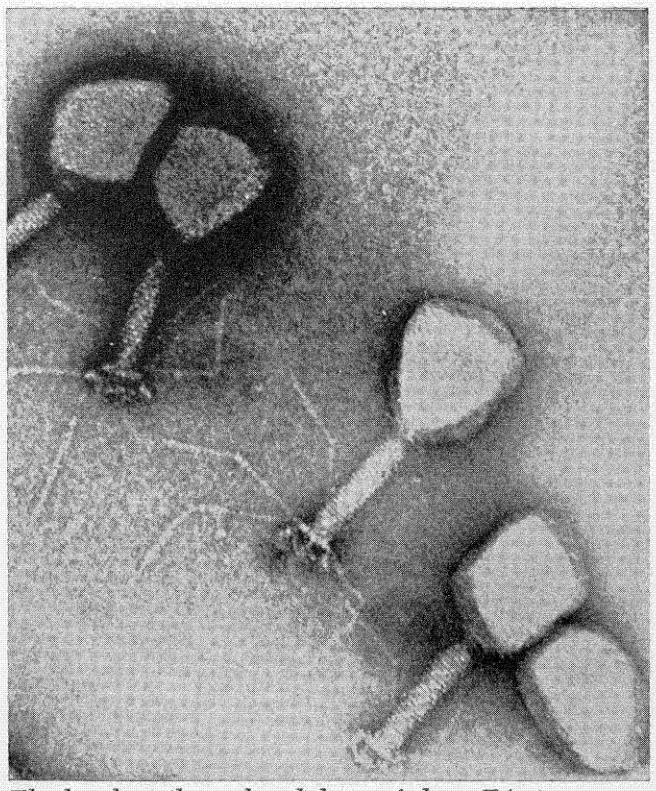
HOW TO BUILD A VIRUS

Caltech biologists take an exciting step forward in genetic research by constructing a virus in the laboratory.

Two Caltech biologists have figured out the orderly sequence of steps involved in the reproduction of a virus and have duplicated this reproduction in the laboratory. Robert Edgar, professor of biology, and William Wood, assistant professor of biology, have put together the virus known as T4, a larger and far more complex virus than the first one created in an artificial environment (tobacco mosaic virus) by biologists at Berkeley ten years ago. Drs. Edgar and Wood based their work on previous investigations by Edgar and collaborators at the University of Geneva.

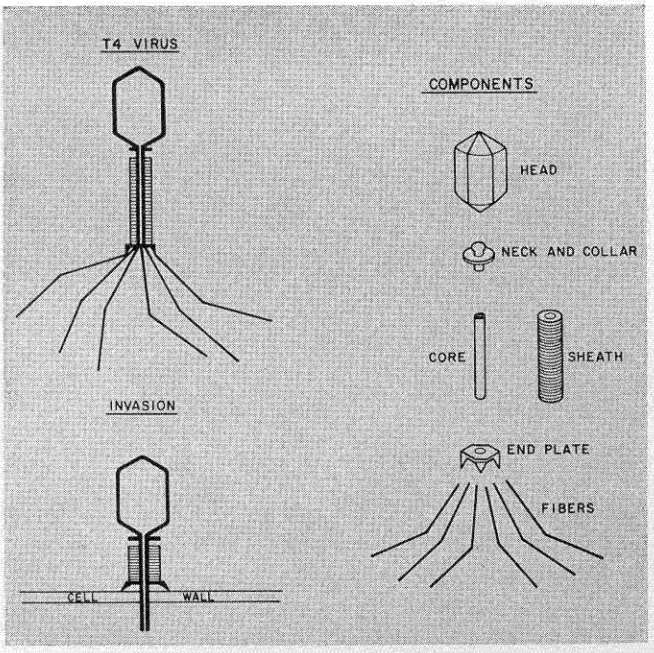


The heads, tails, and endplates of these T4 viruses can be clearly seen in this photograph taken with an electron microscope. The tail fibers are also visible. Magnification is about 200,000.

T4 consists of a head, which contains DNA (the blueprint for reproducing the virus), and a rather complicated tail. The body—head, collar, core, sheath, endplate, and fibers—is no more than a fancy syringe for protecting the DNA and getting it inside another cell. Once the DNA enters the other cell, the machinery of the host translates the plans encoded in the virus DNA into new, finished viruses. In the case of the T4 virus, the host cell is *E. coli*, a harmless, common bacterium found in human intestines.

Inside the *E. coli* the T4 destroys the DNA of its host, allowing its own DNA to take control of the cell. The host cell then starts producing about 100 different kinds of proteins needed to make more T4. After about 30 minutes the cell, filled with T4 viruses, bursts, liberating about 100 live virus particles.

To learn how T4 is assembled, Dr. Edgar and his collaborators at Geneva had to determine the roles played by individual viral proteins. They knew that one way to do this would be to prevent the T4 from making one particular protein, then let the T4 infect a host cell, and observe the consequences of the infection. Since the proteins are made by genes (which are segments of the DNA that encode information), a mutation in one of the 100 genes of T4 could block production of a protein. For example,



To reproduce itself, T4 uses its tail fibers to attach to an E. coli *bacterium, then contracts the sheath, which inserts the core through the wall of the host cell. DNA stored in the virus head is then injected into the* E. coli.

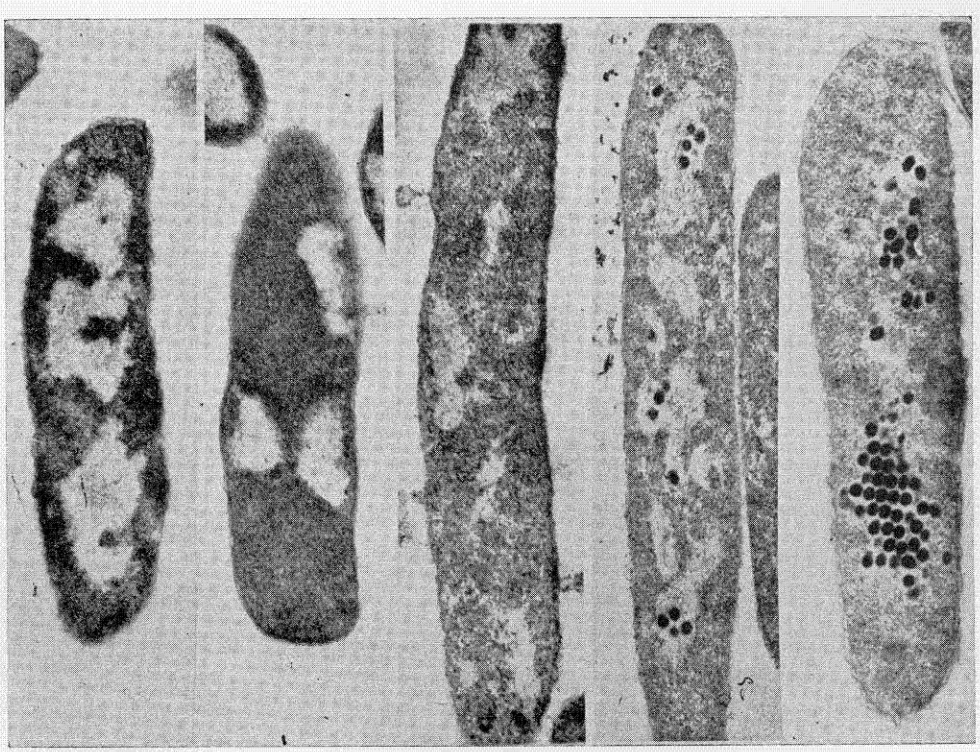
if the DNA had faulty instructions for making a particular protein of the virus head, one might expect that when the host cell burst, tails—but no heads—would be liberated by the host cell.

But this kind of typographic error in the DNA code would be the end of the line (or "lethal") for that virus, because it would be unable to reproduce itself further. Since it is necessary to culture a particular mutant strain through many generations, the scientists had to employ "conditional" lethal mutations—those that are lethal only under certain conditions, which can be controlled.

An example of a conditional (but not lethal) mutation is found in the Siamese cat, which has a gene that controls an enzyme that makes black pigment. But the enzyme that is made is sensitive to temperature, so the pigment is only made in the extremities of the cat's body, where the body temperature is slightly lower than in the rest of the body. Hence, the cat has black ears, tail, and paws. This is known as a temperature-sensitive mutation. Obviously, if that mutation had affected proteins that were necessary for reproduction rather than for pigmentation, then they would be conditional lethal. This is but one example of many kinds of conditional lethal mutations which exist.

Because natural mutations occur infrequently, the T4 virus was treated with chemicals to increase its mutation frequency, and a large number of conditional lethal mutations in T4 were found. These mutations occur randomly among the genes of the virus, and the mutations serve as "tags" for the different genes. The mutations can be sorted out by a variety of different tests and the relative locations of the different tagged genes determined.

To find out what a specific gene does in the process of making T4, Dr. Edgar allowed viruses that were defective in that gene to infect bacteria under conditions where the gene would not work. He then found that in one case no virus DNA was made after infection, suggesting that the defective gene was involved in making the DNA. In other cases he



The infection of E. coli *by T4: (a) Before infection—white areas are DNA of the* E. coli; *(b) After infection—DNA of the T4 destroys DNA of the* E. coli; *(c) DNA of the T4 replaces DNA of the* E. coli—*the infecting particle is still attached to the cell wall at the upper left; (d) Black spots are newly formed T4; (e) T4 continues to form—about 30 minutes after the original infection, the host cell will burst, liberating the new T4.*

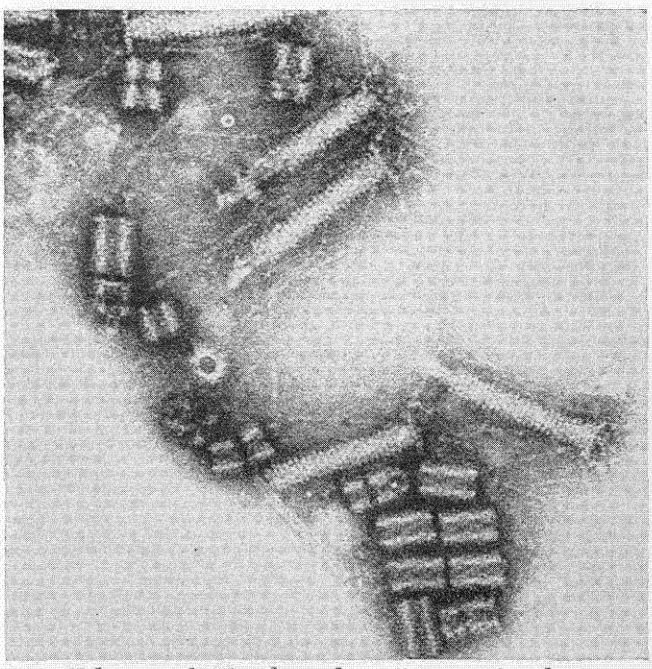
found that the bacterial cell burst open and liberated only heads, or only tails, or perhaps heads and tails not connected.

About 70 different genes in the virus have been identified so far; Dr. Edgar estimates that this is roughly half the total number of genes. This represents a considerable knowledge of the genetics of T4, considering that only about 100 of perhaps 100,000 human genes are known.

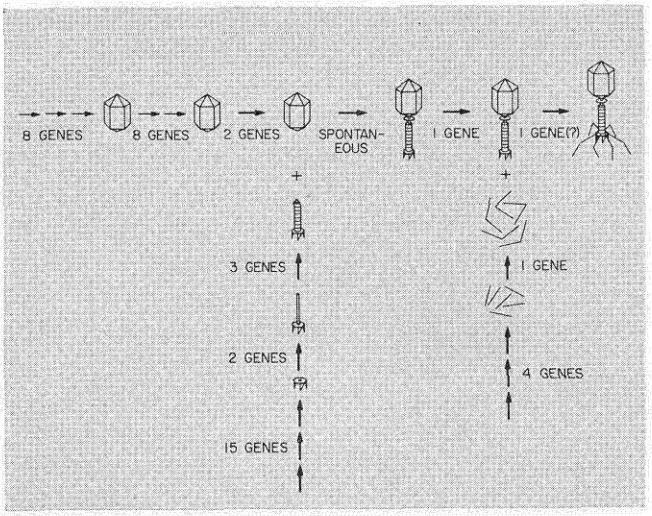
Eventually a genetic map evolved showing the specific or general functions and the relative positions of the identified genes on the DNA. It surprised Dr. Edgar to find so many genes (about 45) that seemed to be concerned with building the virus. Although complex, the virus appeared to be composed of fewer than 45 different proteins. Clearly, the construction of the virus was a more complex process than had previously been envisaged.

To learn more about how the virus used so many genes in its assembly, Drs. Edgar and Wood decided to use the various mutants as a source of virus parts and try to build a virus outside a host cell. They began by taking virus particles which, because of a specific mutation, lacked tail fibers, and mixed these defective particles with an extract from *E. coli* cells infected with another mutant chosen to produce the tail fibers but not virus particles. The result was active virus particles with tail fibers. The next step was to connect heads to tails and then attach fibers, and this too was successful.

From experiments of this type they built up a



Drs. Edgar and Wood used a mixture of tail components like this as a source of supply for building a virus. It contains sheaths with endplates, sections of sheaths showing the center holes for cores, a sheath on end (middle), a core on end (right), and tail fibers.



It takes at least 45 different genes to direct the assembly of a T4 virus, which is put together in an orderly, step-by-step process.

scheme for how the virus is put together. It suggests that the virus is assembled in a step-by-step manner, with each step under control of a different gene. There are at least eight genes involved in building the basic head, with eight more that make proteins for finishing the head. (These probably make the little neck and collar section.) Finally, there are two more that finish the head and make it possible for the head to attach to the tail.

Fifteen genes and, thus, 15 different proteins are used in making the complicated endplate. Two more genes make proteins needed for tacking on the core to the endplate. Once the core is on, three genes make proteins for the sheath, which assembles around the core, finishing the tail. At this stage the assembly will proceed spontaneously, with the heads and tails joining; none of the other steps has yet been found to go spontaneously.

The fibers are built independently of the head and the tail. Four genes are concerned in making half fibers, and a fifth joins the half fibers together. One more attaches the fibers to the tip of the tail.

In spite of the wealth of knowledge now in existence about T4, there is still a great deal of mystery surrounding the processes that Drs. Edgar and Wood have been studying. There are many genes whose functions are not known. Moreover, the manner in which the various genes work in assembling the virus—aside from the general knowledge of what steps in the process they affect—is unknown. Do the genes make different proteins which then join together in sequential steps, or do some genes make enzymes that help to join different parts of the virus? Understanding the chemistry of the assembly process constitutes a substantial task for the future.