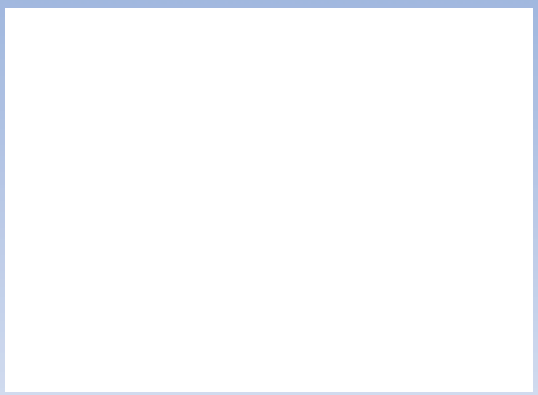
Part IX

# Viruses and Simple Organisms



**Electron micrograph of hepatitis C virus.**

## Discovering the Virus Responsible for Hepatitis C

You may not be aware that our country is in the midst of an epidemic of this potentially fatal liver disease. Almost 4 million Americans are infected with the hepatitis C virus, most of them without knowing it. Some 9000 people will die this year in the United States from liver cancer and chronic liver failure brought on by the virus, and the number is expected to triple in the next decade. In the first years of the new century, the number of annual U.S. deaths caused by hepatitis C is predicted to overtake deaths caused by AIDS.

Hepatitis is inflammation of the liver. Researchers in the 1940s identified two distinct forms. One, called infectious hepatitis or hepatitis A, is transmitted by contact with feces from infected individuals. A second form of hepatitis, called serum hepatitis or hepatitis B, is passed only through the blood. Hepatitis B virus was isolated in the mid-1960s, hepatitis A virus a decade later. This led in the 1970s to the development of tests for the two viruses. Disturbingly, a substantial proportion of hepatitis cases did not appear to be caused by either of these two viruses.

Clearly another virus was at work. At first, investigators thought it wouldn't be long before it was isolated. However, it was not until 1990 that researchers succeeded in isolating the virus responsible for these "non-A, non-B" cases, a virus that we now call hepatitis C virus (HCV).

HCV was difficult to isolate because it cannot be grown reliably in a laboratory culture of cells. Making the problem even more difficult, HCV is a strictly primate virus. It infects only humans and our close relatives—chimpanzees and tamarins. Because it is very expensive to maintain these animals in research laboratories, only small numbers of animals can be employed in any one study. Thus, the virus could not be isolated by the traditional means of purification from extracts of infected cells. What finally succeeded, after 15 years of failed attempts at isolation, was molecular technology. HCV was the first virus isolated entirely by cloning the infectious nucleic acid.

The successful experiment was carried out by Michael Houghton and fellow researchers at Chiron, a California biotechnology company. What they did was shotgun clone the DNA of infected cells, and then screen for HCV.

The genetic material of HCV, like that of many other viruses, is RNA. So the first step was to convert HCV RNA to DNA, so that it could be cloned. There was no need to attempt to achieve entire faithful copies, a touchy and difficult task, because they did not wish to replicate HCV, only identify it. So the researchers took the far easier route of copying the virus RNA as a series of segments, each carrying some part of the virus genome.

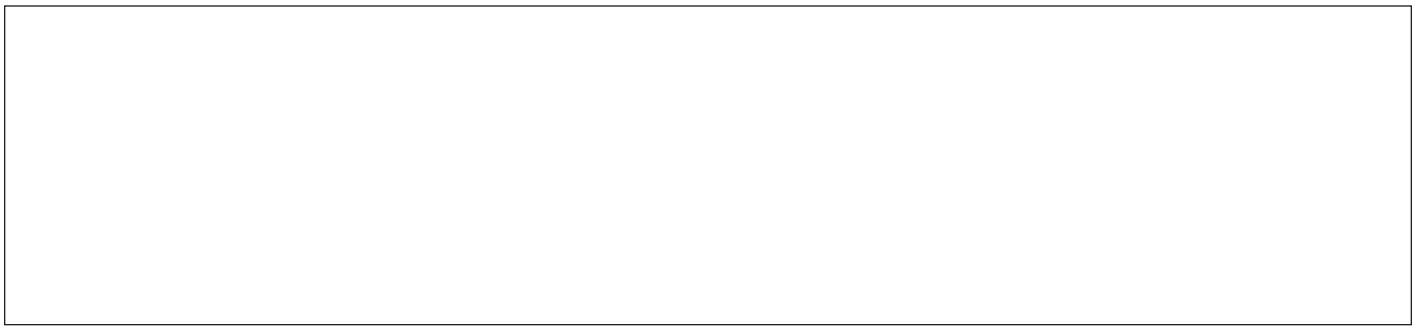
Next, they inserted these DNA copies of HCV genes into a bacteriophage, and allowed the bacteriophage to infect *Escherichia coli* bacteria. In such a "shotgun" experiment, millions of bacterial cells are infected with bacteriophages. The researchers grew individual infected cells to form discrete colonies on plates of solid culture media. The colonies together constituted a "clone library." The problem then is to screen the library for colonies that had successfully received HCV.

To understand how they did this, focus on the quarry, a cell infected with an HCV gene. Once inside a bacterial cell, an HCV gene fragment becomes just so much more DNA, not particularly different from all the rest. The cellular machinery of the bacteria reads it just like bacterial genes, manufacturing the virus protein that the inserted HCV gene encodes. The secret is to look for cells with HCV proteins.

How to identify an HCV protein from among a background of thousands of bacterial proteins? Houghton and his colleagues tested each colony for its ability to cause a visible immune reaction with serum isolated from HCV-infected chimpanzees.

The test is a very simple and powerful one, because its success does not depend on knowing the identity of the genes you seek. The serum of HCV-infected animals contains antibodies directed against a broad range of HCV proteins encountered while combating the animal's HCV infection. The serum can thus be used as a probe for the presence of HCV proteins in other cells.

Out of a million bacterial clones tested, just one was found that reacted with the chimp HCV serum, but not with serum from the same chimp before infection.



**How the hepatitis C virus was discovered.** Michael Houghton and fellow researchers identified the virus responsible for hepatitis C by making DNA copies of RNA from the cells of infected chimpanzees. They then cloned this DNA, using bacteriophages to carry it into bacterial cells. Colonies of the bacteria were then tested with serum from infected chimps. Any colony that produced an immune reaction would have to contain the virus.

Using this clone as a toehold, the researchers were able to go back and fish out the rest of the virus genome from infected cells. From the virus genome, it was a straightforward matter to develop a diagnostic antibody test for the presence of the HCV virus.

Using the diagnostic test, researchers found hepatitis C to be far more common than had been supposed. This is a problem of major proportions, because hepatitis C virus is unlike hepatitis A or B in a very important respect: it causes chronic disease. Most viruses cause a brief, intense infection and then are done. Hepatitis A, for example, typically lasts a few weeks. Ninety percent of people with hepatitis C have it for years, many of them for decades.

All during these long years of infection, damage is being done to the liver. Cells of the immune system called cytotoxic T cells recognize hepatitis C virus proteins on the surface of liver cells, and kill the infected cells. Over the years, many dead liver cells accumulate, and in response the cells around them begin to secrete collagen and other proteins to cover the mess. This eventually produces protein fibers interlacing the liver, fibers which disrupt the flow of materials through the liver's many internal passages. Imagine dropping bricks and rubble on a highway—it gets more and more difficult for traffic to move as the rubble accumulates.

If this fibrosis progresses far enough, it results in complete blockage, cirrhosis, a serious condition which may induce fatal liver failure, and which often induces primary liver cancer. About 20% of patients develop cirrhosis within 20 years of infection.

Luckily, hepatitis C is a very difficult virus to transmit. Direct blood contact is the only known path of direct transmission. Sexual transmission does not seem likely, although the possibility is still being investigated. Married partners of infected individuals rarely get the virus, and its incidence among promiscuous gay men is no higher than among the population at large.

Why not move vigorously to produce a vaccine directed against hepatitis C? This turns out to be particularly difficult for this virus, because antibodies directed against it appear to be largely ineffective. Those few individuals who do succeed in clearing the virus from their bodies gain no immunity to subsequent infection. They produce antibodies directed against the virus, but the antibodies don't protect them. It appears that hepatitis C virus evades our antibody defenses by high mutation rates, just as the AIDS virus does. By the time antibodies are being produced against one version of the virus, some of the viruses have already mutated to a different form that the antibody does not recognize. Like chasing a burglar who is constantly changing his disguise, the antibodies never learn to recognize the newest version of the virus.

To date, attempts to develop a drug to combat hepatitis C virus focus on the virus itself. This virus carries just one gene, a very big one. When it infects liver cells, this gene is translated into a single immense "polyprotein." Enzymes then cut the polyprotein into 10 functional pieces. Each piece plays a key role in building new viruses in infected liver cells. Some of these proteins form parts of the virus body, others are enzymes needed to replicate the virus gene. As you might expect, each of these 10 proteins is being investigated as a potential target for a drug to fight the virus, although no success is reported as yet.

Other attempts to fight hepatitis C focus on the part of our immune system that attacks infected liver cells. Unlike the ineffective antibody defense, our bodies' cytotoxic T cells clearly are able to detect and attack cells carrying hepatitis C proteins. A vaccine that stimulates these cytotoxic T cells might eliminate all infected cells at the start of an infection, stopping the disease in its tracks before it got started. A serious effort is being made to develop such a vaccine.

It doesn't look like an effective remedy is going to be available anytime soon. In the meantime, as the death rates from hepatitis C exceed those for AIDS in the next few years, we can hope research will further intensify.