Title
Hepatitis B Surface Antigen: A Proposal to Study Its Prevalence Among Pregnant Women of Spanish Surname in Salinas, California

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Hepatitis B Surface Antigen:  
A Proposal to Study Its Prevalence Among Pregnant Women  
of Spanish Surname in Salinas, California  

By  
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A.B. (University of California) 1982  

THESIS  
Submitted in partial satisfaction of the requirements for the degree of  
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Approved:  
Chairman  
Date  

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# TABLE OF CONTENTS

List of Tables iv
List of Figures v
List of Abbreviations vi

Section

1. Introduction 1
2. Discovery of the Australia Antigen 4
3. Subcellular Structure of HBV 8
   Overview 8
   Serologic Markers and HBV Infection 11
   Serologic Techniques for the Detection of HBsAg 14
4. Clinical Features of Hepatitis B Infection 17
   Adult Response to Infection 17
   Infant Response to Infection 19
5. Modes of Transmission of Hepatitis B Virus 22
   Introduction 22
   Airborne 24
   Oropharyngeal Secretions 24
   Waterborne 25
   Shellfish-Associated Hepatitis 25
   Foodborne 26
   Contact Transmission 27
   Sexual Transmission 32
   Mother-to-Infant Transmission 35

6. HBsAg Carrier State 46
   HBV as a Cause of Acute or Persistent Infection 46
   Liver Disease in HBsAg Carriers 49
   Serologic Markers in HBsAg Carriers 50
   Age Factor in HBsAg Carrier State 51
   Socioeconomic Status and Carrier State 53
6. HBsAg Carrier State, continued
   Genetic Susceptibility to HBsAg Carrier State 55
   Sex Differences in the Carrier State 59
   Persistence of HBsAg and Immunologic Status 61
   Worldwide Prevalence of HBsAg 63

7. HBV and Hepatocellular Carcinoma 69

8. Postexposure Prophylaxis of Hepatitis B Infection 76

9. Materials and Methods 80
   Background 80
   Rationale 80
   Specific Objectives 81
   Sample Population and Study Population 82
   Sample Size 83
   Measurement Methods 84
   Resources 85
   Projected Budget 86

10. Concluding Statement 87

Appendices
   A. Notice to Prenatal Patients 88
   B. Prenatal Hepatitis B Research Project 90
   C. Code Sheet 91

References 93
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Estimates of HBV Incubation Period</td>
<td>7</td>
</tr>
<tr>
<td>2. Characteristics of HBsAg</td>
<td>7a</td>
</tr>
<tr>
<td>3. Characteristics of HBeAg</td>
<td>7d</td>
</tr>
<tr>
<td>4. Characteristics of HBeAg and DNA Polymerase</td>
<td>7f</td>
</tr>
<tr>
<td>5. Sensitivity of Serologic Techniques for Detection of HBsAg</td>
<td>16</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HBV genome</td>
<td>9</td>
</tr>
<tr>
<td>2. Schematic diagram of HBV and its associated antigens</td>
<td>10</td>
</tr>
<tr>
<td>3. The serologic responses of an individual with a transient HBsAg response and typical acute HB after exposure to HBsAg-positive serum</td>
<td>13</td>
</tr>
<tr>
<td>4. Possible outcomes and sequences of events after exposure to the hepatitis B virus</td>
<td>18</td>
</tr>
<tr>
<td>5. Schematic map of the geographic distribution of HBsAg and primary hepatocellular carcinoma</td>
<td>70</td>
</tr>
<tr>
<td>6. Schematic representation of the possible natural history of HBV infection occurring in infancy</td>
<td>72</td>
</tr>
<tr>
<td>7. Schematic representation of the possible natural history of HBV infection occurring in adulthood</td>
<td>72</td>
</tr>
<tr>
<td>8. Schematic representation of the cycle of HBV infections and liver cancer from generation to generation</td>
<td>74</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab</td>
<td>Antibody</td>
</tr>
<tr>
<td>AHEC</td>
<td>Area Health Education Center</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine amino transaminase</td>
</tr>
<tr>
<td>anti-HAA</td>
<td>Antibody to hepatitis-associated antigen</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>Antibody to hepatitis B core antigen</td>
</tr>
<tr>
<td>anti-HBe</td>
<td>Antibody to hepatitis B e antigen</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>Antibody to hepatitis B surface antigen</td>
</tr>
<tr>
<td>Au</td>
<td>Australia antigen</td>
</tr>
<tr>
<td>CF</td>
<td>Complement-fixation</td>
</tr>
<tr>
<td>CIEP</td>
<td>Counterimmunoelectrophoresis</td>
</tr>
<tr>
<td>DS</td>
<td>Down's syndrome</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
</tr>
<tr>
<td>HAA</td>
<td>Hepatitis-associated antigen</td>
</tr>
<tr>
<td>HB Ag</td>
<td>Hepatitis B antigen</td>
</tr>
<tr>
<td>HBcAg</td>
<td>Hepatitis B core antigen</td>
</tr>
<tr>
<td>HBeAg</td>
<td>Hepatitis B e antigen</td>
</tr>
<tr>
<td>HBIG</td>
<td>Hepatitis B immune globulin</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>HTLV-111</td>
<td>Human T-cell lymphotropic virus</td>
</tr>
<tr>
<td>IEM</td>
<td>Immune electron microscopy</td>
</tr>
<tr>
<td>NMC</td>
<td>Natividad Medical Center</td>
</tr>
<tr>
<td>OMR</td>
<td>Other mentally retarded</td>
</tr>
<tr>
<td>PHC</td>
<td>Primary hepatocellular carcinoma</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
</tbody>
</table>
SECTION ONE
INTRODUCTION

Hepatitis B is one of the most widespread infections; both overt and asymptomatic infections are ubiquitous. The putative etiologic agent of type B hepatitis infection is hepatitis B virus (HBV); it displays morphologic, biochemical, and biologic characteristics rarely seen on other viruses. The host-virus relationship appears to be quite complex; pathogenesis results in a spectrum of immunologic and clinical responses in the host from no response at all to asymptomatic carriage of the virus to fulminant hepatitis. There are a multitude of diverse mechanisms of virus transmission which are not all yet fully understood. These features of HBV infection make it unique in its etiology, pathogenesis, clinical manifestations, and epidemiology.

Rapid advances in hepatitis B research began in the mid-1960s with Blumberg's discovery of the originally called Australia antigen (currently referred to as hepatitis B surface antigen: HBsAg) and later association of this antigen with type B viral hepatitis. There are an estimated total of 200 million chronic carriers of HBsAg; up to 50 million of these chronic carriers may be due to transmission of HBV from carrier mothers to their infants. In areas where HBV is hyperendemic, the role of mother-to-infant transmission of infection may be the most important explanation for the high endemicity of infection.

While most HBV infections in infants are asymptomatic, the spectrum of disease found in infants of HBsAg-positive mothers includes not only chronic asymptomatic HBsAg carriage, but also transient mild acute hepatitis B, chronic active hepatitis B with or without cirrhosis, chronic persistent hepatitis, and fatal
fulminant hepatitis. Increasing evidence suggests that the chronic HBsAg carrier state—especially when acquired at birth or early in life—increases the risk of subsequent liver disease. The liver disease associated with the chronic carrier state includes chronic–active hepatitis, cirrhosis, and primary hepatocellular carcinoma (PHC).\textsuperscript{33,219}

Work has been done to determine the prevalence of the HBsAg carrier state and degree of transmission from carrier mothers to their infants in countries where HBV is hyperendemic. The prevalence of HBsAg is 15–20\% in the general population among Chinese persons; mother–to–infant transmission appears to account for 40–50\% of the carriers.\textsuperscript{21} The life–term risk of death from cirrhosis and/or PHC for Chinese male HBsAg carriers is between 40\% and 50\%; the relative risk of developing PHC among these HBsAg carriers is much higher than among noncarriers (relative risk: 223).\textsuperscript{18}

Increased awareness of these implications of mother–to–infant transmission of HBV infections led to studies in hyperendemic areas such as Taiwan as well as among high risk groups such as the Chinese in the United States to establish the role of prophylactic treatment of infants born to carrier mothers. Data indicates that properly timed combination therapy with hepatitis B immune globulin (HBIG) and HBV vaccine is highly efficacious in preventing infections among these newborns.

The U.S. is considered a low–incidence area for HBV infection. Studies of mother–to–infant transmission of HBV in this country have concentrated on the high risk Asian populations. Little is known about other ethnic groups in the U.S. who may be at increased risk of the HBsAg carrier state than is seen in the general population; Hispanics are one such group of people.

Data indicates that while not at as much of an increased risk for HBsAg carriage as are the Chinese and other high risk populations, Latin Americans are
at higher risk of acquiring HBsAg carrier state than is the general population of the U.S. In addition to advancing our understanding of the epidemiology of hepatitis B, an estimate of the prevalence of HBsAg among pregnant Hispanic women in the U.S. would have important clinical usefulness.

This proposal to study the prevalence of HBsAg among pregnant women of Spanish surname in Salinas, California, was promoted by a desire on the part of clinicians to have this information; knowing this prevalence will enable them to make more informed decisions about screening for HBsAg in pregnant Hispanic women. Such a study has not been done in any Hispanic population in the U.S. Nearly all of the women of Spanish surname who utilize Natividad Medical Center, where the study is being conducted, are from Mexico or are of Mexican descent. Data from Mexico, while limited, indicates that prevalence of HBsAg among the Mexican population may be at least 1.6%,¹³² about four times the level seen in the general population of the U.S.

This paper will present background information about HBV infection including a brief history of hepatitis B, discussions of hepatitis antigens, modes of transmission of HBV, clinical features of infection in infants as well as adults, an overview of the HBsAg carrier state, and the possible causal association of HBV and PHC. The study currently being conducted in Salinas, California, will be described at the conclusion of the paper.
SECTION TWO

DISCOVERY OF THE AUSTRALIA ANTIGEN

The evolution of knowledge about the natural history of type B hepatitis has involved various phases. In the 1940s, evidence about hepatitis infection was based on clinical and laboratory indicators of jaundice. Between 1956 and 1966, subsequent to the development of serum transaminase assays (nonspecific though sensitive markers of liver involvement in hepatitis), serial studies were conducted to learn more about hepatitis infection. These epidemiologic studies allowed identification of subclinical cases of hepatitis as well as cases with persistence of chronic infection when jaundice was absent. The next advance in our understanding of type B hepatitis began in the mid-1960s with the discovery of Australia antigen and its association with hepatitis B.

Baruch S. Blumberg received a Nobel Prize in Physiology/Medicine in 1976 for work begun in 1957 which studied serum protein variants in Basque, European, Nigerian, and Alaskan populations—and later in other ethnic groups as well as in animals—to discover "striking variations in gene frequencies." In 1960, after the biochemical and antigenic variation in serum was well documented, Blumberg and associates found that precipitating antibodies against polymorphic serum proteins had formed in blood donor patients who had received numerous transfusions.

One day in 1963, using the serum of a New York hemophiliac patient, these researchers saw a precipitin band unlike any before noted. This patient's serum formed the precipitin band by reacting with the serum of an aborigine from Australia; this reactant from the Australian aborigine was called Australia antigen.
(Au). After this discovery, the researchers began to look for Au and antibody (Ab) to Au in different human populations. They found it to be rare in normal U.S. population groups (1/1000 positive serums) but common in some tropical and Asian populations. In testing disease groups for the distribution of Au and Ab to Au, they found Au only in transfused patients and in Down's syndrome patients.

In 1966 their studies showed that a Down's syndrome patient originally negative for Au had developed a form of chronic anicteric hepatitis before a subsequent test for Au became positive. Upon testing their hypothesis that Au was associated with hepatitis, the researchers found Au in the blood of many hepatitis patients early in the disease; it disappeared after a few days or weeks.

The association of Au with acute viral hepatitis was confirmed when Barbara Werner, a technician in the laboratory where this research was being conducted, noticed that she was ill and tested her own serum for Au, thus diagnosing the first case of viral hepatitis by a positive Au test. In 1970, in the midst of a prospective study being conducted to assess the role of Au in posttransfusion hepatitis, it became clear that hepatitis was occurring so much more frequently in the recipients of Au-positive blood than in the recipients of negative blood that transfusion of known positive blood was discontinued.79

After screening programs for Au in donor blood were established, posttransfusion hepatitis frequency dropped dramatically (e.g., from 18% to 6% in one early study started in 1969.193 With the establishment of routine testing for Au in blood banks in the U.S. and many other countries, the savings in human suffering as well as money by preventing such cases of posttransfusion hepatitis has been significant.

Also in 1970, Dane and associates proposed that the 42nm diameter virus-like particles that they found in serum samples of some Au-positive hepatitis patients might be complete virus particles; they further suggested that the much
more numerous 22nm particles as well as the long forms of Au were noninfectious
virus-coat material. The 42nm particles were subsequently named Dane par-
ticles. Using detergent treatment on the sera of patients with what was then
referred to as serum hepatitis or as Au-associated hepatitis (currently known
as hepatitis B0, it was shown that in addition to the outer coat of Au, the Dane
particles also contained a spherical inner particle 27nm in diameter, designated
hepatitis B core antigen (HBCAg).3

Agar-gel diffusion and complement-fixation (CF) were the assays used
around 1970 for detecting Au and Ab to Au (at which time they were also called
hepatitis-associated antigen [HAA] and antibody [anti-HAA], respectively) before
more sensitive tests such as passive hemagglutination and radioimmunoassay (RIA)
became available around 1973. Au was redesignated hepatitis B antigen (HB Ag)
in 1972 when its association with type B hepatitis was officially recognized.242
Subsequent to that designation, HB Ag became known as hepatitis B surface antigen
(HBsAg) as it is currently referred to. (This paper will use the term HBsAg, even
when referring to studies done before the nomenclature changed to this designa-
tion.) More sensitive assays were able to detect the presence of HBsAg and anti-
HBs in those cases in which the former, less sensitive tests would not have been
able to.

It is important to keep in mind the laboratory technique employed when
interpreting data which has been compiled over the years. With highly sensitive
RIA, HBsAg was detected as early as six days after parenteral exposure to HBV
using the same serum samples in which HBsAg had been detected 29-43 days after
parenteral exposure, and 67-82 days after oral exposure when examined some
years earlier using CF.111

Table 1 uses reported incubation period of HBV infection following par-
enteral exposure as an example of how the various laboratory methods have af-
### TABLE 1
ESTIMATES OF HBV INCUBATION PERIOD

<table>
<thead>
<tr>
<th>Time</th>
<th>Marker of Infection</th>
<th>Estimate of Incubation Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940's</td>
<td>Jaundice</td>
<td>60 days</td>
</tr>
<tr>
<td>1950's</td>
<td>Serum Transaminase</td>
<td>41 days</td>
</tr>
<tr>
<td>Early</td>
<td>Relatively insensitive</td>
<td>29 days</td>
</tr>
<tr>
<td>1970's</td>
<td>tests for HBsAg (e.g., CF)</td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>Highly sensitive RIA</td>
<td>As low as 6 days</td>
</tr>
</tbody>
</table>

affected interpretation of data over the years.
TABLE 2: CHARACTERISTICS OF HBsAg

Noninfectious HBsAg, the outer envelope of the HBV particle, is found as three distinct morphologic forms: the 22 nm diameter sphere is the predominant form; filamentous forms are also about 22 nm in diameter but 30 to 400 nm or longer; a 42 nm particle containing HBsAg immunoreactive material on its envelope has also been associated with HBsAg. These HBsAg particles consist of viral coat protein but contain no nucleic acid.

**Molecular weight estimated at 2.4 to 4.6 X 10^6 daltons.

**Different morphological forms of HBsAg appear to have no immunological differences.

**Distinct genotypes of HBV specify major subtypes of HBsAg. The relative distribution as well as the numbers of polypeptides are similar among the various subtypes of HBsAg.

The common antigenic determinants a and at least two allelic pairs of subtype determinants (d/y and w/r) are expressed as four major subtypes: adw, ayw, adr, and ayr as well as further categories of these major subtypes and as other incompletely studied determinants. Information about geographic distribution and clustering of subtypes has been added to the epidemiologic data available; however, these subtypes do not appear to correlate with clinical course or outcome of HBV infection. While there does exist evidence for cross-protection between subtypes in chimpanzees, similar results may not be as definitive in people151, p. 55.

** Approximately 20-30% lipid; protein is thought to make up 40-60% of HBsAg.

**Other major components include cholesterol, phosphatidylcholine, sphingomyelin, lysophosphatidylcholine and small amounts of other lipids.

**About 5% of the Ag is carbohydrate; this carbohydrate is thought to stabilize immunoreactivity but not to be necessary for antigenicity.
Table 2 - Continued

**The antigenicity of the particles is not affected by removal of lipid; however, HBsAg appears to be largely dependent on intact disulfide bond conformation for antigenicity. Reduction and alkylation leads to considerable loss of morphological and antigenic integrity.**

**Those enveloped viruses which acquire their outer coats at the surface of the cells which they invade often carry antigenic determinants of the host in these outer coats. It is expected that normal serum components (e.g., albumin) might be closely associated with HBsAg particles (which are synthesized and released by hepatocytes). However, it is not known whether host tissue or serum proteins are components of HBsAg; one suggestion is that some amino acid sequences of the polypeptides may be specified not by the viral genome, but rather by the host. Proteolytic enzyme treatment, however, of HBsAg appears to remove host protein antigenic material which preserving the structural, antigenic, radiolabeled proteins of the particle; therefore it is doubtful that host antigenic material is an integral component of HBsAg. Pre-albumin, albumin, and the $\lambda$ chain of IgG share certain antigenic sites which are physically associated with HBsAg$^{145}$; binding sites for thyroxine and triiodothyronine are also present$^{146}$. It has been postulated that the above-mentioned host proteins are incorporated into those HBsAg particles which have components of these antigenic sites and thereby alter the host immune response to HBsAg$^{236}$. Others suggest that these host proteins may be bound to HBsAg particles as contaminants or because of weak immunologic cross-reactivity$^{40}$. Researchers have not resolved questions about the ability of the host to mount immune response to such host-associated antigens; such information is of importance as it may contribute to not only recovery from acute infection but also to acute liver pathology or even chronic infection if an infective immune response is mounted.

**HBsAg can be stored at room temperature or frozen for years with antigenicity survival.

**HBsAg accumulates to high levels in the circulation, far higher than that of HBV. It is estimated that as many as 3 X $10^{13}$ particles are present in patients infected with HBV and concentrations may reach 20 mg/dl or greater.
Table 2 - Concluded

**HBsAg particles are shown to be in the cytoplasm and close to or on the cell membrane in infected hepatocytes. Particles have also been identified in lymph nodes, spleen, kidney, synovial membrane, blood vessels, and other extrahepatic tissues**\(^{141}\); it is thought that these locations represent hematogenous spread.

**While not infectious, HBsAg does elicit specific antibody formation (anti-HBs).**

**One hypothesis includes circulating HBsAg as instrumental in the immunopathology of HBV infection; it is proposed that hepatocytes synthesize the coat material of HBV (i.e., HBsAg) in excess of whole virus. These HBsAg particles may then enter the circulation and compete with intact virus for cell receptor sites.**
TABLE 3: CHARACTERISTICS OF HBcAg

Infectious Dane particles contain DNA and consist of an outer envelope which contains the major viral coat protein, carbohydrate, lipid (i.e., HBsAg), and HBcAg, a 27-nm nucleocapsid core.

**Molecular weight of the circular, double-stranded DNA of the core is 1.6 X 10^6 daltons.

**HBcAg was first shown to be immunologically distinct from HBsAg using immunoelectron microscopy; further support for this distinction came with the results of lack of humoral or cell-mediated immune cross-reactions to HBsAg and HBcAg.

**In infected hepatocytes, HBcAg is found predominantly in the nucleus.

**Core is present in the liver of carriers of HBsAg or people with HBsAg-positive chronic hepatitis (both considered chronically infected patients); however, it is only infrequently found in the liver of patients with acute hepatitis B infection.

**It is still not known whether the various polypeptides of cores are coded by the virus or by the host.

**It is thought that perhaps: 1) the DNA content of the core has been underestimated; 2) some of the polypeptides are host-specific; 3) the polypeptides share their amino acid composition (i.e., they are not unique) because the size of the core DNA appears too small to code for the proteins isolated from HBsAg and the core.

**HBcAg is found to be localized in the hepatocyte and not in extrahepatic tissue as HBsAg. [It is thought that HBV replication takes place only in the liver; lower concentrations (10^10/ml) of Dane particles accumulate than HBsAg.]

**HBcAg cannot be detected as free antigen in serum; however, detergent treatment of serum with abundant HBV leads to release of HBcAg from the HBsAg coat which surrounds it. The core—if ever a free particle in the serum—is thought to occur only rarely as an artifact of disintegrated whole virus secondary to experimental procedures.
**Anti-HBc is believed to indicate the presence of HBV in infected hepatocytes and to be a marker of HBV replication. It is usually the first antibody detected in serum several weeks to months after circulating HBsAg appears in patients with acute hepatitis B; it usually develops while HBsAg is present.**
<table>
<thead>
<tr>
<th>HBeAg</th>
<th>DNA POLYMERASE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBeAg is found in sera positive for HBsAg but does not itself appear to be an immunogenic component of HBV.</strong></td>
<td><strong>DNA polymerase is specific to the morphologically and immunologically similar core particles.</strong></td>
</tr>
<tr>
<td><strong>Reports show HBeAg to be associated with DNA polymerase in HBsAg-positive serum, with large numbers of HBV particles, and with a high degree of infectivity.</strong></td>
<td><strong>DNA polymerase is not found in association with HBsAg.</strong></td>
</tr>
<tr>
<td><strong>Serum containing anti-HBe seems to have low levels of infectivity.</strong></td>
<td><strong>DNA polymerase's reaction, localized in the core, appears to be dependent on the DNA template associated with the core.</strong></td>
</tr>
<tr>
<td><strong>Anti-HBe has been shown to persist for 1-2 years.</strong></td>
<td><strong>DNA polymerase activity is thought to correlate with the number of HBV particles in the serum and with the presence of HBeAg.</strong></td>
</tr>
<tr>
<td></td>
<td><strong>DNA polymerase activity is believed to be specific to HBV.</strong></td>
</tr>
</tbody>
</table>
SECTION THREE

SUBCELLULAR STRUCTURE OF HBV

Overview

The Dane particle (currently referred to as HBV) is thought to be a DNA-containing virus unique enough, both morphologically and structurally, to be classified unto itself. Electron microscopy reveals a spherical particle, 42nm in diameter, which appears to be double-stranded. The distinguishing features of this unique virus include a 3000 nucleotide length of the DNA genome which is circular and only partly single-stranded; the plus strand is incomplete (see Figure 1). A DNA polymerase is complexed with this incomplete plus strand, thus enabling it to use the complete virus strand as a template.

HBV is found in the serum of patients with acute hepatitis B and in the circulation of some chronic carriers of HBsAg. HBV particles appear to be heterogeneous; this heterogeneity—based on the existence of both high and low density populations—seems to be related to differences in the inner core components. The inner core of HBV, about 27nm in diameter, contains specific antigenic material known as hepatitis B core antigen (HBcAg). This core contains both the circular double-stranded DNA and the DNA-dependent DNA polymerase of the virus. Surrounding the core is a 27nm thick envelope which contains HBsAg, a material apparently identical to that found in smaller particles averaging 22nm. These smaller, somewhat pleomorphic particles circulate in infected patients in extremely large quantities and may take other morphological forms described as tubules or tadpole-like structures (see Figure 2).

HBV has been associated with persistent noncytopathic infections of the
Figure 1. HBV Genome (from the 1984 International Symposium on Viral Hepatitis, San Francisco Hilton Hotel, March 8-10, 1984).
Figure 2. Schematic diagram of HBV and its associated antigens (from Koff, Viral Hepatitis [1976], p. 105).
liver. There is circumstantial evidence that the HBV particle is infectious in humans and in the virus of hepatitis B; however, this has not yet been proved. The presence of DNA and DNA polymerase in the core, intact HBV in serum known to be infected, and the apparently infectivity of high density HBV particles for chimpanzees,\textsuperscript{150} all help to substantiate the idea that the HBV particle is the infectious viral agent of type B hepatitis.

There are two types of particles which accumulate in the blood after being secreted by infected hepatocytes. These two particle types are HBsAg and HBCAg. HBCAg and HBsAg, both associated with whole HBV particles, induce specific Abs (anti-HBc and anti-HBs, respectively, which do not cross react with one another). A third antigen exists which has been designated HBeAg. It too is capable of inducing a specific Ab, anti-HBe; however, the antigen does not appear to be particulate nor has it been identified as a structural component of HBV. HBeAg was identified in 1972;\textsuperscript{122} it is chemically and immunologically distinct from HBCAg and HBsAg.\textsuperscript{147} Thus far it has been detected only in materials positive for HBsAg.

After HBsAg, DNA polymerase and anti-HBc were the next two markers of HBV infection to be reported in the growing body of literature about hepatitis B. Most recently, more sensitive RIA techniques for the detection of HBsAg, anti-HBs, anti-HBc, HBeAg, and anti-HBe have been developed and used to reevaluate findings of previous studies.

**SeroLogic Markers and HBV Infection**

Most acute HBV infections reveal HBsAg in the serum several weeks before onset of symptoms or biochemical evidence of liver damage; in some cases HBsAg appears as early as a few weeks after exposure. During acute illness, HBsAg can be found for a few days up to several months; it is no longer detectable during
convalescence. Both HBsAg and HBeAg have been shown to persist two to five months and occasionally for greater than one year after recovery.\textsuperscript{111}

The appearance of anti-HBs appears in most cases of acute hepatitis after the disappearance of HBsAg; however, in some cases a period of up to several months may occur during which neither HBsAg nor anti-HBs is present. Anti-HBc, the only marker of recent HBV infection, often appears while HBsAg is still present during acute hepatitis. After the disappearance of their respective antigens, both anti-HBs and anti-HBc have been shown to persist for greater than seven years in some cases.\textsuperscript{111} Persistence for many months or years of HBsAg and anti-HBc—seen in a small proportion of adults with HBV infection, but more commonly among children—indicates chronic HBV infection (see Figure 3 for graphic representation of serologic responses).

By prospectively following people who were accidentally inoculated with small volumes of HBsAg-positive blood via needlestick, blood negative for DNA polymerase and HBeAg did not result in hepatitis, whereas DNA polymerase-positive and HBeAg-positive donor blood was significantly correlated with hepatitis in the recipient.\textsuperscript{7} This study did not rule out the possibility that, in large quantities, blood negative for DNA polymerase and HBeAg might be highly infectious; however, the data did suggest that DNA polymerase and HBeAg might be indicators of ongoing viral replication and of the relative infectivity of HBsAg-positive serum.

The following summarizes the ways in which HBV serologic markers are used to differentiate the various stages and consequence of infection:

1. Epidemiologic Markers
   A. HBsAg subtypes
   B. Anti-HBs
   C. Anti-HBc

2. Acute Disease Markers
   A. HBsAg
   B. Anti-HBc IgM
Figure 3. The serologic responses of an individual with a transient HBsAg response and typical acute HB after exposure to HBsAg-positive serum (chronic carrier serum) (from Maxcy-Rosenau, *Public Health and Preventive Medicine* [1980], p. 161).
3. Infectious Virus Markers
   A. HBsAg
   B. HBeAg
   C. DNA polymerase
   D. HBcAg
   E. Anti-HBc

4. Immune Status Markers
   A. Anti-HBs
   B. Anti-HBc

5. Chronicity Markers
   A. HBsAg
   B. HBeAg
   C. Anti-HBc

6. Prognostic Markers
   A. HBsAg titer
   B. HBeAg/anti-HBe

Serologic Techniques for the Detection of HBsAg

The World Health Organization Expert Committee on Hepatitis issued a report in 1953 discussing the important public health problem that the disease represented, and called attention to the limited information available about its etiology and epidemiology. The same committee issued a second report in 1964 which pointed to advances in the control of hepatitis type A (via passive immunization) but was unable to state that such progress had been made for hepatitis type B.

In the late 1960s HBsAg (so-called Au) was recognized as useful for detection of HBV; subsequent to this realization, laboratories throughout the world developed techniques for studying HBV. In 1977, the Department of Health, Education and Welfare (through the Centers for Disease Control, Atlanta, Georgia) published a diagnostic manual in order to help institute worldwide testing for HBsAg. Two years earlier, the World Health Organization (WHO) recommended reverse passive hemagglutination (RPHA) for worldwide screening for HBsAg in blood donors. They also recommended that regional areas provide radioimmu-
noassay (RIA) procedures in order to assess the RPHA results.

RIA and RPHA are the most sensitive techniques available for the detection of HBsAg. Counterimmunoelectrophoresis (CIEP) is less sensitive and specific, but it is an inexpensive and simple procedure used to screen blood routinely for HBsAg. Enzyme immunoassays (EIA), an advanced technique not performed routinely, may have advantages over both RIA and RPHA. A brief discussion of various serologic techniques for the detection of HBsAg follows.

Immunodiffusion technology was the first method used to identify HBsAg. This technique is insensitive and slow, but also specific, simple, and inexpensive; therefore, it is used to identify major antigenic subtypes (adw, ayw, adr, and ayr). From two to ten times more sensitive than immunodiffusion and faster (completion of procedures requires minutes to a few hours) are tests such as CF, CIEP, RPHA, and rheophoresis. Of all the above methods—except perhaps immunodiffusion and rheophoresis—numerous false-positive results are likely to occur in routine tests of large numbers of serum samples.

A second order test, in terms of relative sensitivity, for detection of HBsAg is immunofluorescence microscopy; this method is used primarily in research as it is highly specialized. Immune electron microscopy (IEM) is also a highly specialized procedure used predominantly for research. A more commonly used third order test is RPHA. Immune adherence hemagglutination is used only in some areas of the world.

RIA—both solid-phase and radioimmuno-precipitation methods—and EIA are the most sensitive techniques for the detection of HBsAg. False-negative results are minimized and early false-positive results no longer appear to be a problem. EIA appears as sensitive as RIA and has added advantages of long shelf-life and stable reagents as well as simple equipment. EIA is not yet used on a large scale although it is expected to be more widely applied in the future. The
serum samples of the proposed study will be tested for the presence of HBsAg using RIA.

### TABLE 5

**Sensitivity of Serologic Techniques for Detection of HBsAg**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Relative Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme immunoassay (EIA)</td>
<td>++++a</td>
</tr>
<tr>
<td>Radioimmunoprecipitation</td>
<td>++++</td>
</tr>
<tr>
<td>Solid-phase radioimmunoassay (RIA)</td>
<td>++++</td>
</tr>
<tr>
<td>Reverse passive hemagglutination (RPHA)</td>
<td>+++</td>
</tr>
<tr>
<td>Immune adherence hemagglutination (IAHA)</td>
<td>+++</td>
</tr>
<tr>
<td>Immune electron microscopy (IEM)</td>
<td>+++</td>
</tr>
<tr>
<td>Passive hemagglutination</td>
<td>++b</td>
</tr>
<tr>
<td>Reverse passive latex agglutination (RPLA)</td>
<td>++</td>
</tr>
<tr>
<td>Rheophoresis</td>
<td>++</td>
</tr>
<tr>
<td>Immune fluorescence microscopy</td>
<td>++</td>
</tr>
<tr>
<td>Complement fixation (CF)</td>
<td>++</td>
</tr>
<tr>
<td>Counterimmunoelectrophoresis (CIEP)</td>
<td>++</td>
</tr>
<tr>
<td>Agar gel immunodiffusion</td>
<td>+</td>
</tr>
</tbody>
</table>

*Estimated gradation from most sensitive (++++) to least sensitive (+). These gradations do not give any indication of the relative degree of specificity of the above methods.*

*HBsAg detection by inhibition of passive hemagglutination.*
SECTION FOUR

CLINICAL FEATURES OF HEPATITIS B INFECTION

Adult Response to Infection

Human volunteers experimentally infected with serum contaminated with HBV often experienced a pattern of response as follows: (1) the relatively long incubation period of acute hepatitis with HBsAg appearance in the blood during the prodromal and acute phases along with clinical symptoms; (2) self-limited disease in most cases with clinical recovery and disappearance of HBsAg from four to six weeks after onset; and (3) protection from subsequent infection with HBV in those patients who develop anti-HBs during convalescence.26,216 "Natural" infection, however, less commonly responds in this pattern; instead, a variety of responses are seen subsequent to HBV infection (see Figure 4). These responses include the acute hepatitis described above as well as chronic hepatitis (which includes both chronic aggressive and chronic persistent). Chronic hepatitis may develop from acute hepatitis or may have a more insidious onset; postnecrotic cirrhosis and primary hepatocellular carcinoma are potential sequelae. Other possible responses to HBV infection include asymptomatic infection during which the liver is only minimally damaged or not damaged at all with HBsAg transiently produced and protective titers of anti-HBs developing. Finally, "chronic carriers" of HBV are those people who respond to infection with no symptoms, little or no liver damage, and who persistently produce HBsAg with minimal or no cellular immune response to the antigen and minimal synthesis of anti-HBs.

It is believed that the abnormal immune state associated with hepatitis B infection is not secondary to liver injury; whether these immunologic aberrations
Figure 4. Possible outcomes and sequences of events after exposure to the hepatitis B virus (from Szmuness, Recent advances in the study of the epidemiology of hepatitis B [1975]).
are a direct result of the viral infection or precede the infection (thereby perhaps predisposing to a carrier state) is not known. An evaluation of 69 asymptomatic HBsAg carriers revealed B cell function to be intact, whereas T cell function was markedly impaired.\(^{239}\) The report did not determine whether this abnormal immune status predisposed the individuals to develop a carrier state or whether T cell function abnormalities were a result of HBV infection. The data indicate, however, that liver injury secondary to hepatitis B infection was not responsible for immunologic abnormalities.

Development of the HBsAg carrier state, with or without chronic liver disease, is associated with persistence of HBeAg in acute hepatitis B. It has been suggested that the presence of HBeAg in patients with chronic persistent hepatitis may indicate a serious prognosis; data from 53 patients participating in a study in Denmark indicate that of those patients with chronic persistent hepatitis in the presence of persistence of HBeAg, a significant number developed chronic active hepatitis or cirrhosis.\(^2\)

**Infant Response to Infection**

In a study of 80 children with acute and chronic hepatitis B, the peak frequency among those children less than one year of age was seen between ages two and five months, suggesting perinatal infection.\(^{63}\) Nine of the infants acquired infection associated with contact with their HBsAg carrier mothers; an oral route of infection was postulated based on compatibility of the average time between birth and the appearance of jaundice in the infants (103 days), with average incubation time reported for hepatitis induced by oral inoculation of the MS-2 strain of HBV (98 days).\(^{108}\)

Some studies suggest that infants are more prone to develop chronic persistent hepatitis and become HBsAg carriers.\(^{188,202}\) However, in the above-
mentioned study of 80 children, HBsAg disappeared from the serum within one to five days after onset of jaundice in all of the infants and only three infants developed persistent antigenemia. This study suggested that neonatally infected infants may have adequate immune responses to HBV infection considering that these neonatally infected infants were able to completely eliminate HBV. It has been suggested that the high frequency in certain countries with which neonatally infected infants become HBsAg carriers\textsuperscript{153,202} may not be due to immature immune systems, but rather to genetic (polygenic in nature) and environmental factors.\textsuperscript{130,218}

Most infants infected with HBV have no symptoms of their infection and those who develop anti-HBs are thought to be immune for life with no increased risk of subsequent liver disease. There have been reports of babies, younger than six months of age, capable of completely eliminating the HBV, who died of severe hepatitis B viral infection; some of these infants apparently became infected by their chronic HBsAg carrier mothers.\textsuperscript{62}

Most infants who become chronic HBsAg carriers, acquired from their mothers, show histologic evidence of chronic persistent hepatitis but do not become icteric; they have a transient increase in serum ALT levels.\textsuperscript{90} Follow-up for over ten years of some infants manifesting these changes has shown that they may go on to develop more serious liver disease. Among some such chronic HBsAg carrier children followed, periodic increases in their serum ALT levels have taken place; among eight infants whose initial liver biopsies showed chronic persistent hepatitis, three developed spider angiomas, three developed hepatomegaly, and two developed both angiomas and hepatomegaly.\textsuperscript{190}

While acute hepatitis is an uncommon reaction to neonatal exposure to HBV, there have been isolated cases reported of infants born to HBsAg carrier mothers going on to die of fulminant hepatitis;\textsuperscript{191} however, a far more common
immunopathologic response of neonates exposed to HBV—apparently the most common reaction—is subclinical hepatitis infection. No clinical signs of illness are seen; however, persistent antigenemia and elevated serum transaminase activity has been repeatedly reported.51,135,187,227 Chronic active viral liver disease with cirrhosis has been reported to develop occasionally in some infants following HBsAg-positive neonatal status.46,80,187,243
SECTION FIVE

MODES OF TRANSMISSION OF HEPATITIS B VIRUS

Introduction

Both parenteral inoculation and ingestion of HBsAg-positive blood have been shown to be routes of transmission of HBV.\textsuperscript{78,107} Hepatitis B antigen had been detected in serum, sweat, and ascitic fluid before it was sought and confirmed to be present in vaginal secretions.\textsuperscript{54} The epidemiological significance of other potential routes of infection such as saliva and semen—while being studied—remain in doubt.

HBsAg was found in the saliva specimens of 18 of 24 men whose serum was known to be positive for HBsAg; of those 18 men, 10 also had detectable HBsAg in their semen.\textsuperscript{88} Hepatitis B has been convincingly demonstrated to be transmitted from one sexual partner to another. It remains uncertain, however, whether or not HBsAg-positive saliva and/or semen is capable of transmitting hepatitis B. Such a possibility should not be ruled out; even though the amounts of HBsAg in saliva and semen were small when compared with serum levels, the minute amounts of HBsAg-positive blood which may be infectious point to similar potential in saliva and semen.

In a study of 67 patients (43 males, 24 females) admitted to two London hospitals with acute hepatitis B, nonparenteral spread was more common than parenteral. The definite or most likely source of infection in 40\% of patients was sexual or domestic contact.\textsuperscript{87}

It is believed that contact with HBsAg carriers or jaundiced patients may be—now that the incidence of posttransfusion hepatitis has been lowered by routine
screening for the presence of HBsAg—the single most important factor in the spread of acute type B hepatitis in large, urban areas. Transmission appears likely to occur between sexual partners; there are numerous reports of healthy carrier (asymptomatic, chronic HBsAg-positive) males apparently transmitting hepatitis B to their female sexual contacts. There has been speculation about the roles of promiscuity and homosexuality in sexual transmission of HBV.

Only a minority of those patients with acute hepatitis become jaundiced; therefore transmission by healthy carriers may present an even larger problem than is commonly perceived. In addition to sexual transmission, close contact between individuals is thought to play a role in transmission of HBV.

Work began in numerous areas to search for nontransfusion sources of HBV transmission when it became apparent that some patients with type B hepatitis had no history of transfusions or parenteral exposure. A number of potential routes of transmission, in addition to close contact (including sexual) have been explored. It became apparent that working in a hospital may increase one's risk of developing HBV infection.

Type B hepatitis infection is commonly evident among hemodialysis patients. Data from patients and staff of four hemodialysis units in two metropolitan areas of the southwest United States revealed that 34% of patients and 36% of staff were seropositive for HBsAg or anti-HBs; of all the groups studied, including controls, dialysis patients showed the highest prevalence of HBsAg (6%). None of the patients or staff had any prior history of hepatitis infection. Among staff members, the housekeeping group had the highest prevalence of serological reactivity for HBsAg or anti-HBs, with technicians and nurses following; doctors and clerical personnel showed low prevalence. The authors suggested that these findings may indicate that airborne transmission may not be a major route of spread of infection while handling of blood-contaminated articles may be.
Numerous other routes of transmission of HBV have been examined. Evidence exists suggesting that blood-sucking insects may serve as vectors; however, it is not known whether biological transmission of HBsAg by mosquitoes is possible.\textsuperscript{247} Even tattooing has been implicated in HBV transmission.\textsuperscript{87} A number of potential major routes of transmission have been extensively examined; a more detailed discussion of these potential routes follows.

**Airborne**

Circumstantial evidence exists for airborne transmission of hepatitis B; however, it is not considered an important mode of transmission of the disease. Outbreaks in two separate hemodialysis units following spillage of HBsAg-positive blood supports the hypothesis of airborne transmission;\textsuperscript{4,74} however, studies have failed to demonstrate aerosolization of contaminated blood in a hemodialysis center\textsuperscript{166} or during simulated hemodialysis.\textsuperscript{134}

Contaminated serum from a patient with early icteric illness was directly inoculated into the noses of volunteers resulting in jaundice or elevated serum bilirubin levels in some.\textsuperscript{123} Other studies have failed to transmit hepatitis using similar methods\textsuperscript{155} or by administration of nasopharyngeal washings of patients with acute disease into nasopharynxes of volunteers.\textsuperscript{144}

**Oropharyngeal Secretions**

Some studies have detected HBsAg in the saliva of 75–90\% of patients with acute hepatitis B;\textsuperscript{38,231} the antigen has been detected intermittently in the saliva of chronic HBsAg carriers as well.\textsuperscript{101,167,231,234,240} HBsAg is found in saliva, cervicular fluid, and in mixed saliva specimens in patients with circulating HBsAg. Such HBsAg-positive samples often have occult blood\textsuperscript{233} and one mechanism of entry may be mucosal lesions which leak blood or serum. Duration of HBsAg in saliva appears to be shorter than HBsAg-positivity of serum.\textsuperscript{240}
Subcutaneous injection of contaminated saliva was infectious in gibbons; the same saliva pool inoculated by nasal or oral routes was not infective. Experimental results of nasogastric and oral inoculation of chimpanzees suggest that peroral transmission (gingiva given minute mucosal lesions by toothbrushing prior to spraying the oral mucosa with infectious plasma) spreads infection much more efficiently than does intestinal transmission. Therefore, oral-oral transmission cannot be ruled out and may represent inapparent parenteral transmission via small abrasions of mucosal surfaces; it is not yet known if direct and/or indirect (perhaps via fomites) contact might be mechanisms of preoral transmission. While no direct evidence exists, oral-oral transmission may be possible; current evidence indicates that peroral inoculation of infectious serum may transmit infection.

**Waterborne**

While waterborne transmission of viral hepatitis was recognized as a public health problem as early as 1895, and whereas hepatitis A is clearly implicated in waterborne outbreaks, a role for hepatitis B has not been clearly defined.

It has been reported that HBsAg is stable—for long times—in sewage or activated sludge and that HBsAg could contaminate a city sewage system; however, it is thought that HBsAg infrequently contaminates the feces of infected patients and therefore that HBsAg or other hepatitis B particles probably do not enter sewage from feces in appreciable quantities. Enzymatic fecal antagonists are believed to destroy or disrupt HBsAg and other hepatitis B particles in the gastrointestinal tract. Bacterial species (e.g., *Pseudomonas aeruginosa*) may inactivate hepatitis B in some surface and ground waters.

**Shellfish-Associated Hepatitis**

Hepatitis associated with bivalve mollusk ingestion, both raw and inadequately cooked, has been attributed to hepatitis A virus. The possibility
that hepatitis B can be transmitted by shellfish has not been completely discounted.

HBsAg has been detected in hard-shell clams in closed-system aquariums; these clams were able to transmit HBsAg to other clams.\textsuperscript{105} Hard-shell clams harvested from contaminated, untreated sewage from a small community hospital also harbored HBsAg.\textsuperscript{127}

**Foodborne**

Hepatitis A virus is reported to be the predominant agent of foodborne hepatitis. Hepatitis B outbreaks have not been found to be associated with fecal contamination of food or water. Oral inoculation of serum from children with hepatitis then known to be of long incubation (subsequently known to be HBV) did produce hepatitis in volunteers; contacts of these infected volunteers displayed a low degree of hepatitis B in contrast to hepatitis A which was highly contagious in the same study.\textsuperscript{107}

In spite of this experimental evidence for potential transmission of hepatitis B among volunteers orally inoculated with contaminated serum, enteric transmission by the fecal-oral route has not been substantiated. It has been suggested that the human intestine has inhibitory substances which may antagonize HBsAg such that immunologic reactivity is impaired.\textsuperscript{57} One study, using a variety of methods, found that all HBsAg-positive sera tested became negative subsequent to in vitro incubation with homogenates of human feces or intestinal mucosa.\textsuperscript{114}

Small quantities of HBsAg have been found in the breast milk of carrier mothers;\textsuperscript{36,118} however, one study followed 147 babies born to HBsAg carrier mothers for at least three or more months after delivery and found HBsAg and anti-HBs antigenemis to develop in nearly identical frequencies among breast-fed and nonbreast-fed infants.\textsuperscript{15}
Contact Transmission

As early as 1938, the theory of contact transmission of hepatitis B was supported by evidence of children with hepatitis (due to administration of contaminated measles serum) apparently spreading their infection to two other children; these two children developed jaundice two months after exposure to the children with hepatitis. Later, isolated reports presented associations of hepatitis developing in spouses or close contacts of individuals who had parenterally-transmitted hepatitis B. 

Despite this earlier work which had been done prior to the development of serologic techniques for identifying hepatitis B, the infection was thought to be noncommunicable. This concept—which conceived of type B hepatitis as distinct from contact-associated hepatitis—was formulated from observations that only very few secondary cases resulted from the yellow fever epidemic among U.S. military personnel in 1941, as well as from an inability to transmit the disease either by oral or intranasal administration of contaminated serum. These observations helped to establish the prevailing view of the late 1960s that so-called serum hepatitis was probably not infective orally nor from personal or intimate contact. 

With the subsequent development of serologic techniques for identification of hepatitis B, person-to-person transmission was more closely scrutinized as a potential mode of spread of infection in those 50% of adult patients with hepatitis B who gave no history of parenteral exposure. It is now believed that symptomatic HBsAg carriers may be able to spread hepatitis B via person-to-person contact. Various studies, while oftentimes uncertain about original source of infection, duration of household exposure, and time of initiation of carrier state, have shown high rates of infection within families or households containing carriers of HBsAg. Studies of household transmission of hepatitis B
have correlated oral contact, sleeping together, and close, intimate contact (including sexual contact) in nonparenteral transmission of hepatitis B among family units. 104, 106, 180, 201, 228

An epidemic of hepatitis which occurred in 1963-1964 in Costa Rica was reexamined after serologic tests for the detection of HBsAg became available; the results of testing sera which had been kept frozen as well as restudying the epidemiologic data obtained at the time strongly suggested that the outbreak had been of type B hepatitis. The following observations support a hypothesis of person-to-person transmission of HBV: 230

(1) Coincidence of duration of the epidemic with a severe dry period (perhaps explained by scarcity of water lowering hygienic standards, thereby enhancing person-to-person transmission);

(2) Antigenemia was detected twice as prevalently in urban and densely populated rural areas as in less densely populated rural zones;

(3) Nearly all patients had had contact with another person with hepatitis;

(4) Close personal contact seemed a likely mode of transmission intra-familially as children up to nine years of age appeared to transmit infection to siblings of the same age but not to adults, whereas adults seemed to transmit infection to other adults but not to children; and

(5) Only 20% had histories of either injections or dental procedures within six months prior to onset of illness.

Anecdotal observations began to suggest that HBsAg might be transmitted by nonparenteral or inapparent parenteral routes in household settings. For example, family members and acquaintances of an index patient, a 21 year old male heroin user, developed HBsAg-positive hepatitis B. None of the family members had a history of drug use or needle injection, although they did have close contact, sharing beds, toothbrushes, and the same bathroom. The index patient, his wife,
and one of his brothers were admitted to the hospital with hepatitis during a four month period. An additional brother and the index patient's six month old baby were asymptomatic but had HBsAg present in their serum. Anti-HBs was detected in two siblings of this family; however, it is not known if the antibody was prevalent in the family prior to the epidemic outbreak or if these family members had developed subclinical infection and subsequent anti-HBs in response to infection in their brother, sister-in-law, nephew, or their mother who was HBsAg-positive. It seems most likely that the index patient developed his hepatitis from shared contaminated needles with two friends who were also heroin users and who were also hospitalized with hepatitis.\textsuperscript{138}

In a survey of 449 family contacts from 197 households with an HBsAg-positive blood donor, the prevalence of antigen in these families approached levels seen in institutions for the mentally retarded, suggesting that HBV may disseminate widely in situations of close, long-term contact (only 24% of family contacts have a history of parenteral procedures). This investigation revealed that 6.7% of the family contacts of carriers were HBsAg-positive compared with 0.8% of control households; spouse antigenemia was 3.4%, whereas other genetic family contacts had an 8% prevalence of antigen with the greatest prevalence among siblings at 19.7%.\textsuperscript{213} These figures were counterbalanced by a relatively high prevalence of anti-HBs among spouses and low Ab prevalence among siblings such that combined prevalence of both antigen and antibody was essentially the same in siblings and in spouses. The overall results indicate that in the population at large one would be more likely to detect the presence of HBsAg or anti-HBs in family contacts of H3sAg-positive blood donors than in family contacts of antigen-negative blood donors.

The investigators suggested that long-term, close contact rather than sexual transmission, genetic considerations, or other mechanisms, may have been
the overriding determinant of spread of hepatitis B in the families surveyed. The intent of the study was to assess the frequency of familial clustering in a controlled survey, but the workers acknowledge that the higher prevalence of hepatitis B in the study families compared with the control families may have been due, at least in part, to ethnic and social differences between these two groups; the study sample contained proportionately more nonwhites and low-income families (21%) than did the control group (4%). Environmental factors such as inadequate housing and hygiene as well as overcrowding and close contact with other households may influence the prevalence of hepatitis B infections in families like those included in this study.

In a prospective study conducted in the U.S. of household members exposed to adults acutely infected with type B hepatitis, the secondary attack rate was low except for in the spouse; similar results (i.e., low risk except for spouses) were seen in household members exposed to chronically infected hemodialysis patients (all HBsAg-positive). These observations appear at first glance to be contradictory of reports of high infection rates seen among family members of carriers; however, the observations were of short- and intermediate-term exposure (from 4-40 months). The authors state that more long-term contact (over years) would likely represent more opportunity for transmission; they also report that chances of infection occurring more than doubled in frequency in studies of households with a second carrier.

Data from the Coordinating Group for Research on Hepatitis of the Chinese Medical Association demonstrated that household members in close contact with an HBsAg carrier have an increased risk of developing type B hepatitis; there was a significant difference in hepatitis B morbidity in HBsAg-positive families (51.2%) and HBsAg-negative families (5.3%). While HBsAg carriers were more prevalent in the lower age group, clinical hepatitis cases prevailed in the older
age group. In the HBsAg-positive families, both sexes had similar results; however, the distribution of positive family members ran 80.5% in spouses, 46.2% among parents, 40.8% among children, and 32.5% among siblings. The researchers concluded that close contact probably played more of a role in HBsAg transmission than other potential factors such as genetic predisposition to the carrier state.

Among Vietnamese children recently arrived in the U.S., HBsAg (27.3%) and anti-HBs (30.3%) were found to be highly prevalent. An investigation of members of an adoption agency revealed that 23.1% (6 of 26) of the American children tested were positive for anti-HBsAg, whereas none were HBsAg-positive.228 The adopted American children who were anti-HBs-positive were all members of families that had adopted Vietnamese children; five of the six lived in homes in which the Vietnamese child was positive for HBsAg, suggesting contact transmission. These figures contrast sharply with data showing only 4.1% (3 of 73) of middle- and upper-class American children to be positive for anti-HBs.57 The rates of anti-HBs positivity seen among the American children members of families with adopted Vietnamese children are close to those seen in institutions for the mentally retarded, particularly among children with Down's syndrome.211

A controlled survey of 583 patients and 451 medical personnel of 15 hemodialysis centers in the U.S. found HBsAg in 16.8% of the patients and in 2.4% of the medical staff; 61% of family contacts of dialysis patients with a history of hepatitis B infection had detectable HBsAg or anti-HBs serologic markers.215 Prevalence of HBsAg and anti-HBs was not significantly different between males and females nor among age groups; however, there were differences in relation to ethnic background. Nonwhites had significantly higher cumulative rates of HBsAg and anti-HBs; nonwhite patients had a higher prevalence of anti-HBs and a lower prevalence of HBsAg, while nonwhite staff had an increased prevalence of both HBsAg and anti-HBs.
The authors of this survey suggest that while blood transfusions can serve as a vehicle of HBV introduction into a dialysis center, duration of treatment rather than number of blood transfusions plays a more important role in spread of infection; adjusted rates correlated more strongly with duration of treatment. Nonparenteral routes were also thought to be the major influence of HBV infection among family contacts of infected dialysis patients; very few of these family contacts gave a history of needle sticks or other possible penetration of tissues with contaminated blood.

The question of contact-associated HBV infection is confounded by the possibility that one or more types of minor trauma may lead to percutaneous introduction of infection. Shared razors and shared toothbrushes\textsuperscript{140} and biologic vectors such as mosquitoes,\textsuperscript{58,115,148,175} and bedbugs,\textsuperscript{39,148} have all been suggested as possible routes of transmission.

**Sexual Transmission**

In contrast to spread of infection from asymptomatic HBsAg carriers, there appears to be low risk of household spread of infection from patients with acute hepatitis B who do not become persistent HBsAg carriers, except among spouses and sexual partners, suggesting that intimate, including sexual, contact may be an important mode of transmission of HBV. The period of infectivity in such cases seems to be finite. As with other sporadic evidence suggesting that hepatitis B might be infective by routes other than parenteral, the possibility of sexual transmission—raised by nonconcomitant hepatitis B in a small number of spouse pairs—was largely ignored for some time. Between 1946 and the early 1960s, a number of such cases were reported to develop two to three months after onset or peak of infection in the index patient.\textsuperscript{37,70,105}

In 1885, when the first careful study of a large outbreak of hepatitis among
shipyard workers who had received human serum–containing smallpox vaccine was conducted, no cases of hepatitis among spouses were reported. Since that time, HBsAg has been detected in vaginal secretions, semen, and is postulated to be present in menstrual blood; however, transmission studies have not been done and HBV has not been detected in these fluids.

The prevalence of anti-HBs in prostitutes in Germany was significantly higher than among female age-matched blood donors, suggesting that HBV might be sexually transmissible. The data showed a significantly higher prevalence of anti-HBs in those prostitutes registered (standard procedure by the Public Health Service in the German Federal Republic) longer than three years, especially among the 20–29 year age group, as well as in prostitutes who had more than three recorded venereal diseases (adjusted for age and duration of prostitution).

In order to examine the possibility that the association of anti-HBs and venereal disease might be due to close contact other than sexual, the antibody distribution of an orally transmissible (echovirus type 12) and droplet route transmissible (adenovirus type 10) agent were examined. While the percentage of antibody to both viruses was higher in prostitutes than in age-matched blood donors, there were not significant correlations between those antibodies and length of the registration period nor between antibodies and incidence of venereal disease. The researchers hypothesize that hepatitis B may be transmitted during sexual contact and that such a mode of infection may be more important in highly developed countries where mainly adults acquire the infection.

In Cali, Colombia, 509 persons including four study groups—prostitutes, nuns, persons associated with institutions, and a control group—were examined for HBsAg. The data did not support the hypothesis of venereal transmission of hepatitis B; HBsAg was highest among persons associated with institutions and lowest among prostitutes. Anti-HBs was detected in 20% of the prostitutes,
16% of the nuns, and 12% of the control population group with no statistically significant differences between the groups. Of the prostitutes participating in this study, more than two thirds were under 29 years of age. This may help to explain why the Cali results conflict with the results of the above-mentioned German study; in that study this age category of prostitutes did not exhibit more than 10% anti-HBs. Extended exposure time may be needed for the development of anti-HBs. The nuns in the Cali study worked in establishments involved in health services; people working in health-related fields are known to be at high risk of acquiring hepatitis. Sexual transmission of hepatitis B may not be an important route of spread of infection under certain epidemiologic conditions; in Cali there was a high prevalence of HBsAg and anti-HBs in young people of the control population and a lack of an increase in anti-HBs with increasing age.

Among 974 patients attending a clinic for sexually transmitted diseases in London, 17 were found to be positive for HBsAg while 47 were anti-HBs-positive. The distributions of Ag and Ab had statistically different characteristics; the Ag was found largely in nonpromiscuous heterosexual men of Mediterranean and Middle Eastern countries, whereas Ab was predominantly seen in homosexual men (the two female homosexuals, 22 bisexuals, and 38 others for whom insufficient sexual history was available were excluded from the study), and among promiscuous patients of British origin. Both the HBsAg-positive group and the anti-HBs group had a greater percentage of single men; there were no significant differences in social class distribution. The HBsAg group had a high frequency of gonorrhea and history of viral and fungal infections including genital warts, genital herpes, and monilial balanitis. The anti-HBs group had significant correlations for a history of both gonorrhea and syphilis and a much greater proportion of homosexuals and patients with three or more sexual contacts within the six months prior to examination.
The preponderance of men of Mediterranean origin with HBsAg was not surprising given the high prevalence of HBsAg carriers found in those areas.\textsuperscript{10,28} None of the men in the study had overt hepatitis and all were thought to be chronic carriers. The analysis used the relatively insensitive immunodiffusion test and the authors project that perhaps over 80\% of the homosexuals in their study would have been anti-HBs-positive if hemagglutination had been the method of detection employed. Given that presence of HBsAg is generally considered to be evidence of past infection, the study concluded that of the homosexual patients attending the clinic, a large majority had been infected with HBV at some time in the past. They further suggested that hepatitis B in the U.K. may be primarily a sexually transmitted infection after examining and ruling out the possible roles of lice or scabies mites, menstrual blood, and intravenous drug use in this study population.

In an analysis of 1,650 patients attending a venereal disease department of a London hospital, a rate much greater (i.e., 1.39\%) than that found in blood donor populations in the U.K. was noted.\textsuperscript{98} Highest rates were seen for homosexual patients (3.8\%) and non-European heterosexuals (3.1\%) including patients from Egypt, West Indies, Iran, U.S.S.R., St. Lucia, and Granada. The less sensitive immunoelectroosmophoresis and gel diffusion were used to detect HBsAg; therefore, these results need to be interpreted and compared with other studies with this in mind.

Substantial circumstantial evidence exists suggesting that HBV may be transmitted sexually,\textsuperscript{87,89} the way in which this may take place and the significance that a sexual route of transmission may play in the epidemiology and medical geography of the disease remain largely unanswered.

\textbf{Mother-to-Infant Transmission}

\textbf{Background.} HBV infection can be transmitted from mother to infant;
such transmission has been termed vertical transmission and perinatal transmission. This terminology is not inclusive nor entirely accurate, as most mother-to-infant infections have been shown to result from either maternal-fetal microtransfusions during labor and/or oral ingestion of contaminated vaginal contents by the infant during its passage through the birth canal. While in utero, neonatal (first four weeks after birth), perinatal (shortly before and after birth, generally considered to begin with completion of 28 weeks of gestation and ending one to four weeks after birth), and postnatal (after birth) transmission may occur occasionally, most transmissions appear to take place during birth. For these reasons, this paper will use the designation mother-to-infant transmission of HBV.

In 1862, Saint-Vel reported an epidemic of severe hepatitis in pregnant women in Martinique. Since that time the literature about the effects of viral hepatitis on the fetus and on the course of fetal development has included divergent viewpoints, often with incomplete data.

In 1951, the possibility of transplacental transmission of hepatitis virus was considered in a report of 12 infants who developed hepatitis within the first two months of their lives. In 1954, before tests for serologic markers of HBV were available, evidence of "silent carriers" (a term used by the researchers to refer to asymptomatic carriers with negative liver function tests) was presented. This evidence was partly a circumstantial, retrospective look at the presence of jaundice in recipients of blood of carriers as well as direct evidence from volunteer prisoners who developed jaundice after being injected with serum of suspected HBV carriers. Included in this report—pertinent to the question of mother-to-infant transmission of HBV infection—was evidence for transplacental transmission. An infant developed chronic hepatitis with jaundice at two months of age and died of advanced fibrosis of the liver at age 18 months. The baby was delivered by cesarean section, ruling out infection during passage through the
birth canal; transmission through breast milk was also ruled out as the baby was not breast fed. Serum from this carrier mother and her infant was injected into volunteers, some of whom developed hepatitis.

Various other reports, all prior to availability of laboratory tests for hepatitis B, presented evidence of hepatitis in children within weeks after birth.\textsuperscript{14,192,238} Utilizing the development of tests for HBsAg as a reliable marker of HBV infection, no such marker could be found in cord sera in a number of studies. These early tests were relatively insensitive and only small numbers of patients were used. Subsequently, cord blood of infants born to mothers with acute hepatitis was been found to be positive for HBsAg.\textsuperscript{191} Cord blood from infants born to asymptomatic HBsAg carrier mothers has also been positive for HBsAg.\textsuperscript{189} The placenta appears to act as a partial barrier to the passage of HBsAg in cases of acute maternal hepatitis as well as in the carrier state; this does not seem to be the case with anti-HBs which is more readily detected in cord blood.\textsuperscript{158,191}

While transplacental transmission of HBsAg from carrier mothers to their infants may take place, the Centers for Disease Control in Atlanta estimate that only about 5\% of mother-to-infant infection may occur in utero.\textsuperscript{45} Evidence suggests that most mother-to-infant transmission of HBsAg occurs during delivery rather than during pregnancy or after birth.

\textbf{Transplacental transmission vs transmission during labor and/or delivery.} Transplacental transmission of HBV was first suggested when serum from a mother as well as from her infant led to hepatitis infection in volunteers.\textsuperscript{208} Other cases attributed to transplacental transmission have included that of a woman with acute hepatitis during pregnancy who became HBsAg-negative during the last month of her pregnancy (end at delivery) whose baby had HBsAg in its cord blood.\textsuperscript{188}

Of 11 mother-child pairs observed for more than seven months in Tokyo for the transmission of HBsAg (using immune adherence hemagglutination assay),
antigenemia was detected in eight of the infants within the first six months of their lives.\textsuperscript{153} Incubation periods of 123 and 133 days were consistent with mother-to-infant transmission; however, HBsAg was detected in the serum as early as 5 and 13 days after delivery in other newborns, pointing to the possibility of intrauterine HBV infection.

In 1970 it was first suggested that newborns might be exposed to HBV infection during the time of birth.\textsuperscript{178} These same authors later suggested that HBV transmission might occur parenterally via minor abrasions which take place during delivery. The time frame of the development of antigenemia in these infants is consistent with this hypothesis of parenteral transmission.

In a prospective study of 125 HBsAg carrier mothers and their infants, HBsAg was shown to be almost universally present in the vaginal contents of the mothers during labor and in the gastric aspirates from the babies; presence or absence of HBsAg in the amniotic fluid and cord blood did not correlate with subsequent antigenemia in the babies.\textsuperscript{113} These results suggest that the oral route—while the dose required orally need be 50 times that required parenterally—\textsuperscript{108} may be an important mechanism of transmission while intrauterine infection may not play a major role. It is strongly suspected that the high infectivity rate in infants of certain carrier mothers is related to maternal-fetal microtransfusions during labor and/or infant contact with infectious maternal secretions in the birth canal.\textsuperscript{21,202}

Cases such as one reported of a HBsAg-positive mother delivering a baby in whose cord blood no antigen could be detected by double diffusion or RIA and who had no subsequent contact with the mother from immediately after birth suggest transmission at delivery; the baby was noted to be antigenemic four months later and had remained so when the report was published over two years later.\textsuperscript{188} The authors suggest that during the second and third stages of labor there may
be enough oral contamination of the infant with the mother's blood or feces to account for transmission. As early as 1967 it was shown that oral as well as parenteral transmission of a long-incubation (now known to be HBV) agent can be infective.\textsuperscript{108} Cases suggesting transmission during passage through the birth canal are reported far more frequently than the isolated reports of circumstantial evidence for \textit{in utero} infection.

Numerous studies have reported HBsAg in the cord blood of babies born to chronic carrier mothers.\textsuperscript{35,153,188,202} Results of a prospective study of 18 children born to 13 chronic HBsAg carrier mothers, followed for serological and biochemical evidence of hepatitis B, indicate that HBsAg found in cord blood samples did not result from intrauterine infection but rather from maternal blood contamination of fetal circulation around the time of delivery. The HBsAg present in the 3 of 18 samples of cord blood was found in lower titer than that found in the mother's serum and was cleared rapidly.\textsuperscript{161}

None of 51 neonates from Senegal, an area of high HBV endemicity, born to chronic HBsAg carrier mothers, had detectable anti-HBcIgM (a highly specific enzyme immunoassay was used) suggesting that none had a primary \textit{in utero} immune response to HBV infection.\textsuperscript{82} While the exact route of mother-to-infant transmission remains uncertain, this recent data indicates that \textit{in utero} infection may not play an important role.

It has been suggested that menstrual blood transmission may contribute to the observed maternal effect of the spread of HBsAg.\textsuperscript{129} If menstrual discharge is capable of transmitting the infectious agent of HBV, it is noted that cultural factors would impact on the effectiveness of such a route of transmission. Taboos about sexual contact, child care, and food preparation exist in different cultures to modify behavior relative to menstruation. If menstrual discharge is infectious, such taboos might modify transmission of disease. The suggestion has even been
made that these taboos may have resulted from subconscious adaptation to disease. Mothers with acute hepatitis B as well as asymptomatic carrier mothers can transmit HBV to their infants. Transmission of HBV from mothers who develop acute hepatitis B within two to three months of delivery has been documented. Studies in the U.S. of babies born to women with acute type B hepatitis during pregnancy report 40-50% of the babies developing antigenemia;\textsuperscript{188,189,190} it was suggested that transmission took place about the time of labor and delivery.

It is now recognized that mother-to-infant transmission of HBV to neonates of acutely infected mothers takes place often, particularly when the acute hepatitis occurs late in pregnancy; 16 of 21 (76%) infants became HBsAg-positive when acute illness manifested itself in their mothers during the third trimester or immediately postpartum.\textsuperscript{221} This report found the presence of maternal HBsAg at delivery to be important; all 76% of the babies who became HBsAg-positive were born to mothers positive for HBsAg. In those cases where maternal HBsAg was not detectable at the time of birth, none of the infants developed antigenemia.

In another study of 26 women with acute hepatitis who were HBsAg-positive, 10 of their babies became HBsAg-positive; all of the 10 mothers of these babies had acute HBsAg-positive hepatitis during the latter part of their pregnancy or during early postpartum.\textsuperscript{188}

Most of the early reports found transmission of HBV from asymptomatic carrier mothers to their infants to be rare.\textsuperscript{158,178,189} These studies usually included small numbers of carrier mothers. When larger studies were conducted, they showed that antigenemia frequently developed in babies born to asymptomatic HBsAg carrier mothers.

Of 158 babies born to asymptomatic carrier mothers in Taiwan, antigenemia was detected in nearly 40% (63) of the babies.\textsuperscript{202} These babies were more likely to develop antigenemia when their siblings were HBsAg-positive. Also,
there was a nearly linear increase of positivity among the babies with increasing maternal CF titer. Of the babies in whom antigenemia developed, most became positive within the first six months of life; once the antigenemia was detected, it usually persisted. As with the infants born to mothers with acute hepatitis B, it was felt that transmission took place during labor and delivery.

One study examining both mothers with acute hepatitis B as well as asymptomatic carrier mothers used relatively small sample sizes; nevertheless, a statistically significant difference of 48% vs 5% of chronic HBsAg positivity was seen in the neonates born to 27 women with acute hepatitis B and 21 maternal chronic HBsAg carriers, respectively.\textsuperscript{189} There was a much greater likelihood for the infants born to mothers with acute hepatitis B to become infected if the maternal illness occurred during the third trimester or immediately postpartum. Chronic neonatal infection was seen at similar rates in infants born to mothers who had acute hepatitis in the first two trimesters and to asymptomatic HBsAg carrier mothers. No correlation was seen between detectable HBsAg in cord blood and subsequent hepatitis B antigenemia in the infants.

In areas hyperendemic for HBV and with a high HBsAg carrier rate, such as Asia, transmission of HBV from asymptomatic chronic carrier mothers to their infants may be a more significant mode of transport than that of mothers who develop acute hepatitis B late in pregnancy and thus transfer it to their babies.\textsuperscript{153,202} Infants infected from their carrier mothers are initially asymptomatic and go on to develop a chronic HBsAg carrier state themselves;\textsuperscript{67} many of these babies develop antigenemia within the first two to four months of life. Other possible sequellae for infants infected with HBV at the time of birth include acute hepatitis with recovery and production of anti-HBs, fulminant hepatitis, and progression to chronic active hepatitis and cirrhosis.

High rates of infant carriers result from mother-to-infant transmission.
In the greater Paris area, a survey of 4,452 pregnant women revealed data indicating that asymptomatic chronic HBsAg carrier mothers in low prevalence areas could neonatally infect their infants and that these infants were able to actively eliminate the HBV. The results of this study contrasted with data from earlier reports of low rates of transmission from carrier mothers to their infants in low HBV prevalence areas such as Greece, Denmark, and the U.S. (0-16%), whereas areas with high endemicity of the chronic carrier state had documented higher transmission rates (40-52%). This study in Paris was from an area of low endemicity of the chronic carrier state and reported a high (67%) rate of transmission; however, only 16% of the children who participated in this study became chronic carriers, a proportion similar to that of older children. (The 16% was based on only one out of eight children with HBV infection becoming a persistent carrier.) The investigators observed that they sampled the children much more frequently than had prior studies, and that perhaps this feature alone could account for the high transmission rate and low rate of development of chronic carrier state observed.

Other studies have suggested that neonates are highly likely to develop a chronic HBsAg carrier state. All of 22 infants involved in a study in Los Angeles, who developed HBsAg and were followed for a mean period of more than 24 months, remained antigen positive. In a study from Taiwan, once antigenemia was detected in neonates, it usually persisted; 92% (35/38) of the babies remained HBsAg-positive for an average of 7.5 months of follow-up after their first positive blood specimen.

While the results of the Paris study may not be in strict agreement with other reports, it does supply evidence that infants neonatally infected with HBV are capable of actively eliminating the virus. It has been suggested, based on observations of overt hepatitis in neonatally infected children, that the high rates
of HBsAg carrier infants reported in certain areas (e.g., Taiwan\textsuperscript{202} and Japan\textsuperscript{153}) may be due to environmental or polygenic genetic factors rather than to inadequate immune responses.\textsuperscript{64,130,218} This idea will be discussed more thoroughly.

**Postpartum mother-to-infant transmission of HBV.** Infants, born to carrier mothers, who do not develop antigenemia neonatally, may still be at risk of developing transient HBsAg or of becoming chronic carriers. Two children of maternal HBsAg carriers developed antigenemia, one at six months and another at 14 months of age, remaining HBsAg-positive for three to six months.\textsuperscript{158} Among another group of carrier mothers followed, antigenemia developed in one infant between 4–7 months of age and in another between 8–14 months of age; when reported both children had remained carriers for over one year.\textsuperscript{191} Chronic carrier household members who may also have HBeAg represent another potential risk of HBV infection for these infants.

**Predictive value of serologic markers in mother-to-infant transmission.** Subsequent to the discovery of HBeAg and anti-HBe associated with HBV, it was shown that among HBsAg carriers, the presence of HBeAg was associated with chronic liver disease while the presence of anti-HBe correlated with the absence of liver disease.\textsuperscript{126,150,223} HBeAg, usually associated with a very high titer of HBV particles in the plasma, is almost always present during the first month or two of an acute HBV infection and is never found in the absence of HBsAg. During the first few years of the chronic HBsAg carrier state, one usually also finds the presence of HBeAg; such presence is apparently associated with the potential for mother-to-infant transmission of HBV infection.

Presence of HBeAg has been thought of as a reliable indicator of potential mother-to-infant transmission of infection; presence of anti-HBe has been thought to signal probably absence of transmission of HBV from mothers to newborns. It has been suggested that transmission of HBV from carrier mothers to their
infants is determined not only by the presence of HBeAg but also by the presence of HBV-specific DNA polymerase as well as the absence of anti-HBe. The presence of anti-HBe in carrier mothers was thought to be protective in that the infants born to such mothers appeared not to develop clinical disease.77,154,204 One study of 62 HBsAg-carrier Chinese mothers revealed 32% to be HBeAg-positive with only 2% anti-HBe-positive.16 Studies from the U.S. and Europe report just the opposite, with high anti-HBe prevalence (56-82%) and low HBeAg prevalence (0-1.3%).65,126,223

All of ten babies born to HBsAg carrier mothers also positive for HBeAg developed HBsAg which persisted during an observation period of greater than one year; all of the ten elder siblings of these infants were asymptomatic carriers. None of the seven infants born to carrier mothers positive for anti-HBe developed antigenemia nor were any of their elder siblings chronic carriers in this same cohort from Tokyo.154 Between 85% and 95% of infants born to HBsAg carrier mothers who are also HBeAg-positive became infected perinatally; nearly all of these infants became carriers.16,17,22,154,204 (Some studies have shown slightly lower rates—e.g., 67.6%—of infectivity in HBeAg-positive HBsAg carrier mothers.)24

While a number of studies have indicated that mother-to-infant transmission of HBV did not occur in infants born to HBsAg-positive, HBeAg-negative carrier mothers,199 one must be careful not to assume that because HBeAg correlates so strongly with infectivity that infants of chronic HBsAg-positive, HBeAg-negative women are therefore not at risk of infection or development of chronic carrier state. While the presence of maternal anti-HBe may be associated with much lower rates of mother-to-infant passage of HBsAg than in those cases of maternal HBeAg positivity, this is not categorically the case.

One report of three unrelated infants who died of fulminant massive he-
patic necrosis found that all three mothers were asymptomatic HBsAg carriers with one being anti-HBe-positive. A number of reports have shown that some carrier mothers positive for anti-HBe have babies who develop nonicteric hepatitis B, \textsuperscript{77,195} serologic, not clinical, evidence of transient HBV infection,\textsuperscript{204} or severe acute icteric hepatitis B.\textsuperscript{197} In a report of three infants who developed acute icteric hepatitis B within three months of birth to HBsAg-positive and anti-HBe-positive carrier mothers, all three infants recovered clinically and developed circulating anti-HBs.\textsuperscript{197}

It has also been suggested that the presence of anti-HBc might be a marker of viral replication and therefore of infectivity.\textsuperscript{95} A study of 16 health HBsAg-positive carrier mothers who were all positive for anti-HBc found no evidence of transmission of HBV to their newborns nor infection in 13 older and younger children of these women nor in the husbands followed for four to five years.\textsuperscript{199}

The reasons for such variable response of babies infected with HBV from their chronic carrier mothers is not well understood. Ideas about possible mechanisms for potential differences in the immune response to the transmitted virus are discussed in medical journals.

An estimated 10,000 to 15,000 HBsAg-positive women—both chronic HBV carriers and less frequently those women with acute HBV infection—give birth each year in U.S. hospitals.\textsuperscript{50} This accounts for approximately 0.4% of reported births in the U.S. Of these women, only approximately 3,000 to 5,000 might also be HBeAg-positive. Regardless of what the answers to questions about the variety of responses seen in infants infected with HBV from their mothers might be, it seems clear that immunoprophylaxis is indicated for babies born to HBsAg-positive mothers regardless of the mother's HBeAg status. Infants born to carrier mothers positive for anti-HBe may not be protected as previously thought and HBeAg-negative carrier mothers may also transmit HBV to their offspring.
SECTION SIX
HBsAg CARRIER STATE

HBV As a Cause of Acute or Persistent Infection

Acute infection. Recovery may follow disappearance of HBsAg in cases of acute and severe hepatitis.\textsuperscript{63} Antibodies to HBsAg usually appear after the disappearance of the antigen and are thought to signal recovery. Appearance of anti-HBs correlates with immunity to HBV infection.\textsuperscript{3,94} Spinal fluids examined from HBsAg-positive patients with a variety of clinical syndromes failed to detect the presence of HBsAg; workers suggest that HBsAg does not cross the blood-brain barrier in pathological conditions (including fulminant hepatitis, cirrhosis with encephalopathy, polyarteritis nodosa, and viral hepatitis with meningitis-like syndromes) associated with HBV infection.\textsuperscript{222}

In individuals with acute hepatitis, persistence to HBsAg for longer than 12 weeks is thought to imply a preceding carrier state or chronicity. Some researchers feel that the criterion of HBsAg persistence three months after acute illness as indicative of chronic carrier state is too rigid; follow-up for one or two years has shown that a large proportion of persons may lose their antigenemia.\textsuperscript{181} Others feel that there are only rare cases of HBsAg disappearance with anti-HBs appearance after one to six years; in general they consider persistence of HBsAg beyond four months to be rare and to indicate prolonged carrier state.\textsuperscript{109,181}

HBsAg does circulate for many years in some persons. There is one well documented case (positive tests) of HBsAg carriage in an apparently healthy man for over 20 years.\textsuperscript{246} There is evidence that the prevalence of asymptomatic HBsAg carrier status declines with age,\textsuperscript{218} which has led some workers to specu-
late that the carrier state persists for a limited period (e.g., 15-25 years) in some populations.

With only 5-10% of patients with acute hepatitis B remaining HBsAg-positive longer than three months after their illness and with some of that minority becoming negative with continued follow-up, these acutely ill patients are unlikely to represent a major source of the carrier state. In fact, most of those people identified as chronic HBsAg carriers in serologic surveys give no history of recognized acute hepatitis disease; therefore, anicteric illness may be the most likely source of HBsAg carriage.

Persistent infection. In 1920, when parenteral injections of bismuth and arsenicals were used as treatment of syphilis, it was suggested that an infectious agent might be responsible for the liver damage seen subsequent to such injections. It was not until 1942 that evidence was obtained for the presence of carriers of type B hepatitis in the general population; this evidence accumulated during World War II when the armed services used yellow fever vaccine buffered with human serum. The resultant hepatitis epidemic represents one of a number of times during which the reservoir of carriers in the U.S. was probably significantly increased, thereby amplifying the source of infection for others. In 1941, during this yellow fever vaccine epidemic, an estimated 49,000 cases of hepatitis were seen among U.S. military personnel; the number of subclinical infections that took place is unknown. As hepatitis B virus apparently results in carrier status in at least 5-10% of persons infected, the U.S. reservoir of carriers of HBV may have increased by 5,000 during that epidemic. It seems likely that the pooled plasma so widely used in World War II and the Korean War similarly increased the reservoir of carriers.

Later studies from England of serum pools used in measles prophylaxis and from the U.S. from National Red Cross plasma pools suggest that about
0.2% to 0.5% of the general population of these countries carries HBV in their blood. By 1954 researchers retrospectively recognized that hepatitis seen after administration of donor blood represented presumptive evidence for the existence of a carrier state of hepatitis B; however, one group of researchers felt that confirmation of such circumstantial evidence could only take place by injecting suspected carrier donor blood into volunteers in order to see if hepatitis developed.

They reported that three of five prisoner volunteers developed hepatitis without jaundice when injected with serum from an asymptomatic mother of an infant in whom hepatitis with jaundice had developed soon after birth.209 After this woman's child died at age 18 months with advanced fibrosis of the liver, her serum was injected into five volunteers, of whom two developed hepatitis with jaundice and one hepatitis without jaundice. Parenteral injection of serum of a blood donor (whose name had been removed from the blood donor panel after the association of three cases of jaundice in recipients of his blood) into five volunteers resulted in hepatitis with jaundice in one of the volunteers; the evidence indicated that the donor's blood carried viral hepatitis B over a period of at least ten months.209

With the development of serologic techniques for the detection of HBsAg, some of the data collected in the early 1950s (during human transmission experiments) was reevaluated. When 100 volunteer prisoners were tested in 1970, it was found that 12 had become persistent carriers of antigen; 8 of the 12 volunteers had had mild illness (from the previous inoculation of icterogenic blood products from 1951-1954), while the remaining four had asymptomatic infection.11 This study suggests that the carrier state is more likely to follow mild or asymptomatic illness than overt clinical hepatitis B. Other circumstantial data from studies of HBsAg prevalence among patients in institutions for the mentally retarded has also suggested that very mild, anicteric or asymptomatic infections, in both
children and adults, were responsible for chronic carrier state in the majority of cases.\textsuperscript{211}

**Liver Disease in HBsAg Carriers**

By definition, asymptomatic carriers have no signs or symptoms of liver disease. Serologic surveys have shown that liver chemistries, with the exception of serum transaminases, are usually within normal limits; however, a varying proportion of carriers do have abnormalities ranging from minor changes to severe hepatitis and cirrhosis.

The two pathologic types of chronic hepatitis (i.e., chronic liver disease existing for longer than six months) are persistent and chronic active hepatitis; those at high risk of developing chronic hepatitis include the very young and the elderly.\textsuperscript{194} Chronic persistent hepatitis is associated with HBsAg persistence.\textsuperscript{63} Disappearance of HBsAg has been reported in chronic carriers without clinical symptoms (see Acute Infection, above) but not in individuals with chronic hepatitis.\textsuperscript{181}

Numerous studies have evaluated liver function, some including liver biopsy, in apparently healthy HBsAg carriers. Results indicate that approximately 30% (in those with elevated transaminase values) to 50% of carriers have normal liver histology or only minor alterations (intraplbral or portal tract) which do not indicate chronic active or chronic persistent hepatitis.\textsuperscript{105} The remaining 50% to 70% of asymptomatic carriers have histologic lesions which appear to be those of chronic persistent hepatitis.\textsuperscript{157,183,196,241} From 10% to 40% of carriers may have mildly elevated levels of SGPT and SGOT.\textsuperscript{105} Only a small number of carriers, in particular those with elevated transaminase levels, have chronic active hepatitis.\textsuperscript{247}

One study suggests that intrafamilial spread of HBV may be influenced
by the presence or absence of histologic or clinical signs of chronic hepatitis in
the index case. Of 12 households of carriers tested, there was a higher prevalence
of elevated transaminase or antibody (on liver biopsy) in household contacts of
carriers who had evidence of chronic aggressive or chronic persistent hepatitis
than in contacts of carriers without these changes. However, in another
series of nearly 200 families, those households in which the carrier had elevated
transaminase activity and those in which the carrier had normal values showed
the same frequency of hepatitis B infections. This study could draw no final
conclusions regarding the association between signs of chronic active hepatitis
and infectivity because there is no way of knowing whether the index cases are
the primary cases; however, the authors felt that intrafamilial spread of HBV
was determined by inherited or acquired characteristics of the family members,
particularly the shared common environment, rather than by presence of liver
damage in the index case.

SeroLogic Markers in HBsAg Carriers

Among HBsAg carriers, the concomitant presence of HBeAg is associated
with chronic liver disease, whereas presence of anti-HBe is correlated with the
absence of liver disease. Screening for HBeAg and anti-HBe has been
used to help identify chronic HBsAg carriers who have chronic hepatitis. While
some healthy carriers without liver disease have been shown to have anti-HBe
but not HBeAg, other patients with chronic active HBeAg have been shown to
have HBeAg but no anti-HBe.

Anti-HBc appears to identify recent or continuing virus replication while
DNA polymerase apparently reflects the period of peak replication of HBV; it may be that these markers can help to identify persons—particularly when they
are candidates as blood donors—capable of transmitting HBV even when HBsAg
is not detected by currently available techniques. At least one case of evidence for HBV infection in the recipient of donor blood positive for anti-HBc, in the absence of HBsAg and its antibody, has been documented.\textsuperscript{95} A large investigation of blood donor sera detected anti-HBc in 100% of the samples from chronic HBsAg carriers, in 98% of HBsAg-positive blood donors, and in 1% of HBsAg-negative serum samples.\textsuperscript{95}

**Age Factor in HBsAg Carrier State**

In general, the highest frequencies of HBsAg are detected in different age groups in high prevalence areas (which tend to be tropical, less developed countries) than in those industrialized nations in temperate zones which have significantly lower rates of carriers. The acquisition of HBsAg takes place much earlier in the tropics than in temperate zones. Studies in Africa have revealed that 11% of children under one year of age are chronic carriers; 16% of those 3-6 years old retain persistent antigenemia, with a gradual decline after that.\textsuperscript{214} The prevalence of HBsAg among Chinese persons in the general population is 15% to 20%, rising from birth and peaking between 8 and 12 years of age.\textsuperscript{21} In industrialized countries, young adults 20 to 40 years old, both males and females, display the highest frequency of HBsAg; antigen is detected significantly less frequently in children and adolescents as well as in those older than 40 years.\textsuperscript{218} Detection of anti-HBs, in contrast to HBsAg, constantly increases with age in industrialized nations, reaching a peak among the elderly.

The single most important mode of HBV transmission in high prevalence areas appears to be from carrier mother-to-infant, whereas the degree to which this mode of transmission contributes to the prevalence of carriers in areas of low endemicity is not agreed upon. It was thought that asymptomatic carrier mothers rarely transmitted infection to their babies, but that mothers with acute
hepatitis B prior to or during delivery did transmit infection to their infants. More recent information, however, indicates that chronic carrier mothers do transmit infection to their infants at high rates.

The Willowbrook State School, an institution for mentally retarded children in New York, first noticed so-called infectious hepatitis (hepatitis type A) among its patients in 1949 and by 1953 they recognized the endemnicity of the situation. Using methodology of questionable nature (i.e., artificially exposing susceptible incoming residents of the school to strains of HBV previously shown to be prevalent in the institution), researchers detected antigen in 97% of 40 cases of so-called serum hepatitis (defined as those cases of hepatitis preceded by parenteral inoculation of potentially infectious materials) using the CF test. Persistence of antigenemia was found to be more common in children (from 4 months to 13 years in 36.5% of 104 children followed prospectively) than in adults (15% of 150 adult male retarded patients were carriers).

In contrast to studies which reported a significantly higher incidence of HBsAg among men with Down's syndrome compared with men with epilepsy, the Willowbrook study found the incidence of HBsAg to be nearly the same in men with Down's syndrome (18.6%) and other retarded men (15%); these men had similar exposures to hepatitis virus, having lived in the institution for over six years. However, in young children, the incidence was 43% in those with Down's syndrome, compared to 24.4% in those other mentally retarded children. The authors felt that this difference may have been due, at least in part, to the variations of time spent in the institution by the two groups. The children with Down's syndrome had been admitted at an earlier age, had been exposed to hepatitis longer, and were more ambulatory.

Studies in institutions for the mentally retarded have detected HBsAg in significantly higher proportions of residents with Down's syndrome than in matched
samples of residents with other forms of mental retardation. One study revealed HBsAg in 35.1% of Down's syndrome residents compared to 17.2% of matched residents; the researchers suggested that early age of exposure, compared to later exposure, increased the risk of developing chronic carrier status. It appeared that carrier state in patients with Down's syndrome was of a longer duration while both patients with Down's syndrome and those with other forms of mental retardation had increased resistance to carrier state with age above puberty. Another study detected antigen in 20.4% of patients with Down's syndrome, compared with 7.7% of matched patients with other forms of mental retardation; the higher prevalence of antigen in Down's syndrome was associated with longer persistence of antigenemia. There was also a close relationship between age of admission to the institution and prevalence of the antigen; the younger the patient on first admission, the higher the risk of developing the chronic carrier state for patients with Down's syndrome as well as for patients with other forms of mental retardation.

Work conducted in other closed institutions for the mentally retarded has indicated that the earlier in life that one is exposed to infection, the greater the probability that one will develop a persistent carrier state. Even with similar risk factors, those children admitted at less than five years of age developed a carrier state two to five times more frequently than those who entered the institution at five years of age or older.

Socioeconomic Status and Carrier State

Numerous studies indicate large ethnic differences in prevalence rates of the carrier state in a given population; however, such differences have been shown to diminish in magnitude when adjusted for family size, birth order, and socioeconomic level. Other studies conducted in tropical or semitropical cli-
mates show a positive correlation of poor and crowded living conditions with a high prevalence of neonatal HBV infection.\textsuperscript{105}

Given a higher prevalence of hepatitis B infections among nonwhites (black, Puerto Rican, and Asian) in a large survey conducted of households containing HBsAg carriers in New York, the authors suggest a role for environmental factors such as inadequate housing and hygiene, overcrowding, and close contacts with other households. These environmental factors result from the lower socioeconomic status seen among these families; however, it is difficult—if not impossible—to implicate any single factor such as socioeconomic status, environmental factors, genetic predisposition, sex, or age factors as exclusively responsible for increasing the risk of HBV infection and/or carrier state.

The ethnic distribution of HBsAg in the above mentioned New York study of households containing carriers was complicated by a much higher frequency, within the same environment, of antigenemia detected among blood relatives than among nonblood relatives (i.e., spouses). These findings may imply a genetic predisposition which is supported by prevalences among parents of carriers 2.4 times higher than spouses and of siblings 5.8 times higher, findings which are highly statistically significant. The hypothesis for the role of genetic predisposition suggests that all family members are equally exposed to an antigen carrier within the household, but that primarily blood relatives will develop the carrier state. Data from this New York study supports this idea with detection of HBsAg more frequently among blood relatives than among spouses, whereas spouses had anti-HBs as frequently as, or more frequently than, blood relatives.

The genetic predisposition hypothesis cannot explain all, however, even in this one particular study. Genetic predisposition—if it exists—may interact with other host and/or environmental factors. The increased frequency of detection of HBsAg among siblings than among spouses might be explained by an as-
sumption that the siblings of a carrier were on the average younger than the spouses of the carrier at the time of exposure (the siblings on the average were signifi-
cantly younger than the spouses when the survey was conducted); the development of a chronic carrier state may have been favored in the siblings who developed primary infection at a younger age considering that age at first exposure may be a major determinant of HBsAg persistence.

Genetic Susceptibility to HBsAg Carrier State

It has been suggested that the clustering of chronic persistence of HBsAg, both within families and in different ethnic groups, may be determined by genetically controlled variations in the immune response, both cellular and humoral with cell-mediated immunity playing the predominant role. In 1969 it was first hypothesized that predisposition to the HBsAg carrier state is inherited in an autosomal recessive genetic manner. This hypothesis was tested among 53 families on the Visayan island of Cebu in the Philippines as well as with 617 family groupings, including a total of 1,797 individuals, from the island of Bougainville, Trust Territory of New Guinea; analysis of the data was consistent with the hypo-
thesis of simple autosomal recessive inheritance of predisposition to the asym-
tomatic chronic carrier state. Subsequent large population studies have done similar segregation analyses with results supporting this hypothesis of genetic susceptibility.

Patients with chronic renal disease undergoing hemodialysis have been shown to have an increased prevalence of HBsAg. Other patients have been reported to have continuing hepatitis infection subsequent to renal transplanta-
tion. Among patients undergoing chronic hemodialysis, blacks acquired antigenemia significantly less frequently than did whites. It has been reported that even prior to mass programs screening donor blood for HBV, blacks from
a hospital in Harlem, New York City, rarely developed posttransfusion hepatitis B.48 These distinctive distribution differences of HBsAg prevalence among various populations also led to suggestions that genetic determinants may, at least in part, affect an individual's susceptibility to hepatitis B infection.

Data supporting the hypothesis that host resistance to hepatitis B infection may be influenced by genes associated with the major HLA histocompatibility complex have been reported.34 Among a group of 144 renal patients, of whom 23% had persistent HBsAg and 15% transient antigenemia, the presence of HBsAg was related to HLA locus B types Bw15, Bw17, and Bw35.90 There is a significant possibility that chance categorization could account for apparent associations of disease with various HLA haplotypes because of the large number of different histocompatibility types. In the above mentioned study, none of the three single HLA groups were associated with HBsAg with statistical significance; however, when all three HLA groups combined were compared with all other HLA specificities in the remainder of the population, the association with antigenemia was statistically significant.

Race was a confounding factor with proportionally more black patients who were HBsAg-positive more commonly displaying HLA-Bw17 and HLA-Bw35, whereas HLA-Bw15 was seen exclusively in the white population. After stratification for race, the statistically significant association between HLA group and HBsAg was apparent only in whites. The authors suggest that determinants linked to the HLA complex may influence immune reactions such that antigenemia is suppressed subsequent to a recognition of HBsAg as similar to "self." They acknowledge, however, that this mechanism does not explain the association that they observed of HLA-Bw15 with transient HBs antigenemia.

Anecdotal reports have questioned the simple recessive inheritance hypothesis. One such report was that of both parents HBsAg-positive while their
child was HBsAg-negative even with evidence of past exposure to HBV (i.e., presence of anti-HBs in the child). After such anecdotal reports, and considering that family segregation analyses may not be able to discriminate the roles of environmental versus genetic factors involved with a contagious disease, and noting that the populations analyzed lacked families in which both parents were carriers, another study was designed to test the autosomal genetic hypothesis. Two groups of families were compared for rates of HBsAg among the children: in one group both parents were HBsAg carriers and in the other group the mother was a carrier and the father was positive for anti-HBs. The results predicted by the autosomal recessive genetic theory were not borne out. The results expected by the genetic hypothesis are that all HBV-infected offspring born to carrier parents would become carriers and that a relatively small proportion of children born to carrier mothers and anti-HBs-positive fathers would become carriers (since they would inherit only a single gene for carrier predisposition from their mothers).

Using sensitive RIA techniques, the authors found that the frequency of anemia among the newborns (tested for a mean of 12 months) and their siblings was unrelated to the status of the father's serum (i.e., whether he was HBsAg- or anti-HBs-positive). However, they found that maternal CF titer did influence the baby's response to HBV infection, whether it was the development of anti-HBs, transient anemia, or a chronic HBsAg carrier state.

Original segregation analyses were repeated on a population from the U.S. utilizing more sensitive assays than those used in the late 1960s. Based on 95 families with an index carrier, an excellent fit with the recessive inheritance hypothesis was seen in two groups of families (one parent positive or both parents negative). However, of three family units in which both parents were HBsAg-positive, antigen did not segregate in accordance with the simple recessive inheritance hypothesis in one of the families (a daughter was both anti-HBs- and HBsAg-
negative.)^{218} While isolated pedigrees such as this make it difficult to accept
the genetic hypothesis, the authors point out that it is possible that this HBsAg-
negative daughter either lost the antigen or carried it in amounts not detected.

A report of an identical twin pair discordant for HBsAg also fails to sup-
port the hypothesis of an autosomal recessive trait for susceptibility to HBs anti-
genemia. One of these 10-year old twin boys from Panama had symptoms of hep-
atitis with no HBsAg detectable in his serum (by gel diffusion, counterelectro-
phoresis, complement fixation, or radioimmunoassay), whereas his monozygous
twin did have detectable HBsAb in his serum which persisted for two years during
follow-up; the latter twin was therefore regarded a chronic carrier.^{165}

It has also been suggested that observed family clustering might be due
to maternal or intrafamilial transmission of an infectious agent.^{227} Contrary
to this hypothesis of family infection is data from an analysis of families from
five groups (Cebu, Bougainville, Lau- and Beagn-speaking areas of Malarta, and
Sardinian families from Turin) which showed that different subtypes of HBsAg
can occur among asymptomatic carriers living in the same household.^{130} How-
ever, other studies emphasize exposure patterns as determinants of HBs antigen-
icity rates and provide evidence against genetic determinants.^{182,217}

On the one hand, independent segregation analyses of HBsAg in natural
populations and in families of blood donors have failed to reject the genetic hy-
pothesis which states that susceptibility to the asymptomatic chronic carrier
state is determined by a single autosomal recessive gene, whereas on the other
hand this hypothesis cannot account for the case of one identical twin with HBsAg
and the other with anti-HBs, nor for the presence of anti-HBs or absence of HBsAg
in children whose both parents were HBsAg carriers. One segregation analysis,
using data from the Solomon Islands, was able to reject the genetic hypothesis
as a major factor leading to the HBsAg carrier state in that population. At least
one child was HBsAg-positive from those families investigated in which both parents were HBsAg-negative.\textsuperscript{129a}

There are some inherent difficulties in segregation analysis given that certain assumptions which must be made are probably not valid. Segregation analysis assumes that the genotype is reflected in the phenotype; this may be invalid because some asymptomatic persons may carry the antigen for many years,\textsuperscript{246} whereas some may lose the antigen after a much shorter time.\textsuperscript{214} Segregation analysis also assumes that all healthy people who have HBsAg in their serum are asymptomatic carriers and are homozygous for the susceptibility gene; however, infection can be transmitted to infants from pregnant women with clinical hepatitis but who do not develop chronic carrier status, whereas these infants frequently develop chronic antigenemia.\textsuperscript{189} Finally, segregation analysis assumes that all individuals susceptible to becoming carriers are detected; this is probably invalid because there are some people in all tested populations who show no evidence of having been infected with HBV.

It is difficult to know if some studies are unable to reject the genetic hypothesis while others are able to reject it because genetic factors have a more or less significant role in particular populations, because of the problems of the validity of segregation analysis applied to this type of data, and/or for other reasons. Many workers view genetic determinants—if they do exist—to only partially influence subsequent carrier status. Factors such as geography, ethnicity, socioeconomic status, occupation, sex, age at first exposure to infection, age at testing, sexual promiscuity, sharing a household with chronic carriers, and immunologic deficiency (for reasons other than genetic predisposition) all appear to be associated with the risk of asymptomatic carrier state.

**Sex Differences in the Carrier State**

Higher rates of HBsAg clearance among women than men have been re-
ported in mentally retarded populations\textsuperscript{210} and in African populations.\textsuperscript{214} Response to HBV was examined among patients undergoing renal dialysis at a community-based clinic; they were monitored monthly for HBsAg and anti-HBs. This particular report included only patients who became infected while undergoing dialysis treatment and therefore results do not reflect exposure differences. Data showed that males were more likely to remain HBsAg-positive indefinitely and that females were more likely to convert to HBsAg-negative status and develop anti-HBs. If males, at least those with chronic renal disease undergoing dialysis, are more likely to become chronic carriers of HBV, they would therefore in turn be more likely to develop postnecrotic cirrhosis and primary HCC.

A higher prevalence of persistent HBsAg in males than in females has also been observed in: (a) patients with Down's syndrome in institutions during the first two decades of life;\textsuperscript{120} (b) family studies carried out in the islands of Cebu, in the Philippines, and in Bougainville, New Guinea;\textsuperscript{28} and (c) blood donors.\textsuperscript{102}

No marked sex differences in the carrier rate of HBsAg have been revealed in a number of studies of closed institutions for the mentally retarded;\textsuperscript{93,211} however, other studies have noted such differences. One study did reveal a marked sex difference in the carrier rate; antigen was detected (by counterimmunoelectrophoresis) in 15\% (49 of 319) of males tested, but in only 0.4\% (2 of 467) of females. On wards with both male and female patients, the rates narrowed to 10\% (7 of 67) males and 2\% (1 of 63) females, still significantly different.\textsuperscript{52}

It has been suggested that in populations where sex differences in prevalence of HBsAg or anti-HBs are observed, difference in exposure rather than gender may be responsible.\textsuperscript{131} One study found no significant difference in prevalence of HBsAg or anti-HBs between males and females when comparing age groups less than 20 years and over 20 years; they did detect significantly lower frequencies of Ag and Ab in patients with Down's syndrome who had been admitted after
age 15, while the prevalence of Ag and Ab was greater in long-term residents over the age of 30 than in short-term residents. They matched patients with Down's syndrome who had been admitted after 15 years of age with patients with other mental disorders and detected Ag in 36% of the Down's syndrome patients, compared with 12% of the other patients and conversely Ab in 18% of the Down's syndrome patients compared with 45% of the other group. It is possible that there are immunologic differences in these patients such that patients with Down's syndrome fail to produce anti-HBs and remain carriers while patients with other forms of mental retardation produce anti-HBs and terminate infection.

Sex differences in susceptibility to bacterial meningitis and bacterial septicemia as well as to other bacterial infections have been reported to show a preponderance of males. On the basis of this work, a genetic hypothesis was postulated which attributes these differences to a gene locus on the X chromosome affecting antibody production. Subsequent investigations showing higher antibody response to Rubella vaccine in females, higher serum levels of Escherichia coli hemagglutinins in hospitalized females aged 6-14 months, and highest mean serum levels of IgM in XXX females, intermediate in normal women, and lowest in men support the hypothesis of a gene locus on the X chromosome being involved in greater resistance to infectious disease.

A number of factors have been suggested as being responsible for the apparently higher incidence of HBV among males. Drug abuse is clearly one such factor; however, other factors including environmental need to be considered when examining sex differences among HBsAg carriers.

Persistence of HBsAg and Immunologic Status

Patients in whom an increased frequency of the carrier state has been found include those: (a) undergoing immunosuppressive therapy; (b) with Down's
syndrome; (c) with chronic renal failure; and (d) with lepromatous leprosy. The mechanism of persistence of HBsAg is still being debated; one suggestion has been that complete—or at least partial—immunological tolerance to HBsAg may account for healthy carriage.

Many tropical countries have a high prevalence of asymptomatic carriers; these large numbers of carriers may be related, at least in part, to changes in immunological response. Repeated parasitic infections induce a number of immunological changes; it has been established that malaria has immunosuppressive action. If immunological changes are involved in persistence of HBsAg, then factors such as malnutrition and parasitic diseases known to produce such changes in less developed, tropical countries may influence carrier status in these areas.

One investigation of immunological response of HBsAg carriers, with and without liver damage, concluded that a generalized impairment of immune response is not required for the establishment of the carrier state. However, the two groups studied, healthy blood donor carriers with normal liver histology and no history of liver disease, and patients with acute viral hepatitis which had progressed to chronic hepatitis, numbered only 11 and 7, respectively. Immune deficiency associated with certain clinical conditions may influence the clinical expression of HBV infection as well as its tendency to chronicity. It has been shown that patients with chronic renal failure often develop a chronic carrier state but only rarely develop icteric hepatitis, whereas staff members of dialysis centers develop icteric hepatitis in most cases but rarely a chronic carrier state.

Immune deficiency appears to have a similar effect in other conditions associated with immunologic deficiency including Down's syndrome, leukemia, leprosy, and Hodgkin's disease. Among 95 matched pairs of Down's syndrome (DS) and other mentally retarded (OMR) patients in an institution for the mentally retarded, the prevalence of HBsAg was significantly higher in the DS
group than in the OMR group. Supporting the hypothesis that younger DS patients are immunologically immature, the researchers found lower prevalence and lower levels of anti-HBs in the DS residents than in the OMR residents.

Children appear to become carriers of HBsAg after infection with HBV more frequently than do adults as indicated by studies from the U.S. A number of different mechanisms which may predispose children to carrier state have been proposed: (1) relative immune deficiency secondary to slow maturation of cellular immune response in newborns, resulting in tolerance to HBV (playing a role in mother-to-infant transmission); (2) some data indicates, however, that there was a uniform risk of becoming a carrier among children from one month to 15 years of age; therefore, route and dose of virus may help to explain the disparate rates of chronic infection between children and adults (e.g., low doses of virus by nonparenteral routes in children might favor development of the carrier state by influencing the immune response and severity of infection); (3) there appears to be a higher prevalence of carriers among adults in the U.S. following a mild attack of hepatitis (children generally experience a milder disease and many have subclinical infection; therefore, perhaps an intense immune response is a prerequisite for removal of the virus so that the equilibrium between host and virus seen in the carrier state is avoided).

A number of studies have been done of parenterally inoculated prisoners with materials known to have the potential of transmitting HBsAg-positive hepatitis. The results of one such study suggest that the risk of chronic infection with HBsAg may exist on primary infection (primary antibody responses were seen in some individuals who became carriers) as well as on reinfection (secondary anamnestic-type antibody responses were seen in others).

Worldwide Prevalence of HBsAg

A 1970 study which had used the insensitive Ouchterlong technique (before
CF was available) found HBsAg to be 10 to 100 times more prevalent in the tropical populations surveyed in Africa, India, Pakistan, and Central and South America than in volunteer blood donors in New York City. Awareness of the international health problem of hepatitis B and a growing understanding of the association of HBsAg and viral type B hepatitis led to the development of improved techniques for the detection of HBsAg. By 1972, when there existed a wide variety of sensitivity and specificity of techniques, WHO organized an international study—using standardized techniques and reagents—to determine the prevalence of HBsAg in different parts of the world. Most of the 21 countries employed discontinuous counterimmunoelectrophoresis (DCIE) to assay HBsAg while all of the hepatitis B antiserum was a particular reagent distributed for the WHO study by the National Blood Resources Branch of the National Heart and Lung Institute, Bethesda, U.S.A. Some of the countries used the more sensitive techniques of RPHA and/or RIA. HBsAg was found in higher frequencies among the African, Asian, and Eastern European countries investigated and in lower frequencies among the Americans, Australian, and Western Central European countries studied.

The antigen prevalence seemed to increase gradually during late childhood and early adolescence in countries with a low frequency of HBsAg; in countries with a higher HBsAg prevalence, results suggested that hepatitis infection could be easily spread through families with early contact. While the disparity was small, the frequency of HBsAg did tend to be higher in urban than in rural populations and higher in males than in females.

The rates of prevalence of HBsAg in high risk populations such as medical and dental laboratory workers, blood donors, and various institutionalized groups such as prisoners and military personnel, were significantly higher than in the normal populations in some countries; in other countries, the rates were not greater in high risk populations than in normal groups. Similar results had been obtained
prior to the WHO study, The WHO collaborative effort, as well as other studies, reported a high prevalence of HBsAg throughout the world in patients with acute and chronic liver disease.

Hyperendemicity of HBV, however, is not confined to tropical areas; there have also been reports of high penetrance of HBV in indigenous populations of Greenland and Alaska. In Greenland overall prevalence of HBsAg and anti-HBs ranged from 47% to 81% in a study of 190 sera samples randomly selected. In two remote Alaskan Eskimo villages a 13.9% prevalence of HBsAg and a 40.9% prevalence of anti-HBs for an overall infection prevalence of 54% was found. Infection prevalence was significantly higher in households with a HBsAg carrier than in families with no such carrier.

The degree of endemicity of HBV infection and the prevalence of the chronic HBsAg carrier state in various populations throughout the world are extremely variable. Such variation has been discussed from the standpoint of genetic data, clinical findings, mother-to-infant transmission, and environmental factors favoring contact with blood or other secretions; after considering the current clinical, genetic, and epidemiologic data available, it appears that a variety of mechanisms—and not merely one—may result in transmission of HBV in hyperendemic as well as in nonendemic areas.

Transmission of HBV is apparently common in childhood among Chinese persons, and has been well studied. In Taiwan, 1,510 Chinese preschool children (mean age 29 months) were tested for HBV markers in order to determine the incidence of HBV infections. Relative to HBsAg carrier state, the following observations were reported: An estimated 40-50% of initial infection of Chinese carriers result from mother-to-infant transmission; (2) Age at infection appears to be a major determinant of HBsAg persistence, the proportion of HBsAg carriers among those children infected with HBV was inversely related to age; (3) 86%
of those children who were HBsAg-positive upon enrollment in the study and who were followed up remained positive, whereas only 23% of the children who were infected with HBV (i.e., became HBsAg-positive after enrollment in the study) remained carriers; (4) Of those children who lose HBsAg, the majority developed anti-HBs; (5) 66.3% of the 924 initially susceptible children who were followed up later developed anti-HBs; passively acquired maternal Ab was thought to explain the high frequency of anti-HBs in children younger than one year old in light of the 91% of children initially positive for anti-HBs losing this marker on follow-up.

It has been speculated that the carrier state initiated at birth might in large part explain the large number of chronic carriers for whom no history of hepatitis or other mode of acquisition of HBsAg is apparent.\textsuperscript{135} This hypothesis has been criticized based on a perception that: (1) antigenemia in infants was usually self-limited; (2) overt hepatitis B infection among pregnant women was sufficiently prevalent to account for the high frequencies of presence of HBsAg in some populations.\textsuperscript{158} One large survey of familial clustering of hepatitis B infection supports the idea that mother-to-infant transmission is not of primary importance, at least in the U.S. They found that the frequency of antigenemia in children was not dependent on whether the carrier in the family was the mother or the father; anti-HBs was more prevalent in children of carrier fathers than in children of carrier mothers.\textsuperscript{213} However, numerous studies in the ensuing decade have suggested that the carrier state initiated by mother-to-infant transmission probably does help to explain the large number of asymptomatic chronic carriers who have no history of any other apparent mode of acquisition of HBsAg, certainly in hyperendemic countries and even in the U.S.

Mother-to-infant transmission of HBV infection has been observed to occur with greatly differing rates in various ethnic groups. Studies in Taiwan
have shown that nearly 50% of infants born to carrier mothers become HBs antigenemic within the first year of life,\textsuperscript{202} while observations in the U.S. have suggested 5%.\textsuperscript{189} Rates similar to those seen in the U.S. have been reported in Greece (6%)\textsuperscript{159} and in Belgium (6%).\textsuperscript{198a}

Four ethnic groups were observed for mother-to-infant transmission of HBsAg in the West Midlands region of the United Kingdom. Forty-five percent of the asymptomatic HBsAg carrier mothers were Asian (defined as being from India, Pakistan, and Bangladesh), 28% European, 13% Afro-Caribbean (defined as mothers from the West Indies and Africa, excluding those of Asian and European ancestry), 11% Chinese, and 3% other. The infants who became carriers, however, had a different ethnic distribution: 64% of the Chinese infants became carriers, while 30% of the Afro-Caribbean, 8% of the Asians, and none of the Europeans became carriers.\textsuperscript{56}

With the prevalence of hepatitis B only sporadically reported for Latin America, and because existing data could not be accurately compared due to variable methodologies employed, the Red Cross Latin American Hepatitis B Workshop was helped in 1977 with 13 Western Hemispheric countries participating.\textsuperscript{132} About 500 or 1,000 donor samples were submitted by each country and serologic tests were conducted to detect HBsAg and subtypes of HBsAg as well as anti-HBs, anti-HBe, and anti-HBc. Tests were performed in one location; therefore, results are strictly comparable. RIA was initially used to test all 7,847 serum samples for HBsAg. Subsequent retesting of HBsAg-positive samples was done by immuno-diffusion, counterelectrophoresis, and by RPHA.

Of all 7,847 donor blood samples from the 13 countries, the overall prevalence of HBsAg was 1.6%, ranging from 0.2% (Puerto Rico) to 4.1% (Dominican Republic). Variation was observed in the prevalence of all the HBV markers tested for; overall results suggested extensive exposure to HBV in numerous Latin Amer-
ican populations, the highest at 82.8% of samples from the Dominican Republic with at least one HBV marker.

Of the 500 samples submitted by Mexico, 8 (1.6%) were positive for HBsAg by RIA; 11.6% and 15.5% were positive for anti-HBs and anti-HBc, respectively. Of the Mexico samples, 16.8% had at least one HBV serologic marker.
SECTION SEVEN

HBV AND HEPATOCELLULAR CARCINOMA

Primary hepatocellular carcinoma (PHC) case fatality rates are nearly 100%; in many parts of the world it is one of the most widespread cancers. In certain countries such as Mozambique and Taiwan, it is the most common cancer seen in young men, whereas it is rare in other countries such as the U.S. where it ranks 22nd of malignant neoplasms, and occurs most frequently among Asians. In general, PHC is common in Asia, Oceania, and black Africa, rare in Australia, Western Europe, and South America. Evidence supportive of a causal relationship between HBV and PHC includes a strong geographic correlation between the incidence of PHC and the prevalence of the HBsAg state (see Figure 5).

Case control studies showing a higher frequency of HBsAg among PHC cases than in controls have strengthened the evidence for a causal association between HBV and PHC suggested by the striking geographic distribution of HBsAg and PHC. A controlled study of hepatoma conducted in Senegal, West Africa, an area hyperendemic for HBV infection, found 71% of the mothers of hepatoma patients to be HBsAg-positive, whereas only 14% of the mothers of the control individuals without hepatoma were positive for HBsAg. The estimated relative risk of developing PHC if the mother was a chronic carrier, controlling for various factors in an unmatched analysis, was 12.3; using a matched pair analysis, the estimated relative risk was 5. These results are representative of numerous similar studies done in areas with a high incidence of PHC in Asia and Africa; HBsAg was detected 10-15 times more frequently in patients with PHC than in controls with a range between 37% and 90% of PHC patients positive for HBsAg.
Figure 5. Schematic map of the geographic distribution of HBsAg and primary hepatocellular carcinoma (from Szmuness, Hepatocellular carcinoma and the hepatitis B virus: Evidence for a causal association [1978]).
Additional evidence for a causal relationship between HBV and PHC comes from reports of tumor tissue which has integrated the viral genome into the DNA of the hepatocyte.99

Dr. R. P. Beasley stated: "In 1982, the evidence that the hepatitis B virus causes primary hepatocellular carcinoma is overwhelming."19 One year earlier Beasley had reported a significantly increased risk for PHC among Chinese men in Taiwan who were carriers of HBsAg relative to noncarriers.18 This prospective study of the general population included 22,707 men of whom 3,454 were found to be HBsAg carriers and the remaining 19,253 served as controls; of the 71 cases of PHC that developed, 70 were in the HBsAg-positive group, giving them a relative risk of 390 of developing PHC as compared with the controls. This is a tremendously high relative risk when one considers that the relative risk of developing lung cancer for cigarette smokers is only approximately 10-12. PHC and/or cirrhosis accounted for 57% of deaths among HBsAg carriers compared with less than 2% among noncarriers.

Beasley also published a report of a hepatoma seen in a HBsAg carrier whom they had documented as having been infected perinatally; the 7-year old patient—who died one day before his 8th birthday—was the son of one of the 10,670 pregnant women included in a prospective study of mother-to-infant transmission of HBV.20 Hepatomas have been circumstantially associated with mother-to-infant HBV infection in other cases as well. Multiple members of families with clustered HBs antigenemia in which mothers were uniformly HBsAg-positive suggest prior transmission from the mothers.152 It is believed that this may be a more common phenomenon than has been recognized; however, the interval between birth and onset of hepatoma is usually 40-50 years.

The following schematic representations of the possible natural history of HBV infection occurring in infancy (Figure 6) and in adulthood (Figure 7) and
Figure 6. Schematic representation of the possible natural history of HBV infection occurring in infancy.

Figure 7. Schematic representation of the possible natural history of HBV infection occurring in adulthood. (From Beasley, Hepatitis B virus as the etiologic agent in hepatocellular carcinoma [1982]).
of the cycle of HBV infections and liver cancer from generation to generation (Figure 8) are those proposed by Dr. Beasley for Asia; more information about the possible natural history of HBV infection and its relation to liver cancer in other parts of the world is needed. While the prospective data evaluating the risk of PHC in relation to HBV infection is available for Taiwan, no such studies have yet been done in other countries.

In addition to the relationship between HBV and PHC having been demonstrated in African and Asian populations, it has also been reported in a Caucasian European population. Among Greek patients with PHC, compared with age and sex matched controls, the relative risk for PHC associated with active HBV infection (defined by HBsAg or anti-HBc positivity with no anti-HBs) was 10.4; among patients with metastatic liver cancer, such an association was not seen (relative risk 1.2). Patients who had recovered from hepatitis B and were anti-HBs-positive (without markers for HBV infection) had low risk for PHC (relative risk 0.8).225

Results of a study of serologic and tissue markers of HBV in 50 patients who died at the University of Southern California Liver Unit (USC–Liver Unit) with PHC confirmed at autopsy indicate an extremely high prevalence of HBV infection among PHC patients with nonalcoholic chronic liver disease in the U.S. While earlier reports from the U.S. indicated relatively low coincident rates of HBsAg and PHC,6,139,173,200 they failed to consider the relatively large numbers of cases of PHC related to alcoholic cirrhosis. In the study of the 50 patients who died at the USC–Liver Unit, HBV markers in patients with PHC arising in alcoholic cirrhosis were compared to patients with PHC in nonalcoholic chronic active liver disease. The infection rate in the 22 patients with alcoholic cirrhosis was similar to that seen in 63 control alcoholic cirrhotic patients (9.1% and 7.9%, respectively), whereas 75% (15 of 20) of patients with PHC in nonalcoholic chronic active liver disease showed evidence of HBV infection.156
Figure 8. Schematic representation of the cycle of HBV infections and liver cancer from generation to generation. (From Beasley, Hepatitis B virus as the etiologic agent in hepatocellular carcinoma [1982]).
Quantities of dietary aflatoxins, like distribution of HBsAg carriers, has a good geographic correlation with incidence of PHC.\textsuperscript{5,163} It has been hypothesized that the extremely high incidence of cases of HCC in Asia and Africa compared with the low incidence in the U.S. may be related to a cocarcinogenesis between chronic HBV infection and aflatoxins; however, this study from the U.S. indicates that the prevalence of HBV in patients with PHC in nonalcoholic cirrhotic patients is about the same as that seen in Asia and Africa. While the data from Asia and Africa do not preclude the possibility of an etiological interaction between HBV and aflatoxins or other factors, they do suggest—especially the prospective data from Taiwan—that HBV alone may be able to cause PHC.

A higher frequency of HBsAg has been observed among mothers than fathers of PHC patients.\textsuperscript{112,202} Given that mother-to-infant transmission of HBV may account for about 40\% of the carriers in the general population of Taiwan and Japan,\textsuperscript{19} and that HBV infection appears to be an antecedent to PHC, some investigators believe that prophylaxis of infants at birth may significantly decrease the numbers of cases of PHC seen later in life. The risk of PHC from infections in infancy may be much greater than that resulting from infections in adults, considering that approximately 95\% of infected infants become carriers contrasted to less than 10\% of adults.
SECTION EIGHT

POSTEXPOSURE PROPHYLAXIS OF HEPATITIS B INFECTION

Sexual partners living with index patients who had acute hepatitis B participated in a double-blind study which used hepatitis B immune globulin (HBIG) and a control globulin deficient in anti-HBs. Results indicated that HBIG was efficacious (85%) in preventing overt hepatitis B infection among the partners cohabiting with the index individuals; HBIG appeared effective in suppressing disease as well.180

Infants of HBeAg-positive HBsAg carrier mothers participated in a randomized blind controlled trial of HBIG plus hepatitis B vaccine in Taipei, Taiwan (Republic of China). The trial assessed the prevention of HBsAg carrier state; HBIG plus vaccine was efficacious in 94% of cases,25 compared with 45% (single dose) of 75% (multiple dose) efficacy of HBIG alone, or 75% efficacy of vaccination alone.17 The authors believe that most of the 6% of infants who were not protected by the HBIG and hepatitis B vaccine combination prophylaxis had been infected in utero and therefore had established infection at birth.

Current recommendations for use of HBIG and hepatitis B vaccine in neonates is based on the high postnatal infectivity of HBsAg carrier mothers. HBIG is initially administered as immediately after birth as the infant is physiologically stabilized; this reduces the HBsAg carriage which so often results from the transmission of infection during labor and/or delivery. HRIG is preferably given within 12 hours of birth because its efficacy decreased markedly when treatment is delayed beyond 48 hours.45 One would certainly want to administer HBIG within 48 hours; HBsAg has been detected as early as six days.111
While some of these infants given HBIG develop passive-active immunity, many remain susceptible to infection later. For this reason, vaccination is introduced before passive antibodies wear off. It is recommended to give the vaccine initially within seven days of birth; it can be given at the same time as the HBIG but at a separate site. The passive antibody response to HBIG disappears in three to four months after an initial dose given at birth;\(^{24}\) therefore, vaccine is repeated at one and six months of age.

Figures from Taipei hospitals revealed that 38.1% of infants protected with HBIG during the first year of life subsequently became infected (over the next 17.5 months); nearly half of these infected children became carriers.\(^{24}\) As with perinatal transmission, infants of HBeAg-positive carrier mothers are at highest risk of infection, while those with mothers negative for both HBeAg and anti-HBe are at moderate risk, and the babies of anti-HBe-positive mothers are at lowest risk.

Hepatitis B vaccine was not expected to be highly immunogenic because most newborns infected with HBV become carriers and because babies have not responded well to many vaccines in the past. Studies have shown that not only do most infants respond well but that passive antibodies, whether maternally transmitted or given as HBIG, do not inhibit response to the vaccine.\(^{23,114,128}\) In controlled trials of hepatitis B vaccine to determine its efficacy in preventing early HBsAg carrier state in children of an endemic area (Senegal), anti-HBs of maternal origin was found not to interfere with immunization.\(^{128}\)

Follow-up data is not available for how long infants are protected (duration of anti-HBs) by hepatitis B vaccine, but according to projections from antibody decay curves in older children and adults, anti-HBs may remain detectable for four to five years. It is further postulated that even after loss of detectable anti-HBs antibodies, the children may still be protected as they may be able to restim-
ulate an immune response during the 2-3 month incubation period of HBV infection.

In June 1984, the Centers for Disease Control, Atlanta, published recommenda-
tions for HBV prophylaxis. The following is a list that they compiled of women for whom prenatal HBsAg screening is recommended:45

(1) Women of Asian, Pacific Island, or Alaskan Eskimo descent, whether immigrant or U.S.-born;

(2) Women born in Haiti or Sub-Saharan Africa;

(3) Women with histories of (a) acute or chronic liver disease; (b) work or treatment in a hemodialysis unit; (c) work or residence in an institution for the mentally retarded; (d) rejection as a blood donor; (e) blood transfusion on re-
peated occasions; (f) frequent occupational exposure to blood in medico
dental settings; (g) household contact with an HBV carrier or hemodialysis patient; (h) multiple episodes of venereal disease; or (i) percutaneous use of illicit drugs.

Questions have arisen about the possibility that an agent for acquired immunodeficiency syndrome (AIDS) may be transmissible through vaccines for HBV. While hypotheses about an etiologic role for HBV in AIDS have been postu-
lated,133,179,245 such a relationship remains uncertain; however, HBV is strongly associated with AIDS.8

Circumstantial evidence to date indicates that one most likely cannot contract AIDS from hepatitis B vaccine. It is suggested that human T-cell lym-
photropic virus III (HTLV-III) is the agent of AIDS; HTLV-III recently has been isolated in a high proportion of AIDS cases and antibody to HTLV-III has been detected in most cases.73,185

Serum from homosexual men in New York City was tested in a trial of the efficacy of hepatitis B vaccine; the same serum was also tested in order to consider the safety of the vaccine relative to AIDS. Their results show that the hepatitis B vaccine produced in the U.S. does not contain transmissible HTLV-III,
does not induce an antibody response to HTLV-III, and does not protect against infection with HTLV-III. Prior to this study, follow-up studies had shown that the frequency of AIDS in homosexual men who had received hepatitis B vaccine was similar to that seen in a group of unvaccinated homosexual men.
SECTION NINE
MATERIALS AND METHODS

Background

Current recommendations for whom prenatal HBsAg screening is suggested include women of Asian, Pacific Island, or Alaskan Eskimo descent, as well as women born in Haiti or Sub-Saharan Africa. These groups are known to be at increased risk of being asymptomatic chronic carriers of HBsAg. Information about the relative risk of Hispanic women for being chronic carriers is not available. Given the paucity of literature about the prevalence of HBsAg in Hispanic populations, clinicians have wondered if the prevalence is high enough to warrant routine screening for HBsAg as a part of the prenatal care of these pregnant women. If the prevalence is significantly higher than that seen in the general U.S. population, such screening might be indicated.

Data from Mexico from 500 blood donors showed 1.6% of the sample population to be positive for HBsAg, whereas the HBsAg carrier rate in the U.S. population ranges from 0.1% to 0.5%. It has been suggested that it would be reasonable to routinely test for HBsAg among pregnant women who are members of groups with carrier rates of 1% or greater.

Rationale

The aim of this proposed study is to determine the prevalence of HBsAg in women of Spanish surname receiving prenatal care at Natividad Medical Center (NMC). These women have a relatively low socioeconomic status, sometimes live under poor sanitary conditions, and may come from areas with higher endemic
levels of HBV than those in the U.S.; all of these features are associated with increased rates of HBV infection. Specific data about HBsAg prevalence would be useful to public health agencies as well as clinicians in making choices about prenatal HBsAg screening policy. Health care personnel at NMC have expressed eagerness to participate in this project and have indicated that they would use the results in their clinical decisions about screening. The patient population seems ideal for this study in that over 50% of prenatal patients seen are of Spanish surname.

**Research question:** What is the prevalence of HBsAg in pregnant women of Spanish surname being seen for prenatal care at Natividad Medical Center in Salinas, California?

**Specific Objectives**

The objectives of the present study are as follows:

1. To screen prenatal visitors at NMC for HBsAg until data on a minimum of 500 samples are available.

2. To estimate prevalence of HBsAg in this population and estimate what percentage of HBsAg-positive samples are also HBeAg-positive.

3. To concurrently obtain historical information about place of birth (country, state, and town), and trimester during which blood is drawn on a sample large enough to estimate relative numbers of women born in the U.S. and Mexico and specific location of birth.

4. To inform the physicians of the women positive for HBsAg so that appropriate steps can be taken.

5. To retrospectively gather information about patient's risk factors for hepatitis B from those women who are HBsAg-positive.

6. To make the results of the study available to all interested parties at NMC as well as elsewhere.
Sample Population and Study Population

The Central Coast Area Health Education Center (AHEC) of California includes Monterey, San Benito, San Luis Obispo, and Santa Cruz counties, and is located midway between the metropolitan areas of San Francisco and Los Angeles along the California coast; it encompasses 8,434 square miles. In the 1980 census, 19.7% of the area population identified itself as being of Spanish origin; this includes all indigenous and migrant farmworker Hispanics.

The migrant and seasonal farmworkers of the Central Coast AHEC totaled 29,000 in 1978. The Raza Health Alliance wrote of these farmworkers in their October 1979 Raza Health Plan. They found that 87% were Hispanic with one fourth migrant and three fourths seasonal, and living near their employment. Their average income was less than $5,000 per year and they often lived in overcrowded conditions. Poor environmental conditions such as inadequate sanitation and indiscriminate use of pesticides was found to be not uncommon among these farmworkers.

Most of California's farmworkers work in the San Joaquin, Sacramento, Imperial, and Salinas valleys. Natividad Medical Center in the Salinas Valley (Salinas, Monterey County) serves as a primary care facility of the minority population of the Central Coast; the minority population of this region totals 24.1% of whom 71% are Hispanic. Approximately 60% of the patients seen at NMC are Hispanic; of this population, 30% are monolingual Spanish speaking, most of whom were born in Mexico. The physicians caring for pregnant Hispanic women at NMC would like to know what proportion of these women are asymptomatic chronic HBsAg carriers in order to make a more informed decision about screening for HBsAg among the prenatal patients seen at their facility.

This cross-sectional study will examine a group of subjects once, usually during a prenatal visit. Both inpatients and outpatients who go to the laboratory
at NMC with prenatal blood screening orders will be asked if they would like to participate in the HBsAg prevalence study. A consent form (see Appendix A)—in English and Spanish—will be presented to these women in the lab; they may deny to participate if they so choose. No signatures will be required either to participate or to deny participation. An aliquot of 2cc of serum will be spun off and frozen for each participant; no additional blood than would have been drawn for the prenatal screening panel ordered will need to be drawn for the purposes of this study. The test tubes will be labeled with the patient's name and medical record number as well as the date that the blood was drawn. A log sheet with patient record number, date, and doctor's name will be kept in the lab as well (see Appendix B). Occasional samples of blood drawn during labor and sent to the lab may be included in the study.

Serum samples will be shipped to Abbott Laboratories, North Chicago, Illinois, where RIA for the detection of HBsAg will be performed. The serum samples sent will be coded with a number which will be used to record results of all samples (see Appendix C). If any serum remains after the tests, it will be frozen for potential future tests.

Sample Size

Since the expected prevalence of HBsAg positivity is low, a relatively large sample size will be necessary in order to obtain enough positives to give a reliable estimate of prevalence. If the true population prevalence is 1.5% (close to the level estimated in Mexico), a sample size of 500 would have a 78% chance of giving an estimated prevalence of 1.0% or greater. A result of 1.0% in a sample size of 500 would have a 95% confidence interval ranging from 0.4% to 2.5% (by the Fleiss "exact" method). If the true population prevalence is only 1.0% (midway between the 0.4% seen in the U.S. and 1.6% estimated in Mexico), a sample size
of 500 would have a 76% chance of giving an estimated prevalence of 0.6% or greater. A sample size of 500 thus appears to be an appropriate minimum. A larger sample size would, of course, give narrow confidence intervals for the estimated prevalence.

**Measurement Methods**

Abbott Laboratories will be using Austria II-125, their registered trademark for a third generation test for the qualitative radioimmunoassay (RIA) of HBsAg in serum or plasma. Negative results (i.e., those specimens nonreactive by the Austria II-125 test) are not tested further.

False-positive results occasionally occur with different methodologies for testing serum for the prevalence of HBsAg; therefore, it has been stressed that RIA-positive results be confirmed.176 Those specimens which are reactive will be retested by the Austria II-125 test; if repeatability reactive, the specimen will be confirmed using Austria II-125 Confirmatory Neutralization Test before considered positive.

Workers from the Department of Virology, Abbott Laboratories, published the methodology for the first commercially available RIA for the detection of HBsAg, Austria-125, in 1972.117 In 1974 Abbott Laboratories introduced the first coated bead RIA for HBsAg, Austria II-125, in order to improve sensitivity and specificity. Austria II-125 "sandwich" RIA uses beads coated with guinea pig anti-HBs and incubates them with serum or plasma and appropriate controls (negative control: recalcified human plasma, nonreactive for HBsAg and anti-HBs; positive control: human plasma, positive for HBsAg). Any HBsAg present binds to the solid phase antibody. The unbound material is aspirated, the bead is washed; human 125I-Anti-HBs is allowed to react with the Ab-Ag complex on the bead and unbound 125I-Anti-HBs is removed by washing. A gamma scintillation counter
is used to count the radioactivity remaining on the beads.

\[ \text{\ensuremath{\text{\textarrow{ Anti-HBs } \text{ HBsAg}}} \text{\ensuremath{\text{\rightarrow}}}} \]

\[ \text{\ensuremath{\text{\textarrow{ Anti-HBs \cdot HBsAg } \text{\textarrow{ 125I-Anti-HBs}}}}} \]

\[ \text{\ensuremath{\text{\textarrow{ Anti-HBs \cdot HBsAg \cdot 125I-Anti-HBs}}}} \]

Specimens are considered negative for HBsAg if the counts per minute are equal to or greater than the cut-off value determined by multiplying the negative control mean count rate by a factor.

A reactive specimen of a screening procedure is considered repeatedly reactive for HBsAg if repeats are greater to or equal to the cut-off; the original result is classified as nonrepeatedly reactive if repeat testing shows specimens to be less than the cut-off.

A donor or patient cannot be told that he/she is an HBsAg carrier until specificity analysis is performed. A repeatedly reactive specimen is considered HBsAg-positive after confirmation by neutralization with human anti-HBs or other licensed HBsAg tests. The results of this study will not be used diagnostically. Repeatedly reactive specimens will not be confirmed in this study. The physicians of any of the patients who test positive will be informed of the results; however, these test results cannot be used to inform the patients that they are HBsAg carriers. Additional screening of their blood will have to be performed in order to make that determination.

Resources

The Andrew W. Mellon Program in Clinical Epidemiology at U.C.S.F. offers a wide range of expertise in clinical epidemiology and research methodology. The faculty hold appointments in the Department of Epidemiology at the U.C.S.F.
Medical School. Biostatisticians and computer facilities are also available. Dr. Norman Hearst is a Senior Fellow in the Epidemiology program. Joan Baumbach is a medical student (U.C. Berkeley/U.C.S.F. Joint Medical Program) who began work on this project as a Summer Research Fellow with the Mellon Program.

Natividad Medical Center is a county hospital in the Salinas Valley of California which is affiliated academically with the U.C.S.F. Medical School. The patient population which it serves consists largely of Mexican farmworker families. Dr. John Midtling, M.D., M.S., Residency Director and Vice Chairman, Division of Family and Community Medicine, and Dr. Bob Levin, Chief of Pediatrics, Pediatrics/Infectious Disease, will be instrumental in coordinating the physicians, nurses, and laboratory personnel to be involved in the study.

Abbott Laboratories will be performing the radioimmunoassays for the detection of HBsAg (using Austria II-125) as well as testing for HBeAg in any serum samples positive for HBsAg. In addition to performing these tests, Abbott Laboratories will pay for up to $2,400 of additional expenses.

Projected Budget

Transportation (including round-trips from San Francisco to Salinas and Federal Express shipment of sera from Salinas to North Chicago, IL) $1,230.00

Supplies
   Lab (test tubes, labels, etc.)
   Office (stationery, photocopy, postage, etc.) 540.00

Telephone 630.00

Total $2,400.00

Time of all investigators will be donated.
SECTION TEN
CONCLUDING STATEMENT

To date, 129 serum samples have been tested for the presence of HBsAg. Five of the results required retesting for HBsAg; all five samples were negative on retest as well as negative for HBeAg which was also tested for. These 129 samples represent only about 20% of the total projected sample size of 600; therefore, it is only speculative to comment about a possible trend in the results. If the trend seen thus far continues and there are no positive samples, or fewer than would be statistically significant, we could state with a fair degree of certainty that in this particular population there is not a higher prevalence of HBsAg than is seen in the general population.

If it turns out that this population does not have a higher prevalence of HBsAg than the level seen in the general population, these women would not represent a group that would require special screening for HBsAg. However, any of these pregnant women who fall into a high risk category for HBsAg—for whatever other reason—must be screened for HBsAg regardless of conclusions of prevalence studies attempting to determine the relative risk for carrying HBsAg.

If, by the time the results of the entire sample planned are in, the trend changes such that this population of pregnant women appear to be at increased risk for the carrier state, a larger study would be useful in order to more accurately determine the prevalence of HBsAg among pregnant Hispanic women in this country.
APPENDIX A

NOTICE TO PRENATAL PATIENTS

This clinic or hospital is helping scientists at the University of California study blood from pregnant women for evidence of hepatitis, a serious infection which can be passed from mothers to their unborn babies. If your blood is being drawn today for routine prenatal tests, a test for hepatitis may be done (at no charge to you) in addition to the usual tests. No more blood will be drawn from you than would have been taken otherwise.

Since this information is rare, we expect very few women to have a positive test. Those who do will be contacted later and asked a few questions about how they might have caught it. If you are one of these women, the advantage to you is that we will notify you and your physician so that appropriate treatment can be given to you and your baby. The only possible disadvantage is that if further tests show that you do indeed have this infection, you might be denied work in a restaurant.

This study is important because it will help determine how much hepatitis there is in pregnant women in the Salinas area. We appreciate your cooperation. If you decide for any reason that you do not want your blood tested for hepatitis, just tell the blood drawer, or your doctor or nurse, or call Mary Lou Vasquez at 757-0487, and we will make sure that your blood sample is not included in this study. Mary Lou Vasquez speaks Spanish and English.
NOTICIA A PACIENTES PRENATAL

Esta clínica o hospital está ayudando científicos en la Universidad de California estudiar sangre de mujeres embarazadas para evidencia de hepatitis, una infección que las madres pueden pasar a sus niños recién nacidos. Si su sangre fue tomado hoy por exámenes prenatal, un examen para hepatitis posiblemente sería hecho (sin cobro a Usted) con los otros exámenes. No necesitaremos tomar más sangre que tomamos para los otros exámenes.

Esta infección es rara, por supuesto pocas mujeres tendrán resultados positivos. Les llamamos las mujeres que resultan positivas; les preguntaremos unas cuestiones acerca de la infección. Si Ud. resulta positiva, la ventaja es que nosotros les notificaremos a Ud. y a su doctor para dar tratamiento a su nene. Lo único desventaja es que si otros exámenes indican que Ud. verdaderamente tiene esta infección, es posible que podría ser negado empleo en restaurantes.

Este estudio es importante porque indicara cuanto hepatitis hay en mujeres embarazadas en la área de Salinas. Agradecemos su cooperación. Si decide Ud. que por cualquier razón no quiere que su sangre sea examinado por hepatitis, digale a la persona que está tomando su sangre, o a su doctor o enfermera, o llame a Mary Lou Vasquez al 757-0487 y no usaremos su sangre en este estudio. Mary Lou Vasquez habla Español e Ingles.
APPENDIX B
PRENATAL HEPATITIS B RESEARCH PROJECT

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APPENDIX C

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