr. R.C. Valentine.



An adenovirus, which causes respiratory disease in man, magnified 500,000 times in the electron microscope.



A model of the structure of an adenovirus, an icosahedron with fibers at the 12 vertices. Many animal viruses have icosahedral symmetry.

microns in length. There are 25,400 microns to the inch. Viruses are many times smaller, ranging between 20 and 250 nanometers in diameter. A nanometer is a thousand times smaller than a micron, making 25,400,000 nanometers to the inch. Anything this small is not visible in the light microscope, and it is only since the development of electron microscopy that virologists at last have a means of making viral structure visible.

WHAT DO VIRUSES LOOK LIKE?

Viruses are relatively simple in construction, because they have a limited number of protein molecules with which to form a protective shell around the nucleic acid. Many identical copies of only a few protein types are used, and their placement around the nucleic acid follows certain geometric principles. One popular model for virus construction is the icosahedron, a Euclidian solid that has 20 faces, each of which is an equilateral triangle. (A more familiar example of icosahedral construction is the geodesic dome.) Two common icosahedral viruses infecting humans are poliovirus (the infectious agent in infantile paralysis) and adenovirus (which causes a respiratory infection and was first isolated from human adenoids).

Another model for virus construction is the helix, in which the protein subunits are wound around one another like the steps of a spiral staircase. The helix is coiled very tightly, so that from the outside a helical virus looks like a long rod. One commercially important helical virus is tobacco mosaic virus (which infects plants of the nightshade family, especially tobacco).

A number of viruses go one step further and have a lipid-containing envelope, a membrane, wrapped around the protein-nucleic acid core, which in these cases can have either icosahedral or helical symmetry. These enveloped viruses include the herpesviruses, such as herpes simplex virus, which causes fever blisters; genital herpes virus, which causes similar eruptions on the genitalia and has been implicated in cervical carcinoma; and varicella virus, which is responsible for chicken pox. Influenza virus, mumps virus, rubella virus (German measles virus), and a host of other human viruses are also enveloped.

Another group of viruses has a curious bullet-shaped structure — round on one end and blunt on the other. These viruses have a modified helical core in which the helix is wound into a very tight coil like a ball of string, and is surrounded by a lipid envelope. Two such viruses are rabies virus and vesicular stomatitis virus, a virus of cattle and pigs that has been extensively studied in the laboratory.

In addition to the animal and plant viruses that have either helical or icosahedral symmetry, there are a number of bacterial viruses that have an even more elaborate struc-

ture in that they have a tail. Max Delbrück pioneered the study of these tailed bacteriophages, as they are called, here at Caltech 40 years ago. Bacteriophages have a head with cubic symmetry properties (usually roughly hexagonal in outline but not strictly icosahedral) and a tail with helical symmetry. At the end of the tail may be found a base plate with long fibers attached to it. They are much more complicated in their construction and have many more types of proteins than the other viruses I have discussed.

HOW DO VIRUSES REPLICATE?

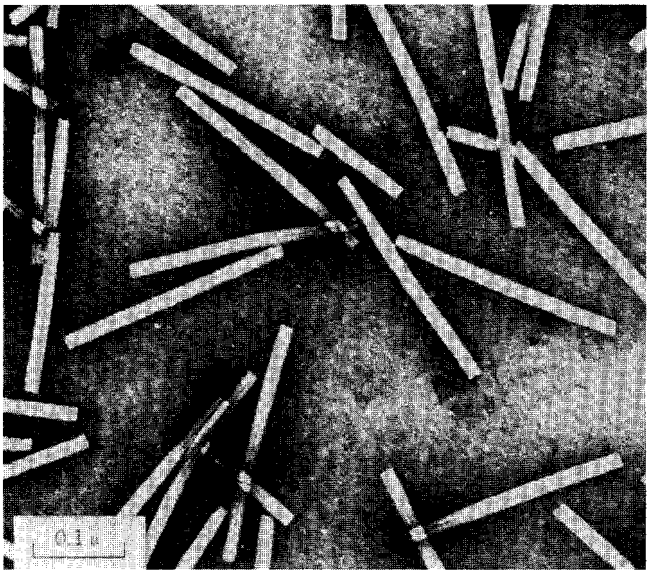
From the point of view of the virus, its sole function in life is to persuade a cell to make many more viruses just like itself. To do this, the first step is to ensure that the viral nucleic acid and its genetic information are introduced inside the host cell.

In the case of the tailed bacteriophages, the virus attaches to the bacterium through its tail, and a contraction of the tail forces the nucleic acid inside the cell. Animal viruses, however, do not have tails and have evolved other strategies to enter a cell. In one strategy the virus attaches to a specific receptor on the outside of a cell, and then the cell engulfs it and takes it inside. Cells normally take in a variety of substances, such as metabolites, and respond to molecules attached to their surfaces like chemical stimulants and hormones. The virus probably makes use of these mechanisms and tricks the host into internalizing it. Enveloped viruses, on the other hand, can enter a cell by fusing their lipid membrane with the lipid membrane of the cell, which puts the core into the cellular cytoplasm. In this case also the virus first attaches to a specific receptor on the cell surface before entry.

Once a virus's nucleic acid gets inside a cell, it can begin to replicate, and that's the crux of the whole issue — how does a virus make more of itself? In short, the virus introduces a few new genes inside the cell, and these new genes take over the host cell's metabolic machinery, causing the cell to reproduce the virus instead of reproducing itself. A cell that literally is performing millions of functions before infection stops doing all the things it normally does and instead produces hundreds of thousands of virus particles. The time required for virus replication depends upon the virus and the cell. For bacterial viruses, 30 minutes suffices. For animal viruses, hours and even days are required.

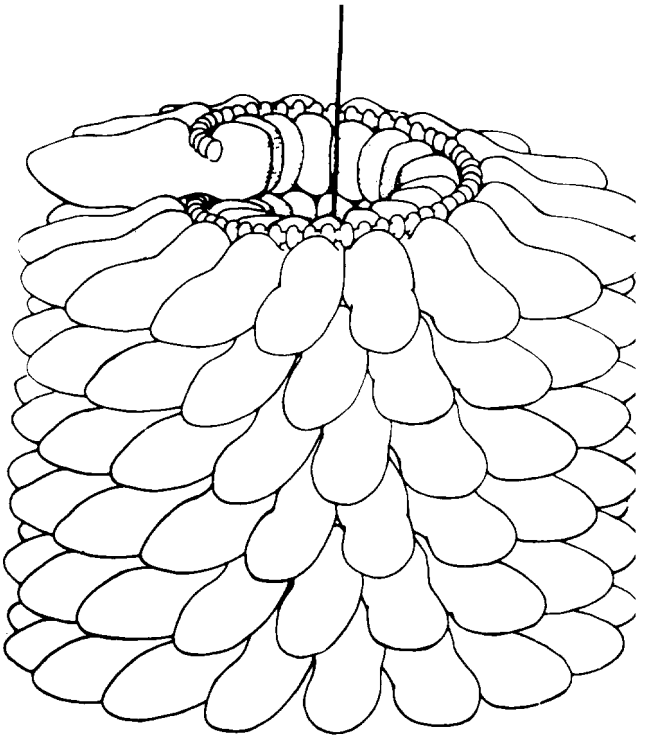
As an example of virus replication, consider Sindbis

From *General Virology*, by Luria, Darnell, Baltimore, and Campbell. Original courtesy of J. T. Finch.



In the electron microscope, tobacco mosaic virus particles appear as rigid rods with a hole down the middle. These viruses are magnified about 150,000 times.

From *Viral and Rickettsial Infections of Man*, Horsfall and Tamm, eds. J. B. Lippincott Company, 1965.



A model of the structure of tobacco mosaic virus shows an inner coil or helix of nucleic acid, encased in globular protein molecules that are arranged like the steps of a spiral staircase. This drawing shows about 1/20th of a full-length virus rod.

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virus, a virus that has been intensively studied in my laboratory for several years. Sindbis virus, named for the town of Sindbis, Egypt, infects a wide range of animals in nature, including mosquitoes, birds, and man. In the laboratory we study the replication of the virus in tissue culture cells derived from chickens, hamsters, mice, monkeys, or mosquitoes. After infection, the RNA of the virus goes to the ribosomes of the host cell, which are the factories for protein synthesis inside a cell. There the RNA is translated into protein. These new proteins are able to replicate the viral RNA, specifically using that viral RNA as a template to make new copies of it. In addition, a second virus messenger RNA is produced and translated into a second set of proteins — those that make up the protein shell of the virus particle. These proteins assemble with viral RNA to form progeny virus.

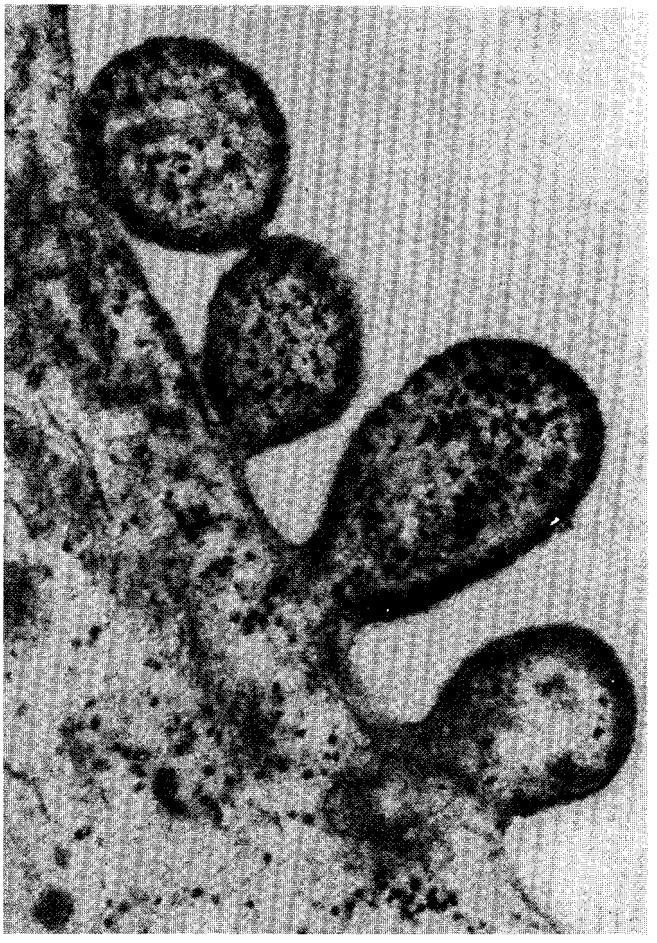
All viruses replicate by these methods. Messenger RNA is made and translated into proteins. These proteins are of two types: those that replicate the virus's nucleic acid and those that make up the structure of the virus particle. If you have a DNA-containing virus rather than an RNA-containing virus, you simply have one more step. The viral DNA must replicate, and it must be transcribed into messenger RNA.

HOW ARE VIRUSES RELEASED FROM THE INFECTED CELL?

Once a virus has infected a cell and replicated there, it must get outside so it can find another cell to infect and start the cycle all over again. There are two ways for this to happen. One is a process of lysis, or cell destruction, in which the cell simply falls apart and releases its contents. In the case of bacterial viruses, lysis is an active process. The viruses actually make something that causes the cell to disintegrate. In the case of animal viruses, usually the virus simply kills the cell by causing it to stop growing. As soon as the cell's normal maintenance functions are no longer performed, the cell dies, disintegrates, and spills its contents into the medium.

In addition to lysis, viruses can get out of the cell through the process of budding. Sindbis virus buds, and my laboratory has taken advantage of this to study the processes of membrane structure and assembly. When viruses bud out, they may kill the cell, but they don't have to. Instead, they may set up a persistent infection. Perhaps the classic example is rubella virus. This is the well-known German measles virus that causes developmental abnormalities in a fetus when a woman is infected with it within the first three months of pregnancy. It is thought that maybe the virus causes developmental abnormalities because it grows in the embryo without killing the cells that it infects. Perhaps because the cells are virus-infected, they may grow more slowly or not divide at the right time. Perhaps because the virus is budding, new surface proteins — antigens — are introduced into the surface of the cell and cause the embryo to develop improperly.

From *General Virology*, by Luria, Darnell, Baltimore, and Campbell. Micrograph courtesy of S. Rozenblatt and C. Moore.



Measles virus budding from the surface of an animal cell is magnified about 500,000 times. The helical nucleocapsid migrates to the surface of the cell and buds out as a completed enveloped virus.

THE EPIDEMIOLOGY OF VIRUS INFECTION, OR HOW DOES A VIRUS GET FROM HOST TO HOST?

Getting the virus outside the cell is not the end of the story, obviously, because in order to continue its lifestyle, the virus must find another living organism to infect. If we're talking about a human virus, it must get from one human to the next, and there are a number of mechanisms by which it can do so. One is the oral/fecal route. This

usually involves viruses that grow in the gut, which are spread by contamination of food or water supplies. Polio and hepatitis are examples of these kinds of viruses.

A second method of getting around makes use of blood-sucking arthropods — mosquitoes, ticks, and the like — as an intermediate. There are quite a number of viruses that are able to grow in humans or in other higher vertebrates and that are also able to grow in mosquitoes or ticks. Sindbis virus is such a virus. An infected mosquito transmits the virus when taking a blood meal. The virus then grows and circulates in the blood of the vertebrate and can be in turn transmitted to an uninfected mosquito. In nature these viruses thus alternate between vertebrate and invertebrate hosts. It is interesting that there are viruses of plants that have the same sort of life cycle. They can grow in both plants and in insects that feed on plants, such as leafhoppers and aphids.

A third method for getting around, which is used by respiratory viruses, is human contact. These are the viruses we know most about in personal terms. The simple act of breathing excretes cold viruses, influenza viruses, and other respiratory viruses because they grow in the upper respiratory tract. Since these viruses are likely to cause coughing and sneezing, the efficiency of their transfer from one host to another is often increased.

We usually think of respiratory viruses as being those that spread chiefly in wintertime. The very name "cold" implies that. It turns out that probably colds are more numerous in wintertime *not* because people are more susceptible to them then, and *not* because the weather is cold, but simply because human beings behave differently in the winter than they do in the summer. They stay indoors more, under more confining conditions, and so they are exposed to viruses more. Conversely, there are viruses, such as poliovirus, that are epidemic in the summer.

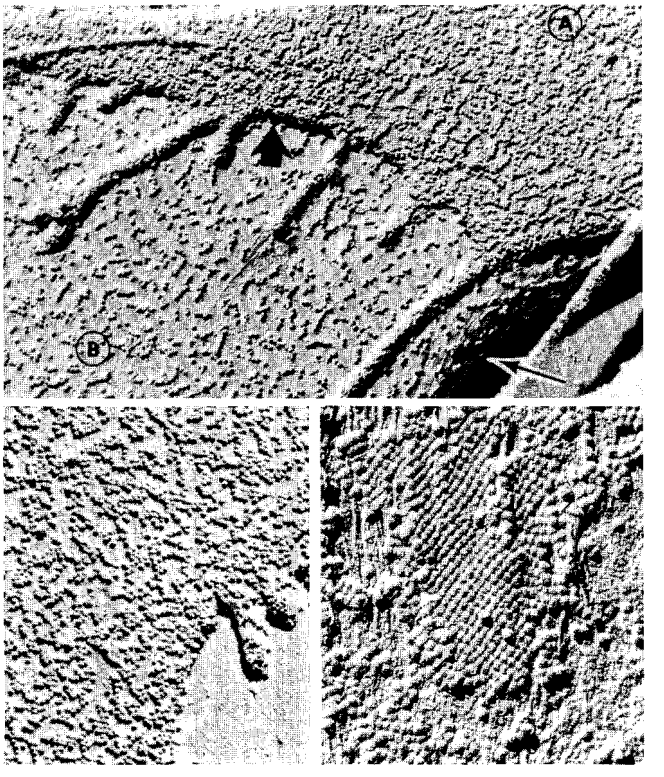
WHAT CAN WE DO TO CONTROL VIRUS INFECTIONS?

The first line of defense against virus infection is the body's immune system. When the body is infected by a foreign agent like a virus, it responds by producing antibodies against the virus in order to inactivate it. It's been known for centuries that infection with, say, smallpox — if a person survives the disease — confers permanent immunity. This principle has been used as a method for preventing viral diseases for 200 years, since Jenner reported that contraction of cowpox, which causes an innocuous infection in humans, confers immunity to smallpox. The recent campaign by the World Health Organization to vaccinate the entire susceptible population of the world has succeeded in eradicating smallpox as a disease agent in humans.

Many other viruses have now been controlled through immunization — polio, measles, mumps, and so forth — using either live virus or killed virus vaccines. Prior to 1964 there were on the order of 100,000 cases of measles in this country every year. In 1963 measles vaccine was licensed, and a mass eradication program was initiated in 1966. It has succeeded in virtually eliminating measles virus as a severe pathogen in the United States.

One virus that has resisted immunization up to now is that of influenza, because the virus changes very rapidly. The surface proteins of a virus, the antigens, are what the body's immune system recognizes, and these mutate when the virus replicates. As these proteins become less related to what they were through mutation, it becomes harder and harder for the immune system to recognize them and so to

From "Distribution of the Receptor Sites for Sindbis Virus on the Surface of Chicken and BHK Cells," by Charles R. Birdwell and James H. Strauss. *Journal of Virology* 14 (1974): 672-678.



Sindbis virus adsorbed at 4°C to the surface of chicken cells in culture. In the upper panel the solid arrow and the black-and-white arrow indicate the edges of cells A and B, respectively. Each cell has receptors for many thousands of particles, but not all cells have the same density of receptors. When adsorption is performed at this temperature, the virus tends to cluster on the surface (lower left), at times forming paracrystalline arrays (lower right).

successfully counteract the virus particle. Thus there are recurrent epidemics of influenza, and no vaccine can be introduced that is effective for more than a year. Last year's vaccine doesn't work against this year's virus.

Every so often, every ten years or so, there is a dramatic change in the antigenicity of these proteins that is thought to occur by means of recombination between a human influenza virus and an influenza virus of other animals. When this change takes place, no one is immune to the new virus, so it sweeps through the human population with the speed of jet travel, causing literally hundreds of millions of cases of influenza.

It is important to remember that because all viruses do mutate they will adapt to changing circumstances, so we can't become completely complacent about the fact that a number of virus diseases have been controlled by our having a vaccine against them. It's always possible that new virus strains will arise in nature and infect the human population. For instance, even though smallpox virus has been eliminated, there are lots of other pox viruses in nature — pox viruses of monkeys and other primates as well as many other animals. It's always possible that variants of monkey pox could arise, which could infect humans and spread. So it's necessary to continue our efforts to learn as much about the disease agents as we can.

A second approach to the control of virus infections is to try and find some chemical that suppresses virus growth. We are all aware of the tremendous success achieved in controlling bacterial infections with antibiotics. Pneumonia, for example, was once a great killer, but is now easily controlled. Antibiotics are compounds that are for the most part produced by molds as a form of chemical warfare against bacteria, which share the same habitat. But antibiotics make use of the fact that a bacterium is a free-living organism whose metabolism differs in many ways from that of a mold or from that of humans. The antibiotics are directed against that different aspect, so that you can in effect poison the bacterium without poisoning a human or a mold.

The concept of an antibiotic is very different for a virus, which replicates inside the cell as an integral component of it. The only promising compound for the suppression of virus growth that has been identified so far is a protein called interferon. Interferon is synthesized by animals in response to virus infection, and it does inhibit the growth and replication of many viruses. The fact that people don't make inteferon all the time but only in response to virus infection probably means that the compound is not completely atoxic. It represents an emergency response to a virus infection in order to try to control it until the body's immune system can take over. But if human interferon were available in quantity, its potential for the control of virus disease is enormous.

A number of clinical studies have shown that interferon has great potential also as a therapeutic agent in treating certain cancers. These studies indicate that interferon is useful in treating osteosarcoma, a form of bone cancer, and multiple myeloma, a cancer of the immune system.

The problem has been to get enough interferon to study. For one thing, only human interferon is effective in humans. For another, it is produced in exquisitely minute quantities, as is shown by the fact that the cost of interferon for the clinical trials referred to above was about $70,000 per patient. Getting enough of the material even to study its chemical properties has proved all but impossible up to now, but perhaps with the development of new microsequencing techniques in the laboratories of Leroy Hood and William Dreyer at Caltech it may be possible to learn more about the chemical structure using the small amounts of material that are available.

One promising approach to obtaining more human interferon for study makes use of the new techniques of recombinant DNA technology in the cloning of genes. A number of laboratories, including some at Caltech, are cloning human genes, which simply means taking a small part of a human chromosome and amplifying it enormously in bacteria. The purpose is to get enough material to study and thus try to learn what the structure of the human chromosome is. These studies may make possible the treatment of many human diseases, such as thalassemia (a form of anemia). In the case of interferon, it may be possible to clone the human interferon gene and use the bacteria as a factory to produce enough interferon for study and perhaps for therapeutic treatment.

VIRUSES AND CANCER

It is known that some human cancers are caused by viruses. For instance, a certain rare form of nasopharyngeal cancer is probably caused by a virus called Epstein-Barr virus, which also causes mononucleosis. It is probable that human cervical carcinoma is caused by a strain of herpes virus. It is also known from certain animal studies that leukemias are caused by viruses that are infectious agents. These viruses can spread the disease — through the cat population, for example. For this reason and for many others, a continuing study of viruses should lead to improved human health. It may also help to satisfy human intellectual curiosity, specifically about the lifestyle of these fascinating creatures called viruses, and more generally about the nature of living processes. □