The Hepatitis B Virus

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The disentanglement of the complex of diseases collectively called hepatitis and the elucidation of their epidemiology can be counted as one of the more brilliant achievements of American epidemiology. Less than 40 years ago our understanding of this complex was so primitive that for at least one of its components, hepatitis A, the name acute catarrhal jaundice was applied, and its causative agent was subject to rampant conjecture.

The ensuing 40 years have witnessed one of the most intriguing adventures in epidemiology. The epidemiologic separation of serum hepatitis, or hepatitis B, from infectious hepatitis, or hepatitis A, has clarified to a great extent the confusion that existed for years among a set of diseases whose principal physical findings were jaundice and liver function abnormalities. Epidemiologic approaches have in great part also reduced the confusion as to sources and modes of transmission. In more recent years epidemiologists have found the presence of the hepatitis B surface antigen (HBsAg) circulating in the blood of patients with viral hepatitis an extremely useful tool in the separation of hepatitis A from B and in projecting the recognition of other viral agents as causes of hepatitis by the designation of a non-A-non-B group.

Dr. Baruch Blumberg speaks to the epidemiologic contributions in this area of disease and particularly on the discovery of the hepatitis B antigen and its impact on our understanding of the viral hepatitides. Dr. Blumberg is an eminent scientist with the Fox Chase Cancer Center's Institute for Cancer Research. His pioneering work in the discovery of the B-antigen and its serendipitous contribution to the etiology and epidemiology of hepatitis B is an awe-inspiring saga that rivals the best of those in "The Microbe Hunters." For this work he was awarded the Nobel Prize. The role of B-antigen has been extended to considerations of the etiology of leukemia and, with persistent infection with the hepatitis B virus, primary hepatocellular carcinoma.—LEONARD M. SCHUMAN, MD

THE DISCOVERY OF AUSTRALIA ANTIGEN, as it was initially called, and of this material's relation to hepatitis B virus followed an unusual pattern in terms of the identification of an infectious agent. The research project that resulted in the discovery did not start with the direct intention of finding the cause of viral hepatitis; rather the discovery was a consequence of an interest in the inherited differences between people that are reflected in the serum proteins.

History of Early Studies

For many years the existence of inherited antigenic differences between the red blood cells had been known. Beginning about 1955 with the introduction of the

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starch gel electrophoresis method, it became possible to separate serum protein into many more fractions than had previously been possible. Some of this protein variation was found to be under genetic control, and we postulated that some of the differences were antigenic. If this were the case, in patients who had received large numbers of transfusions, antibodies might develop against antigenic specifications that the patients themselves had not inherited or acquired. These antibodies could then be used to detect inherited or acquired serum protein variants. Shortly after initiation of our study, an antibody was found in the serum of a transfused patient that reacted with a specific family of serum proteins, the low density lipoproteins. (This protein is involved in a variety of chronic illnesses, including arteriosclerosis, heart disease, and stroke.) The antiserum was able to distinguish inherited antigenic differences between lipoproteins; that is, the prediction derived from our hypothesis had been fulfilled. The antiserum was referred to as the Ag system.

Since this first series of experiments had been successful in identifying an inherited protein system, the search was continued. Soon another antibody was discovered with characteristics different from those of the Ag system. The new antibody was found in the blood of a transfused hemophilia patient from New York. Because in the initial studies, it reacted only with the serum of an Australian aborigine that was present in the panel against which it was tested, the unknown antigen was given the name Australia antigen (Au). This was a geographic name without any implications about the function of the newly discovered antigen, and therefore it encouraged a wide variety of hypotheses.

We then set out to find out why a precipitin band had developed between the serum of a hemophilia patient from New York and that of an aborigine from Australia. At the outset we had no set views on where this path might lead, although our investigation was guided by our prior experience with the Ag polymorphism. In preparing this "history" of the discovery of antigen Au, I constructed an outline, based on a hypothetico-deductive structure, showing the actual events that led to the discovery of the association of Au with hepatitis. From this outline it is clear that we could not have planned the investigation in advance so as to find the cause of hepatitis B. This experience does not encourage an approach to basic research for the solution of biological problems that is based exclusively on programs directed at specific goals.

The next step was to collect information on the distribution of Au and antibody to Au in different human populations and disease groups. We had established a collection of serum and plasma samples, which was later to become the blood collection of the Division of Clinical Research of the Institute for Cancer Research and to number more than 300,000 specimens. The antigen was very stable; blood that had been frozen and stored for 10 years or more still gave strong reactions for Au. In some instances, blood had been collected from the same person for 6 or more successive years. If the serum test results were positive on one occasion, they were in general positive on subsequent testings; if negative initially, they were consistently negative. The presence or absence of Au appeared, at least in the early experiments, to be an inherent characteristic of a person.

We were able to use our stored serums for epidemiologic surveys, and in a short time, we accumulated a considerable amount of information on the worldwide distribution of Au. It was very rare in apparently normal populations of the United States; only 1 of 1,000 serums tested was positive. However, it was fairly common in some tropical and Asian populations (for example, 6 percent in Filipinos from Cebu, 1 percent in the Japanese, and 5 to 15 percent in certain Pacific Ocean populations).

In order to search for more antiserums to Au, our technician, Sam Visnich, had been asked to select from our collection the serums of patients who had received transfusions. He decided, however, to use these serums both as potential sources of antibody and also in the panels against which antiserums to Au were tested. Among the transfused serums were specimens from patients with leukemia who had received transfusions. A high frequency of Au, rather than antiserums to Au, was found in this group. We subsequently tested patients with other diseases, but found Au only in transfused patients.

On the basis of these observations, we made several hypotheses. One hypothesis stated that although Au may be rare in normal populations, leukemia is more likely to develop in persons who have Au than in persons who do not have the antigen. That is, there is a common susceptibility factor that makes it more likely for certain people both to have Au and to develop leukemia. We also suggested that Au might be related to the infectious agent (virus) that is said to be the cause of leukemia.

A corollary of the susceptibility hypothesis is that persons in whom there is a high likelihood of leukemia developing would be more likely to have Au. Leukemia is more likely to develop in children with Down's syndrome than in other children; estimations of the increased risk vary from 20 to 2,000 times that of children without Down's syndrome. We tested the serums of Down's syndrome patients resident in a large institution

and found that Au was very common in this group (the results of approximately 30 percent of the serum tests were Au positive); the prediction generated by our hypothesis was fulfilled by these observations, an encouraging finding (*1*). The presence of the antigen in people living close to Philadelphia also made it possible to study persons with Au more readily. Until this time, all the persons with Au who had been identified either lived in Australia, or some other distant place, or were sick with leukemia.

Australia Antigen and Hepatitis

Down's syndrome patients were admitted to our clinical research unit (located in our sister institution, Jeanes Hospital) for clinical study. The presence or absence of Au seemed to be a consistent feature of a person. If Au was present on initial testing, then it was present on subsequent testing; if absent initially, it was not found later. In early 1966, one of our Down's syndrome patients, James Bair, whose test result had originally been negative, was found to have Au on a second test. Since this finding was aberrant, we admitted him to the clinical reseach unit. There was no obvious change in his clinical status. Because a "new" protein apparently had developed in his blood, and since many proteins are produced in the liver, a series of liver chemistry tests were performed. These showed that between the first testing (results negative for Au) and the subsequent testing (results positive for Au), this patient (J. B.) had developed a form of chronic anicteric hepatitis.

On the day of J. B.'s admission to the clinical research unit, my colleague, Alton Sutnick, wrote the following dramatic note in the patient's chart:

> SGOT [serum glutamic oxaloacetic transaminase] slightly elevated! Prothrombin time low! We may have an indication of [the reason for] his conversion to Au+.

Sutnick's prediction proved correct. The diagnosis of hepatitis was clinically confirmed by liver biopsy on 20 July 1966, and we then began to test the hypothesis that Au was associated with hepatitis (*2*). First, we compared the transaminase (SGPT—serum glutamic pyruvic transaminase) levels in males with Down's syndrome who had Au and those who did not. The SGPT levels were slightly, but significantly, higher in the Au(+) males. Second, we asked clinicians in Pennsylvania to send us blood samples from patients with acute hepatitis. W. Thomas London and others in our laboratory soon found that many hepatitis patients had Au in their blood early in their disease, but that the antigen usually disappeared from their blood after a few days or weeks.

Another dramatic incident added to our urgency in seeking to determine the nature of the relation of Au to hepatitis. Barbara Werner, the first technician in our laboratory in Philadelphia, had been working on the isolation of Au by extensions of the methods devised by Alter and me (*3*). Early in April of 1967, Werner noticed that she was not in her usual good state of health. Well aware of our observations that Au was related to hepatitis, one evening she tested her own serum for the presence of Au. The following morning a faint but distinct line appeared, the first case of viral hepatitis diagnosed by the Au test. She subsequently developed icteric hepatitis, but fortunately went on to a complete recovery.

By the end of 1966, we had found that Au was associated with acute viral hepatitis. In our published report (*1*), we said:

> Most of the disease associations could be explained by the association of Au(1) with a virus, as suggested in our previous publications. The discovery of the frequent occurrence of Au(1) in patients with virus hepatitis raises the possibility that the agent present in some cases of this disease may be Australia antigen or be responsible for its presence. The presence of Australia antigen in the thalassemia and hemophilia patients could be due to virus introduced by transfusions.

That is, we made the hypothesis that Au was (or was closely related to) the etiological agent of "viral" hepatitis, and we immediately set about to test it. Our original publication did not elicit wide acceptance; there had been many previous reports of the identification of the causative agent of hepatitis, and our claims were naturally greeted with caution. Indeed, an additional paper on Australia antigen and acute viral hepatitis (*2*), which extended our findings that we had first published in 1967, was initially rejected for publication on the grounds that we were proposing another "candidate virus," and there were already many of these.

Confirmation of our findings and the first definitive evidence on the relation of Au to posttransfusion hepatitis came soon. Okochi, then at the University of Tokyo, and his co-workers had followed a line of inquiry similar to ours. They had started investigation of antiserum to Ag (lipoprotein), and we had corresponded on this subject. They then found an antiserum in a patient with chronic myelogenous leukemia that was different from the precipitins in antiserum to Ag. They also found that this antiserum was associated with liver damage. Okochi sent the unusual antiserum to us to compare with antiserum to Australia antigen; we found that they were identical. The Tokyo investigators confirmed our finding of the association of Au with hepatitis and then proceeded to do the first definitive study of transfusion. They found that Au could be transmitted by transfusion and that it led to the development of hepatitis in some of the people who received

it; also, that in some transfused patients, antibody to Au developed (*4*).

We had made several preliminary observations in Philadelphia, in collaboration with John Senior of the University of Pennsylvania, about the transfusion of donor blood that was found to contain Au. We then drew up a protocol for a controlled, long-term study to determine whether donor bloods that had Au were more likely to transmit hepatitis than those that did not. In 1969 we heard from Okochi that he had already embarked on similar transfusion studies. In June of that year he visited our laboratory in Philadelphia and showed us his data. These, in his (and our) opinion, demonstrated with a high probability that donor blood containing Australia antigen was much more likely to transmit hepatitis than donor blood that did not contain the antigen. We immediately stopped the experimental study, and in the hospitals where we were testing donor units, estalished the practice of excluding donor bloods with Australia antigens.

This is a dramatic example of how technical information may completely change an ethical problem. Until Okochi's data became available, determination of the consequences of transfusion blood containing Australia antigen was a moral necessity; and it had to be done in a controlled and convincing manner, since major changes in blood transfusion practice were consequent on the findings. As soon as the conclusion of Okochi's well-controlled studies were known to us, the administration of donor blood containing Australia antigen became untenable.

It was, however, possible to evaluate the efficacy of Au screening on posttransfusion hepatitis with the use of historical controls. Senior and colleagues had completed an analysis of posttransfusion hepatitis in Philadelphia General Hospital before the advent of screening and had found an 18 percent frequency of posttransfusion hepatitis. In the fall of 1969, we started testing all donor blood and excluding Au-positive donors. Senior and others undertook a similar followup study 1 year after this screening program was in progress. They found that the frequency of posttransfusion hepatitis had been reduced to 6 percent, a striking improvement (*5*).

The practical application of our initially esoteric finding had come about only 2 years after publication of our paper on the association between Au and hepatitis (*1*). In retrospect, one of the major factors contributing to the rapid application of the findings was the simplicity of the immunodiffusion test. Another was our program of distributing reagents containing antigen and antibody to all investigators who requested them. We continued this practice until this function was assumed by the National Institutes of Health.

After the association of hepatitis with Australia antigen had been confirmed, a large number of studies were published, and in a relatively short time the routine use of the Au test in blood banks became essentially universal in the United States and many other countries. It has been estimated that the annual saving resulting from the prevention of posttransfusion hepatitis amounts to about half a billion dollars in the United States.

PHC and Hepatitis B Virus

Primary hepatocellular carcinoma (PHC), or hepatoma, is a relatively rare cancer in this country, amounting to about 6,000 cases per year. However, in some regions of the world, including sub-Saharan Africa, parts of Oceania, Southeast Asia, Taiwan, Korea, and the People's Republic of China, it is a major cause of cancer in men. In these regions it represents a major public health problem, particularly in men. (HBV is also associated with chronic liver disease, a life-shortening disease that is a major cause of morbidity and mortality in these heavily populated regions.) Unfortunately, no treatment for PHC is effective, with the possible exception of very early surgery.

There is now a large body of evidence that persistent infection with hepatitis B virus is necessary for the development of primary hepatocellular carcinoma. Nine independent lines of evidence support this hypothesis, which I will briefly summarize. (This summary is based in large part on reference 6.)

1. PHC occurs commonly in the same regions where chronic carriers are prevalent.

2. A common epidemiologic method for investigating whether some factor is associated with a disease is the case-control study, in which identified persons with the disease are compared with appropriate controls without the disease (usually matched by age, sex, and location) for the presence or absence of the suspected factor. Such studies and related ones have shown that up to 80 percent of the blood serums of patients with PHC living in areas where PHC is endemic are HBsAg(+) and 50 to 87 percent are anti-HBc(+) (table 1). In the same areas, controls have lower frequencies of HBsAg (0 to 24 percent) and anti-HBc (0 to 36 percent). Even in the United States, patients with PHC have significantly higher frequencies of HBsAg (15 percent), and especially anti-HBc (49 percent), in their blood than controls. That is, serological evidence of persistent infection with HBV is significantly more common in the patients with PHC than in the controls.

3. Eighty percent or more of the cases of PHC arise in a liver already affected with cirrhosis or chronic

Table 1. Frequency of hepatitis B surface antigen (HBsAG) and antibody against hepatitis B core (anti-HBc) in blood of patients with primary hepatic carcinoma and in controls in selected countries

County	Serums of patients with primary hepatic carcinoma		Serums of controls	
	Number tested	Percent positive	Number tested	Percent positive
	Hepatitis B surface antigen			
Greece	189	55.0	106	4.7
Japan	260	37.3	4,387	2.6
Mozambique	29	62.1	35	14.3
Senegal	291	51.9	100	12.0
Singapore	156	35.3	1,516	4.1
South Africa	138	59.5	200	9.0
Spain	31	19.3	101	2.0
Taiwan	84	54.8	278	12.2
Uganda	47	47.0	50	6.0
United States	34	14.7	56	0
Vietnam	61	08.3	94	24.5
Zambia	19	63.1	40	7.5
	Antibody to hepatitis B core antigen			
Greece	80	70.0	160	31.9
Hong Kong	37	70.3	58	36.2
Senegal	291	87.3	100	26.0
South Africa	76	86.0	103	31.7
Spain	31	87.0	101	14.8
United States	33	48.5	56	0

NOTE: Table is based only on studies using radiommunoassay or a test of equivalent sensitivity for HBsAG and in which controls were included.

active hepatitis, or both. If chronic hepatitis and cirrhosis are steps on the way to the development of liver cancer, then case-control studies of these two diseases should also show higher prevalences of chronic infection with HBV in the persons with these two diseases compared with appropriate controls. Studies in Africa, Taiwan, and Korea have demonstrated that almost all such patients are chronically infected with HBV (HBsAg(+) or anti-HBc(+), or both).

4. Since PHC is a cancer of the liver, one would expect to find signs of HBV infection in the hepatic tissues of patients with the disease. HBV proteins can nearly always be demonstrated in such tissues by histochemical stains or immunological techniques. It is interesting that HBsAg and HBcAg are either not detected or only found in small quantities in the tumor cells themselves. Rather, the viral antigens are found in the nonmalignant cells adjacent to the expanding tumor. These viral proteins are not found in the livers of uninfected persons or in persons with anti-HBs in their serum.

5. If persistent HBV infection "causes" PHC, such infection should precede the occurrence of PHC. To test this hypothesis, it is necessary to identify "healthy" chronic carriers of HBV and controls who are not carriers and to follow the persons in such groups over a period of several years to see whether PHC develops. Two studies of this prospective design are currently in progress; one in Japan directed by Dr. K. Sakuma and colleagues (H. Ohtake, K. Okada, and M. Mayumi) and a second in Taiwan conducted by Beasley of the University of Washington and Lin of the National Taiwan University (7). In the study in Japan, all employees of the Japan National Railway in the Tokyo district between the ages of 40 and 60 had a blood sample drawn as part of their annual physical examination. The workers who were found to have HBsAg in their blood were tested again 6 months later. If results were still positive, the workers were designated chronic carriers. Three hundred and forty-one such persons were identified, compared with 17,843 HBsAg(—) noncarriers. Over the succeeding 6 months to 3½ years, three cases of PHC occurred among the carriers, whereas none were observed among the noncarriers.

Beasley's prospective study in Taiwan was conducted on male civil servants, also between the ages of 40 and 60 (7). A total of 3,518 carriers were identified in a fashion similar to that used in Japan. The controls were 19,223 noncarriers, or HBsAg(—). By January 1980, each group had been followed for an equivalent interval of about 5 years. Forty-three cases of PHC had occurred during the 3 to 4 years of followup, and all but one of these cases developed in the chronic carriers:

Disease status	Number of persons	Deaths	PHC cases
HBsAg(+) chronic carriers	3,815	71	42
HBsAg(—) controls	19,223	140	1

These two studies, particularly the Chinese study, provide strong support for the hypothesis that chronic infection with HBV is etiologically related to PHC.

6. As stated previously (point 3), PHC usually develops in a liver affected by cirrhosis or chronic hepatitis, or both. Some investigators have argued that any hepatotoxic agent (for example, alcohol or liver parasites like *Schistosoma mansoni* and *Clonorchis sinensis*) which causes cirrhosis is associated with an increased risk of PHC and that hepatitis B virus is "just" one more hepatotoxic agent. Therefore, a particularly rigorous test of the hypothesis that chronic infection with hepatitis B virus imparts an increased risk of PHC beyond this infection's role in the production of cirrhosis is to compare the incidence of PHC in patients with cirrhosis who are and are not chronic carriers of HBV.

Such a study is being carried out in Japan by Obata and colleagues at the Tokyo Women's Medical College (*8*). Beginning in April 1973, patients with cirrhosis were categorized as HBsAg(+) or HBsAg(−), were carefully evaluated to be sure that they did not have PHC on admission to the study, and were then followed for 1 to 4½ years for the development of PHC. During a 3- to 4-year followup, the patients who were HBsAg (+) were at about four times the risk of developing PHC as the HBsAg(−) patients, as the following table shows. PHC developed in nearly a quarter of the patients in whom the cirrhosis was associated with HBV, but in only 6 percent of the patients whose cirrhosis presumably was due to other causes (*8*).

Disease status	*Number of patients*	*PHC cases*	
		Number	*Percent*
HBsAg(+)	30	7	23.3
HBsAg(−)	85	5	5.9
Total	115	12	10.4

7. In populations where HBV is endemic, there is good evidence that many of the persons who become chronic carriers do so as a result of infection transmitted from their mothers early in life (at the time of delivery, in the period after birth when the mother and child have considerable close contact, or perhaps, prenatally). That is, the mothers themselves are chronic carriers and all of their offspring born at times when the mothers are infectious are likely to become chronic carriers. The potential infectiousness of the mothers appears to be associated with the presence of intact hepatitis B virions, DNA polymerase, and HBeAg in their peripheral blood. Among members of a population, persons infected at birth will have been chronic carriers of HBV longer than chronic carriers of similar age infected later in life. Therefore, if the duration of the carrier state is related to the likelihood of PHC developing, one could predict that the mothers of patients with PHC would be more likely to be chronic carriers than the mothers of controls of similar age who do not have PHC. In collaboration with Barbara Werner, Edward Lustbader, and our French and Sengalese colleagues Bernard Larouze, Gerard Saimot, Marc Sankale, and Maurice Payet, we conducted such a study in Dakar, the capital of Senegal. We found that about 70 percent of the mothers of patients with PHC were HBsAg(+) compared with only 6 percent of the mothers of controls. Even when the mothers of PHC patients were compared with mothers of HBsAg(+) carriers without cancer, the mothers of the cancer patients were significantly more likely to be HBsAg(+) (*9*). In collaboration with Chung Yong Kim of the National University Hospital and our colleague Dr. Hie-Won Hann, we have carried out similar studies in Seoul, South Korea, whose results are consistent with the African studies (table 2).

If chronic carrier mothers give birth to children who become chronic carriers, and such children have an increased risk of PHC developing, then, also, other children born to the same mothers should have an increased risk of becoming carriers and having chronic liver diseases develop, including PHC. Studies by Hann and colleagues in South Korea support this notion (*10*). About 60 percent of the brothers and 40 percent of the sisters of patients with PHC are HBsAg(+), and the brothers appear to have an increased prevalence of chronic active hepatitis, cirrhosis, and PHC.

We have also detected a paternal effect. Husbands of chronic carriers generally have a high prevalence of anti-HBs (from 70–90 percent in our studies). Fathers of patients in Korea and Senegal with PHC or other chronic liver diseases had significantly lower frequencies of anti-HBs than men of similar age in the general population, despite chronic exposure to carrier wives or carrier children, or both. The nature of this paternal factor is not clear at this time.

8. HBV DNA is present in the liver cells of a majority of patients with PHC. Most studies of the relationship of viruses to cancer have been done in experimental animals or tissue culture systems. It is thought that the genome of such viruses becomes integrated into the genome of the host cell and that the product of a viral gene is required to produce malignant transformation of the cell. Whether HBV conforms to this model of viral carcinogenesis is uncertain.

Summers of the Institute for Cancer Research in Philadelphia and colleagues (*11*) have tested this hypothesis. They isolated HBV DNA, and using DNA polymerase from *Escherichia coli,* they made radioactive copies of the HBV DNA which, in turn, could be used as probes to see whether viral DNA was present in PHC tissue, that is, whether viral DNA would hybridize with DNA extracted from liver tumor cells. Their studies demonstrated viral DNA in the liver cells of patients with PHC who showed evidence of HBV infection in their blood. The viral DNA, however, was located within protein cores and did not appear to be integrated into the genome of the tumor cells. Because it is not certain that a given block of tissue removed at autopsy is free of nontumor cells and blood, it is difficult to know whether the viral DNA was extracted from malignant or nonmalignant hepatocytes. In any event, Summers was unable to demonstrate integration of the HBV genome into malignant or nonmalignant hepatocyte genomes.

Table 2. Frequency of HBsAG, anti-HBc, and anti-HBs in blood of mothers of patients with PHC (primary hepatic carcinoma) compared with frequency in blood of controls, Senegal and Korea

HBV response	Serums of mothers of PHC patients		Serums of controls	
	Number tested	Percent Positive	Number tested	Percent positive
	Senegal [1]			
HBsAg	28	72	28	14
Anti-HBc	28	72	28	14
Anti-HBs	28	11	28	54
	Korea [2]			
HBsAg	9	44	114	4
Anti-HBc	9	100	46	52
Anti-HBs	9	44	114	45

[1] The controls were mothers of persons who did not have PHC, but who were usually carriers of HBsAg.
[2] The controls were other women in the same population (*9,10*).

Recently, Marion and Robinson of Stanford University studied a unique tissue culture preparation that had originally been developed by Dr. Jennifer Alexander of South Africa from the liver of an African patient who had died of PHC (*12*). They found that part of the genome of HBV was integrated into the DNA of the chromosomes of the liver cells growing in the tissue culture. It is not clear at present how this finding relates to the studies on liver cells from biopsies and autopsies.

9. One hundred and forty years ago, Jacob Henle suggested four criteria for assessing whether an infectious agent causes a particular disease. These criteria, which were applied by Robert Koch to the identification of the bacterium that causes tuberculosis, have since been called "Koch's postulates," or more correctly, "Henle-Koch postulates." They are: (*a*) the agent should be isolated from the human or animal with the disease; (*b*) the agent should be cultured in artificial media; (*c*) the agent grown in the artificial media, when inoculated into an experimental animal, should reproduce the original disease; (*d*) the agent should be isolated from the animal with the experimentally induced disease. It has been difficult to satisfy all of these criteria for many virus-induced acute diseases, and rarely have they been met for the virus-related chronic diseases. For example, HBV has not been grown in tissue culture; it can infect a few higher primates like chimpanzees but not the routinely used laboratory animals, and even in chimpanzees, inoculations of HBV isolated from human blood have induced only slight liver damage, not clinical hepatitis. Hence, approaches other than simply trying to satisfy Koch's postulates must be taken.

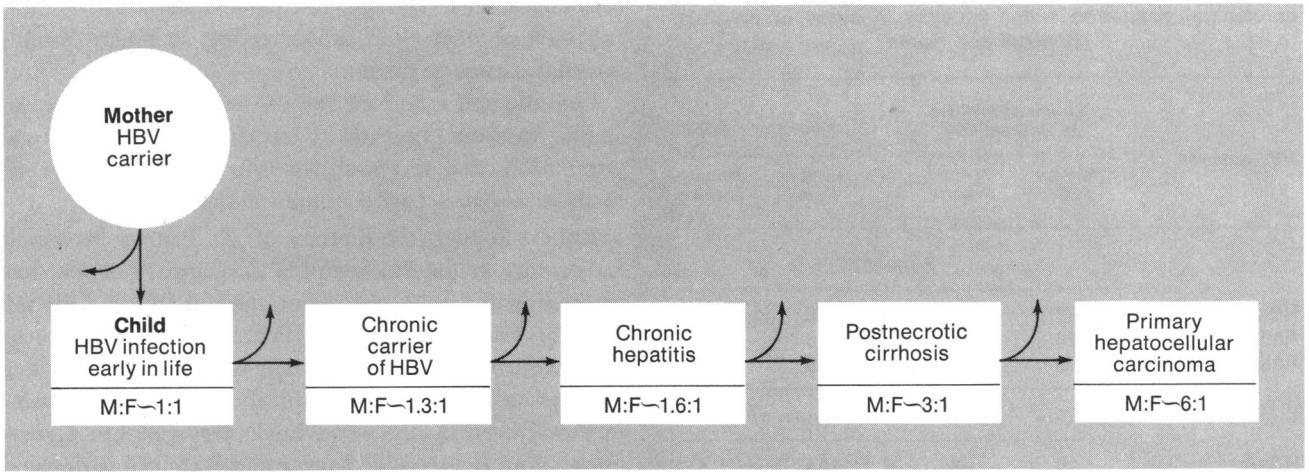
One method is to look for the occurrence of diseases similar to those observed in humans in natural animal populations and to search for infectious agents similar to those associated with human diseases.

Robert Snyder, the director of the Penrose Research Laboratory at the Philadelphia Zoological Garden, has trapped in the wild and maintained at this zoo for the past 18 years a colony of Pennsylvania woodchucks (*Marmota monax*). The animals were permitted to live in cages until they died of natural causes. In the laboratory environment, the woodchucks survived 3 or 4 years longer than in the wild. Snyder and Ratcliffe performed postmortem examinations on 102 of these animals; 23 had primary liver cancer, and 3 had chronic active hepatitis. They noted that as in humans with PHC, the tumors in the animals were usually associated with chronic hepatitis and, sometimes, cirrhosis (*13*).

Several years ago we had examined the serums of some of these animals for the presence of the surface marker of the hepatitis B virus, HBsAg, but did not detect it. Summers of our institute and colleagues studied these serums again, this time with more rewarding results. Their aim was to see if the serums would contain nucleic acid structures and a DNA polymerase similar to that found in HBV. HBV has an unusual circular, double-stranded DNA genome with a single-stranded region and a DNA polymerase capable of filling in the single-stranded region to make a fully double-stranded circular DNA. They assayed serum samples from the woodchucks for particles containing a DNA polymerase with this activity; about 15 percent of the serum samples had such particles. Summers and colleagues (*14*) went on to study three animals in great detail: two that had died of liver cancer and one that had died of a myocardial infarction. They found that the two animals with liver tumors had particles in their serum much like the three types of particles associated with HBV; the animal without a tumor did not have such particles. No virus, other than HBV, had previously been associated with these types of particles. The viral DNA was, as predicted, similar to HBV in size and structure.

Recently, Werner of our laboratory and colleagues (*15*) found cross-reactivity between the surface (WHsAg) and core (WHcAg) antigens of the woodchuck hepatitis virus (WHV) and the comparable antigens on human HBV. Antiserums against HBcAg precipitate the cores of WHV, and antiserums against WHV cores (anti-WHc) precipitate HBcAg. There is

Scheme for progression from infection with hepatitis B virus to primary hepatocellular carcinoma



also cross-reactivity of the surface antigens, but it is less than for the core antigens. Antibody against HBsAg (anti-HBs) will not precipitate WHsAg or vice versa, but antibody to WHsAg (anti-WHs) will agglutinate red blood cells coated with HBsAg, and anti-HBs antibodies will agglutinate erythrocytes coated with WHsAg.

A carrier state for WHV in *Marmota monax* that appears to be analogous to the HBsAg carriers in humans has been found. The frequency of carriers among Pennsylvania and New Jersey woodchuck populations is 10 to 20 percent, similar to that found for HBV in several human populations.

Liver cancer in the woodchuck is not what is generally thought of as a "laboratory model" of a human disease. It was not designed or "created" by an investigator for his purposes; rather, it is a naturally occurring disease related to a naturally occurring virus, both of which have a remarkable number of features in common with their human counterparts. It may be possible by careful study of the relationship of WHV to primary liver cancer in the woodchuck to understand the mechanisms that relate HBV to human liver cancer. For example, it appears that the woodchucks that develop PHC are chronic carriers of WHV, and it is likely (although not proved) that animals which develop antibody to the surface antigen of WHV are protected from PHC. Thus, the factors that result in either of these two polar responses to WHV can be investigated and identified. Factors that appear relevant in the development of the chronic carrier state and PHC in the woodchuck can be studied epidemiologically in humans—and vice versa; factors associated with PHC from epidemiologic studies in humans can be investigated experimentally in woodchucks. Through this back-and-forth process, the intent of the Henle-Koch criteria may be satisfied without performing the exact experiments prescribed 100 years ago.

The Icron. Early in our studies we recognized that HBV had unusual characteristics of structure and behavior. As a heuristic device, we suggested in 1971 (*16*) a group term to designate HBV and other infectious agents that might be found in the future with sufficiently similar characteristics to be grouped with HBV. The word "Icron" is an acronym formed from the name of the Institute for Cancer Research in Philadelphia (where our studies had been done) with a neuter Greek ending (-on) added. Woodchuck hepatitis virus appears to be a member of this group, and other similar agents have now been reported in other animals. This raises the probability that similar viruses related to other human cancers may be found in the future.

Sequence of Events in Liver Cancer

The preceding nine pieces of evidence, in addition to supporting the hypothesis that persistent infection with HBV is required for the development of most cases of PHC, also provide insight into a sequence of events that may begin at birth, or before birth, and progress ultimately to cancer (see chart). HBV is transmitted from an infected mother to her child early in life. The child becomes a chronic carrier of the virus, and over a period of many years an inflammatory disease of the liver—chronic active hepatitis—develops; this leads to regeneration of liver tissue and proliferation of fibrous tissue (cirrhosis); and finally, by mechanisms that are currently unclear, cancer can arise from the regenerating liver tissues.

This sequence can be considered the life history of one person in whom PHC develops. On a population basis, however, the sequence is more like a pyramid. As a result of maternal and other means of transmission of the virus, many chronic carriers (5 to 20 percent of the population) are present in areas where it is endemic.

Although the proportion of carriers accounted for by each route of infection is not known, estimates from Japan suggest that maternal transmission accounts for about 40 percent. Chronic active hepatitis develops in some of the chronic carriers, but not in the majority; cirrhosis develops in some proportion of the people with chronic active hepatitis, but not in the majority; lastly, PHC develops in some people with cirrhosis, but again not in the majority (~75 percent from the Japanese study). Therefore it is essential to investigate which factors lead to the progression from one stage to the next and which lead to the arrest of the process or even to its reversal.

Prevention of Chronic Liver Disease and PHC

The data presented here and in other studies now being done support the hypothesis that persistent infection with HBV is necessary for the development of chronic liver disease and PHC. If the present and future studies continue to support this hypothesis, then it would follow that prevention of infection with hepatitis B virus or modification of the host response after infection, or both, could lead to a decrease in the incidence of these deadly diseases. To effect control, it is necessary to know the modes of transmission and the factors related to host response. HBV is a very adaptive organism, and several modes of transmission are known. This characteristic could be construed as unfortunate, since it might be difficult to control all the methods of transmission. Another view is that the existence of many routes of infection allows for multiple strategies for control; that is, there are numerous vulnerable places in the scheme of pathogenesis where preventive techniques could be applied, and these are discussed in detail elsewhere (*17*).

Millman and I have developed a vaccine (*18*) that is currently being tested in field trials. If it proves to be effective and safe, then it could find important applications in public health programs.

Several of the subjects of epidemiologic interest relating to the discovery of the hepatitis B virus and its application to preventive medicine that I have discussed here are described in greater detail in other publications (*17,19,20*).

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