Hepatitis B Vaccine Passes First Major Test

Unusual vaccine protects at least 92 percent of recipients, may provide some protection against liver cancer

An unusual new vaccine has passed its first major clinical trial in this country, providing nearly complete protection against the debilitating disease hepatitis B. Other major trials here and abroad are also proceeding successfully, and the vaccine could be available for general use in as little as 2 years. In addition to its activity against hepatitis B, there is also evidence suggesting that the vaccine might also provide protection against liver cancer. That would make it the first successful vaccine against cancer.

Hepatitis B, formerly known as serum or postransfusion hepatitis because a major mode of transmission is through the blood, is characterized by inflammation of the liver, fever, weakness, loss of appetite, malaise, headache, and muscle pain. It is generally considered the most dangerous form of hepatitis, particularly since many of those exposed to it are already ill. An estimated 80,000 to 100,000 cases of hepatitis B occur in the United States each year, with a fatality rate of 1 to 2 percent, but the incidence is as much as ten times greater in some other parts of the world.

The foundation for the present work on vaccines was laid by Nobel laureate Baruch S. Blumberg and Irving Millman of the Institute for Cancer Research in Philadelphia. In 1964, they observed a viruslike particle, now called the hepatitis B surface antigen (HB_sAg), in the blood of an Australian aborigine and, subsequently, in the blood of hepatitis B patients. That particle is now known to be part of the protein coat of the hepatitis B virus; the complete virus was identified in 1970 by D. S. Dane of the Bland-Sutton Institute in London.

As many as 10 percent of hepatitis B patients become chronic carriers of the virus, displaying relatively high concentrations of HB_sAg in the blood serum. It has been postulated that such individuals have an immune defect that prevents them from clearing the viral infection, but such a defect has never been demonstrated. There are an estimated 800,000 carriers in the United States and as many as 200 million throughout the world.

Since the hepatitis B virus cannot yet be grown in the laboratory, Blumberg and Millman suggested that excess antigen in the blood of carriers could be separated, purified, and used as a vaccine; they even patented the method. Shortly thereafter, Saul Krugman and his colleagues at the New York University Medical Center proved the feasibility of the concept. Krugman obtained blood containing HB_sAg, diluted it with water, and heated it to inactivate residual viruses. He then injected this preparation into children scheduled for admittance to the Willowbrook State Institution in New York, where hepatitis B was endemic. The crude preparation protected about 70 percent of the children.

Buoyed by these results, Maurice R. Hilleman and his colleagues at the Merck Institute for Therapeutic Research in West Point, Pennsylvania, began preparation of a commercial vaccine. A similar approach was pursued by Robert H. Purcell of the National Institute of Allergy and Infectious Diseases (NIAID) and John L. Gerin of the NIAID-Atomic Energy Commission Molecular Anatomy Laboratory in Rockville, Maryland. Both vaccines were quickly shown to be safe and effective in animals (*Science*, 11 April 1975, p. 137). It is the Merck vaccine that has been studied in the clinical trials.

Production of the Merck vaccine takes 65 weeks—the longest production and testing cycle of any vaccine now manufactured. The process begins with the collection of blood plasma from specially licensed blood donor centers in major metropolitan areas across the country. The major manufacturing steps then include (i) precipitation of protein from the plasma with ammonium sulfate, (ii) purification and concentration of HB_sAg by centrifugation and column chromatography, (iii) digestion with the enzyme pepsin to remove additional protein and treatment with urea to facilitate removal of extraneous human liver cell and plasma components, (iv) sterilization by filtration, (v) inactivation of residual viruses with Formalin, (vi) pooling of the antigen, and (vii) adsorption of the antigen onto an aluminum hydroxide adjuvant to assure maximal antibody response. Between the last two steps, a 6-month safety test is conducted in chimpanzees. This elaborate procedure is necessitated, at least in part, because of the widely expressed fears of investigators such as Arie J. Zuckerman of the London School of Hygiene and Tropical Medicine that vaccines containing host cell components or proteins may themselves be deleterious to the liver.

The newly reported clinical study, directed by Wolf Szmuness of the New York Blood Center, was conducted among 1083 homosexual males in New York City. This group was chosen because the incidence of hepatitis B is unusually high within it (as great as 12 percent per year), and because these individuals are readily accessible through gay organizations and have been cooperative in previous studies. Three 40-microgram doses of vaccine were given to about half the men during a 6-month period, while the remainder received a placebo preparation containing no HB_sAg. Neither doctors nor patients knew who received which.

Side effects of the vaccination were minimal. The most common was arm soreness reported by 15.8 percent of those receiving the vaccine. The percentages of those reporting other side effects, including rash, nausea, joint pain, and low-grade fevers, were virtually identical in both the vaccine and control groups and were lower than the incidence reported for other Formalin inactivated, alum-adsorbed vaccines. In light of the previous warnings, says Jules L. Dienstag of the Massachusetts General Hospital, "the vaccine's safety is especially reassuring."

After the third dose was administered, 96 percent of those who received the vaccine developed antibodies against HB_sAg. None of the individuals who developed antibodies contracted hepatitis. Overall, no more than 3.4 percent of those who received the vaccine contracted hepatitis, and most of those developed it within the first 5 months after the initial vaccination, suggesting that the infection occurred before vaccination or before protective antibodies were established. In contrast, 27 percent of those who received the placebo vaccine developed hepatitis.

A significant reduction in the incidence of hepatitis was observed within 75 days of the first injection, suggesting that the vaccine is at least partially ef-

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fective even after the recipient has been exposed to the virus. The only other vaccine for which such a phenomenon is observed is the rabies vaccine. In both cases, it appears that the long incubation period of the virus allows time for protective antibodies to be developed before the infection becomes manifest. This effect apparently occurs only during the incubation period, however. A preliminary study by Szmuness among 16 chronic carriers of HB_sAg revealed no positive effects of the vaccine.

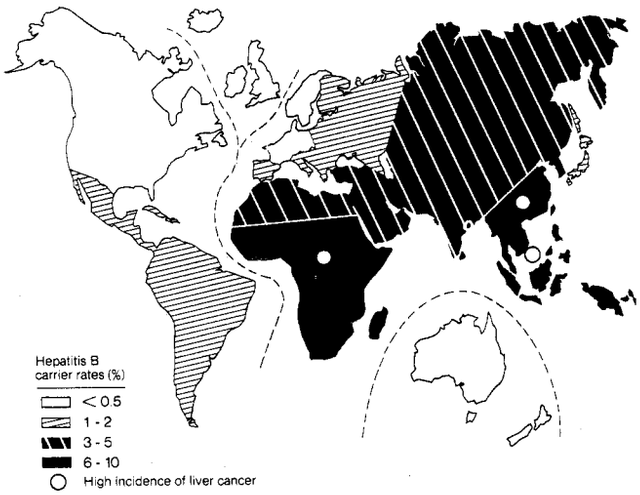
Szmuness is also directing two other large studies to test the efficacy of the vaccine. The first will study nearly 1000 dialysis patients who are at high risk of developing hepatitis because of residual contamination of the dialysis machines. A second study is being conducted among 800 staff members of dialysis centers, who have a somewhat lower, but still high, risk of developing the disease. Results from these studies should be available next year. Meanwhile, Donald P. Francis of the Center for Disease Control's hepatitis laboratory in Phoenix is conducting a similar study among 1200 homosexuals in five western cities. In this study, only 20-microgram doses of vaccine are being used. Results from this study should also be available next year.

The New York group is also undertaking some clinical trials abroad. They are just now starting a trial among children ages 2 to 7 in Taiwan, which has one of the highest incidences of hepatitis B in the world; nearly 15 percent of the population is affected. The group will also soon begin a study on children born to mothers who are carriers of HB_sAg. A similar study will be launched about the same time in the area surrounding the border between Mozambique and South Africa.

Purcell and Gerin have been proceeding much more slowly in the development of their version of the vaccine. "The government is not in the business of developing commercial vaccines," says Purcell. Instead, they have been studying the effects of small changes in both the manufacturing process and the adjuvant on the immunogenicity of the vaccine. So far, they have found that immunogenicity can be increased by minor changes in certain manufacturing steps. The two investigators have studied the efficacy of antibody formation (in a strange juxtaposition to the Merck studies) in small groups of Trappist monks and scientists at NIAID. Both groups were chosen because they are unlikely to have had prior exposure to hepatitis B infection.

A much faster pace of development is being achieved by Philippe Maupas of the Institute of Virology in Tours, France, who has also developed a vaccine based on HB_sAg. That vaccine is now in the final stages of a clinical trial being conducted among dialysis patients at the Pasteur Institute in Paris. Maupas is also conducting trials in 36 villages in West Senegal; in these trials, half the patients receive the vaccine and half receive the DPT (diphtheria, pertussis, tetanus) vaccine. Results from these studies should be available next year.

The incidence of hepatitis B and PHC.



Despite similarities between the U.S. and French vaccines, "We think ours is much better," says Szmuness. He and other American investigators are very critical of the design of the Senegal trial because it is neither random nor double-blind. Both factors lend themselves to potential bias in the interpretation of experimental results. Nonetheless, the Maupas vaccine can be licensed in France solely on the basis of the Pasteur study, while licensing in the United States cannot be achieved until confirmatory results have been obtained from the other studies now in progress. The Maupas vaccine will thus most likely be commercially available next year, while the Merck vaccine will probably not be available for another 18 to 24 months.

The next major step in vaccine production, pending an unexpected breakthrough in growing the virus in the laboratory, will probably be production of HB_sAg in bacteria. Last year, Nobel laureate Walter Gilbert of Harvard University and colleagues at the University of Edinburgh and the University of Heidelberg reported that they had inserted hepatitis viral genes onto *Escherichia coli* so that the bacteria produced small quantities of both HB_sAg and a protein from the virus core. More recently, William J. Rutter and his associates at the University of California at San Francisco have found that the bacteria can produce substantial quantities of HB_sAg if the antigen is fused to a bacterial protein so that it can be secreted unharmed. Rutter suggests that the bacteria-produced antigens could be used for a commercial vaccine in 5 years or less.

In addition to reducing the incidence of hepatitis B, the vaccine might also provide protection against primary hepatocellular carcinoma (PHC), a severe, generally fatal form of liver cancer and one of the two or three most common cancers in the world. A large and growing body of evidence suggests that infection with hepatitis B is an important precursor of this form of cancer, says Blumberg, and "the evidence is now sufficiently impressive to conclude that the hypothesis is much more likely to be supported than rejected, and actions consequent on its validity . . . are warranted."

The supportive evidence for this hypothesis takes many forms. The late P. H. Steiner, Szmuness, Blumberg, and others have shown, for example, that there is a strong geographic correlation between hepatitis B infections and PHC. In the United States and Western Europe, the incidence of HB_sAg carriers in the population is only about 0.25 percent and the incidence of PHC is correspondingly low—about 5000 cases per year in the United States. In Southeast Asia and South Africa, in contrast, the incidence of chronic carriers is about 10 percent and PHC is generally the most common form of cancer. In Taiwan, which has a population of only 17 million, there are 10,000 cases of PHC each year. Furthermore, investigators have shown that as many as 90 percent of PHC victims in the high incidence areas have HB_sAg in their blood.

At least 80 percent of all cases of PHC

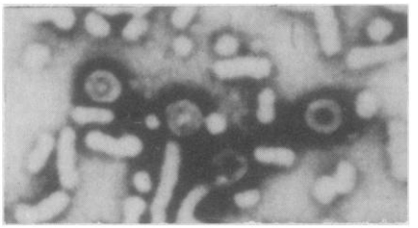
arise in a liver already affected by cirrhosis (a hardening caused by excessive growth of connective tissue followed by contraction), chronic active hepatitis, or both. Studies in Africa, Taiwan, and Korea have shown that nearly all such patients have a chronic hepatitis B infection. Furthermore, proteins associated with the hepatitis B virus can nearly always be demonstrated in patients with PHC, whereas such proteins are not found in livers of uninfected individuals.

If chronic infection is a prerequisite for development of PHC, says Blumberg, it should be possible to show that chronic carriers develop PHC at a high rate. Two such studies are currently in progress. K. Sakuma and his colleagues at the Chiba University School of Medicine in Japan have identified 341 employees of the Japan National Railway who are chronic carriers of HB_sAg. Sakuma is monitoring their health and that of 17,843 controls whose blood does not contain the antigen. In the 3.5 years since the study began, three cases of PHC have been observed among the carriers and none among the noncarriers.

Similarly, Palmer Beasley of the University of Washington and C. C. Kim of the National Taiwan University are monitoring 3000 male civil servants in Taiwan who are chronic carriers, 3000 carefully matched controls, and another 15,000 controls who are matched only by age. After a little more than 4 years, 42 cases of PHC have been observed among the chronic carriers and only one in the control group. These results strongly suggest that chronic hepatitis B infection is a precursor of PHC.

Several investigators have found that children of mothers who are chronic carriers are themselves likely to become chronic carriers of HB_sAg. Bernard Larouze of France has also found that about 70 percent of the mothers of PHC victims are chronic carriers, compared with only about 14 percent of the mothers of controls. This is a major reason why one of the first studies of the hepatitis B vaccine will focus on children of chronic carriers.

Persuasive evidence of the link between PHC and hepatitis B was presented this past year when four groups independently reported that they had found DNA from the hepatitis B virus integrated into the genome of cells from liver cancers. Previous investigators had been able to find the viral DNA associated with cellular protein, but not in the DNA. Three groups used cultured cells derived from the liver tumor of a Mozambican male by Jennifer Alexander of South Africa. Rutter, William Robinson



The hepatitis B virus

The round particles are the virus, the long ones are HB_sAg.

and P. L. Marion of Stanford University, and David Shafritz and his associates at the Albert Einstein College of Medicine reported that at least one copy, and perhaps as many as six copies of the complete DNA sequence of the hepatitis B virus—consisting of about 3250 to 3300 base pairs—is integrated into the genome of the Alexander cells. Rutter and his associates have subsequently also found the genome integrated into tumor cells from other patients. Similarly, Pierre Tiollais and his colleagues at the Pasteur Institute reported that the viral DNA is integrated into the genome of both tumor cells and a cell line derived from the tumor of a man from the Ivory Coast.

Rutter had previously found messenger RNA in the Alexander cells coding for the formation of HB_sAg. Shafritz's group also found three RNA molecules specific for the hepatitis B virus sequences. These results indicate that the viral genome is not merely hidden away among the cellular DNA, but that at least part of it is being expressed and thus participating in the control of the cell. Until now, the integration of viral DNA in the genome of human tumors had been confirmed only for the Epstein-Barr virus in Burkitt's lymphoma and some nasopharyngeal tumors, although the phenomenon is apparently common in animal models of tumor systems.

A final piece of evidence is the existence of an animal model for the relationship between hepatitis B and PHC. Robert Snyder of the Philadelphia Zoological Garden maintains a colony of Pennsylvania woodchucks (*Marmota monax*). During the past 18 years, postmortem examinations by Snyder on 102 woodchucks showed that 23 had primary liver cancer and three had active chronic hepatitis. He further noted that the tumors were generally associated with chronic hepatitis and, sometimes, cirrhosis.

Two years ago, Jesse Summers of the Institute for Cancer Research reported that the woodchuck hepatitis virus has a DNA polymerase similar to that found in the hepatitis B virus. More recently, Barbara G. Werner of the institute found immunological cross-reactivity between the surface (HB_sAg) and core antigens (HB_cAg, protein from the interior of the virus) of the hepatitis B virus and the corresponding antigens from the woodchuck hepatitis virus. These findings suggest that the two viruses are closely related but not identical. Interestingly, the surface antigen of the woodchuck hepatitis virus is found in 10 to 20 percent of woodchucks found in the wild in Pennsylvania and New Jersey, indicating that the proportion of chronic carriers in woodchucks is about the same as in the human population in endemic areas.

Subsequently, Marion has identified a similar hepatitis virus in the Beechey ground squirrel, and Summers and William Mason have identified similar viruses in Pekin ducks. The preliminary data suggest that these viruses are all very similar and represent a new class of virus. Blumberg has tentatively named the members of this class Icrons in honor of his institution.

The mechanism by which the hepatitis B virus might interact with a cell to produce a tumor is still largely a mystery. The closest analogy is found in avian virus-tumor systems, where it is known that the virus is also integrated into the cellular genome and that the key product of the viral genes is a protein kinase that preferentially phosphorylates the amino acid tyrosine. It has now been shown that the hepatitis B virus has similarly been integrated into the cellular genome, and Robinson has found that it produces an appropriate kinase. Beyond this point, however, little is known. In particular, there is little understanding of the long latent period between infection and development of a tumor, of why males are more susceptible to the disease than females, and of why some individuals become chronic carriers while others recover completely. There is also little understanding of the role of other factors, such as chemical carcinogens, in the development of the disease.

Such an understanding may not be necessary for effective action, however. If hepatitis B infection is a necessary prerequisite for the development of PHC, then interference with that infection should also interfere with tumor initiation. Scientists are thus hopeful that widespread use of the hepatitis vaccine will reduce the incidence of PHC. It will take at least 15 years to prove that such an effect is occuring, but an all-out vaccination effort seems justified. Even if the vaccine should have no effect on PHC, a reduction in the incidence of hepatitis B would, in itself, be a very worthwhile achievement.—THOMAS H. MAUGH II