Where Is the Hepatitis C Virus?

Non-A, non-B hepatitis is apparently caused by one or more viruses, but getting a handle on them has proved exceptionally difficult

In the words of the late John F. Kennedy, life is unfair. No sooner do you get one problem solved than another one, often worse, rises up to take its place. Consider hepatitis. By the mid-1970's, investigators had developed conclusive tests to identify blood serum containing either hepatitis A or hepatitis B viruses, and many investigators hoped that post-transfusion hepatitis would become a thing of the past. But it was not to be. Between 7 and 10 percent of the 3 million Americans who receive transfusions each year still develop hepatitis.

As many as 90 percent of these new cases of hepatitis are not related to the hepatitis A or B viruses, or to the four or five other viruses that occasionally produce liver disease. The new form was thus identified as what it is not, and has become known as non-A, non-B (NANB) hepatitis. The acute phase of NANB hepatitis is less severe than that of hepatitis B (*Science*, 14 November, p. 760), and the disease is rarely fatal. More than a third of the individuals who contract NANB hepatitis, however, develop a chronic form of the disease in which most may remain infectious indefinitely. This chronic state may lead to cirrhosis of the liver and, some scientists speculate, eventually to liver cancer. In contrast, only about 5 percent of individuals in this country who contract hepatitis B develop evidence of chronic liver disease.

The first important breakthough in the search for a cause of NANB hepatitis occurred in 1978 when two groups of investigators independently produced the disease in chimpanzees by injecting them with blood serum or plasma from patients with hepatitis and patients whose blood had previously been shown to transmit the disease to other humans. One group was headed by Harvey J. Alter of the Clinical Center at the National Institutes of Health and Robert H. Purcell of the National Institute of Allergy and Infectious Diseases; the other was headed by Edward Tabor and Robert J. Gerety of the U.S. Food and Drug Administration's Bureau of Biologics. These studies showed that there is, indeed, an infectious agent for the disease and that a chronic carrier state exists in humans; they also established an animal model. Tabor and Gerety demonstrated that the agent was like conventional viruses when they treated one of their donor samples with Formalin—a chemical that inactivates viruses—and found that it no longer produced the disease.

Similar results with chimpanzees were subsequently reported by teams headed by Daniel W. Bradley of the Center for Disease Control (CDC) in Phoenix, Arie J. Zuckerman of the London School of Hygiene and Tropical Medicine, Blaine Hollinger of the Baylor College of Medicine, and Makoto Mayumi of the Tokyo Metropolitan Institute of Medical Science.

At least ten different laboratories have reported obtaining electron micrographs of virus-like particles in liver tissue or serum from infected chimpanzees and humans; these particles have ranged in size from 20 to 80 nanometers. "Unfortunately," says Tabor, "it is very easy to find things that look like viruses in serum or liver tissue." Perhaps the most convincing results, some investigators concede, have been obtained by the CDC group. Bradley and his colleagues have observed particles ranging in size from 25 to 33 nanometers in liver sections from chimpanzees given a pooled plasma fraction and in those infected by blood from the former group. They have also observed crystalline arrays of virus-like particles, a phenomenon that is normally associated with a viral disease. Recently, Mayumi's group has obtained electron micrographs of particles that appear to be similar in size and appearance to Bradley's.

Despite these initial successes, many investigators now profess discouragement. Says Alfred Prince of the New York Blood Center, who has obtained electron micrographs of a virus-like particle isolated from his own blood: "It's easy to see such particles, but we can't be sure they are associated with the disease if they don't have an immunological marker." The chief thrust of most investigators recently has thus been to find an immunological or biochemical handle for the virus.

At least four different laboratories have so far developed tests in which fluorescent antibodies are used to detect putative antigens associated with the NANB virus. Another eight laboratories have developed tests with either agar gel diffusion or counterelectrophoresis techniques to detect presumed antigen-antibody systems resulting from the viral infection.

The results of the tests are very controversial. Many investigators argue that their own test is specific for the virus and that the other tests are nonspecific, detecting autoantibodies or other liver components. The difficulties are compounded because the presumed NANB virus apparently produces much less antigen than does the hepatitis B virus, even when the disease is in the acute stage; furthermore, both of these types of assays are estimated to be a hundredfold less sensitive than the radioimmunoassays used for detecting hepatitis A and B antigens.

One attempt to resolve some of the controversy occurred earlier this year when a panel of coded serums (blood samples from chronic NANB hepatitis carriers, from hepatitis A and B patients, and from patients with no liver disease) was distributed to the laboratories that have developed tests to see whether they could pick out the NANB samples. Results presented this past July showed that there was a wide discrepancy among laboratories and that the tests did not appear to be specific, but the presence of only one negative control (blood from a healthy donor) clouded the results. A new panel of serums is now being put together by Alter and his colleagues and will be distributed in about 2 months. The results should be available in March.

If one or more of the tests should prove to be specific, a valuable research tool would become available; nonetheless, the tests would have limited value for screening blood donors. The fluorescent tests are too expensive and too complicated, and both types of tests are too insensitive to be of great value. They could, however, provide a first step toward the development of a radioimmunoassay.

Despite the evidence of viral involvement in NANB hepatitis, scientists have been reluctant to label the disease hepatitis C, primarily because more than one virus may be implicated. Several lines of evidence suggest this possibility,

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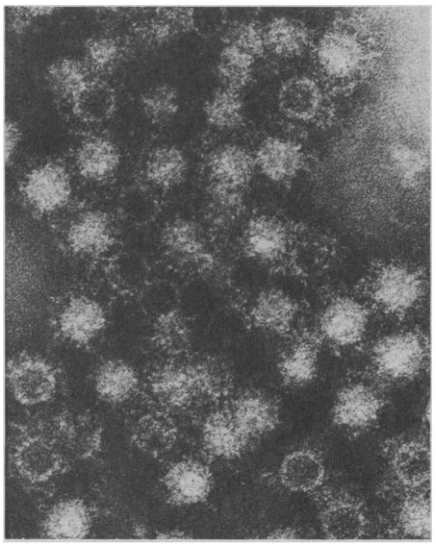
although some evidence is firmer than others. NANB hepatitis has, for example, been found to have widely varying incubation periods, ranging from 2 to 26 weeks. In general, though, the incubation periods fall into two ranges, very short (2 to 4 weeks) and longer (8 to 12 weeks). This suggests that two viruses may be involved.

Similarly, the pattern of elevation of liver enzymes during the acute phase of the disease is variable. In some cases, it is monophasic, showing a sharp increase several weeks after exposure followed by a gradual decrease. In other cases, it is biphasic: a modest increase in enzyme concentrations is sustained for a short period followed by a sharp increase. This also suggests the involvement of two viruses.

Last summer, Purcell and Alter reported that they had apparently induced two different types of NANB hepatitis (termed strains F and H) in chimpanzees, as determined by electron micrographs. In the animals given strain F, the two investigators consistently observed "peculiar tubular structures" in the cytoplasm of liver cells. In animals given strain H, they observed aggregates of virus-like particles 20 to 27 nanometers in diameter. Each type of structure was seen only in animals infected with a particular inoculum, suggesting that two viruses are involved. Some other investigators have subsequently observed the tubular particles in their own chimpanzees. Others, though, argue that the two structures are simply different manifestations of infection by one virus.

Other evidence favoring two viruses is perhaps more persuasive. James W. Mosley and Allan G. Redeker of the University of Southern California School of Medicine have closely examined a number of subjects who had multiple bouts of hepatitis; several of the patients had two distinct episodes of NANB hepatitis in addition to hepatitis A, hepatitis B, or both. Since recovery from a viral infection generally precludes a subsequent infection by the same virus, these results indicate either that the NANB virus is very unusual or that there are at least two strains of the virus. Using inoculums from different sources, Zuckerman, Tabor and Gerety, and Hollinger have succeeded in inducing two separate bouts of NANB hepatitis in chimpanzees. The results could have alternative explanations, but the most likely one is that two viruses are involved. Most investigators have not been able to achieve this result, however, so one virus may be more common than the other.

Perhaps the best evidence is provided by the cross-challenge experiments conducted by Bradley. His group inoculated eight chimpanzees with one of three agents: (i) blood factor VIII, a pooled clotting agent used in his laboratory to induce NANB hepatitis in chimpanzees; (ii) factor IX, another clotting agent used by Zuckerman; and (iii) Purcell and Alter's strain H. After the animals had recovered from the disease, Bradley then challenged them with an infectious dose of different origin. He found, for example, that animals previously infected with factor VIII material developed a second case of hepatitis when exposed to factor IX material, but not when exposed to strain H material.



Is this hepatitis C?

Electron micrograph of a crystalline array of virus-like particles from the blood of a chimpanzee with NANB hepatitis. [Source: Daniel W. Bradley]

Similarly, a chimpanzee previously exposed to factor IX material developed a second bout of hepatitis when exposed to factor VIII material. These and other findings suggest that the factor VIII and strain H materials share a common etiological agent, but that factor VIII and factor IX may contain two distinct agents. They also observed crystalline arrays of virus-like particles in cells from some of the animals injected with either factor VIII materials or strain H plasma, and tubular structures in cells from all the animals except one.

Whether NANB hepatitis is caused by one virus, two, or more, however, the primary concern of investigators now is to find some way to screen blood donors to identify carriers of the disease. In the absence of a specific test for NANB hepatitis, there is growing sentiment that nonspecific tests could be used. The proposed use of such tests, however, is also controversial.

Some members of the Transfusion Transmitted Virus Study Group—a multihospital cooperative association of hepatitis investigators—argue, for example, that assays of liver enzymes might be useful. The concentrations of some of these enzymes, such as the readily measured alanine aminotransferase, are frequently elevated in the blood of patients with active hepatitis. It would thus theoretically be possible to use enzyme concentrations to screen out hepatitis carriers from among potential donors. Recent results by Tabor and Gerety from a retrospective study, however, show that only about 20 percent of blood donors who transmitted NANB hepatitis have elevated enzyme concentrations. Other investigators, furthermore, have found that the enzyme levels can be increased by extraneous factors, such as heavy drinking. But an even more recent prospective study by the Transfusion Transmitted Virus Study Group has shown that use of an enzyme screen might prevent between 40 and 50 percent of the potential cases of transfusion hepatitis, while eliminating only 2 to 5 percent of healthy donors.

Another possibility has been offered by Gary L. Gitnick of the University of California at Los Angeles. Gitnick and his colleagues have found that the concentration of CEA [carcinoembryonic antigen, a glycoprotein that was once thought to be a specific biochemical marker for colon tumors (*Science*, 5 August 1977, p. 544)] is elevated in the blood of at least 60 percent of chronic carriers of NANB hepatitis. Unfortunately, CEA is also elevated in individuals who smoke tobacco or who have one of several other conditions. Its use as a screening agent for NANB hepatitis is thus subject to the same problems as the use of enzymes. A third possibility, also suggested by Gitnick, is that the concentration of bile acids may be raised in patients with NANB hepatitis; these bile acids could be monitored with sensitive radioimmunoassays that have already been developed. Very little is yet known about the specificity of such an assay for hepatitis.

And so the race to find a test for NANB hepatitis continues. Investigators are mindful not only of the suffering undergone by patients who receive contaminated blood during transfusions but also of the fact that development of a test for hepatitis B brought a Nobel Prize to Baruch S. Blumberg of the Institute of Cancer Research in Philadelphia. If a successful test is found, lightning might very well strike twice.

—THOMAS H. MAUGH II