# CHAPTER II

# LITERATURE REVIEW

## 1. History of Hepatitis B Virus

Viral hepatitis is a term that is reserved for infections of the liver caused by one of at least five distinct hepatitis viruses. The most notable sign of this disease is jaundice, an orange-yellow discoloration of the scleroproteins of the skin and conjunctivae seen with an increased plasma bilirubin resulting from faulty excretion of bile pigment by damaged hepatocytes.

The existence of a parenterally transmitted form of hepatitis was documented in 1885 (Lurman, 1885) but not until the late 1930s that the existence of hepatitis transmitted by parenteral inoculation of human serum was firmly established. In 1937, it was reported from England (Ministry of Health, 1937) that approximately 40% of 109 individual receiving inoculations of a single batch of human measles convalescent serum developed jaundice after incubation periods of up to 114 days. This was followed by an outbreak in British troops given filtered human mumps convalescent plasma (Beeson, Chesney, and McFarlan, 1944). In 1942 (Fox et al., 1942), subsequently implicated human serum as the vehicle of transmission. They also observed that the incubation period of parenterally transmitted hepatitis was much longer than that described for infectious hepatitis. It is assumed that these outbreaks were caused by hepatitis B virus (HBV) infection.

Between the late 1950s and early 1970s, Murray (Murray, 1955) and Krugman (Krugman et al., 1967) were instrumental in further defining the seroepidemiologic relationships between hepatitis A and B, which eventually led to the evaluation of new methods for the diagnosis and immunoprophylaxis of these diseases.

The chain of events that culminated in the discovery of HBV and an effective vaccine for the prevention of hepatitis B was a tortuous one. It began in 1963 when Baruch Blumberg began examining thousands of blood samples from diverse populations in a study designed to look for inherited polymorphic traits in different geographic areas of the world (Blumberg et al., 1982). To detect novel antigens, sera

from multiply transfused hemophilia patients were used because it was postulated that such sera might contain antibodies against these unique proteins. In the course of the investigation discovered that a serum sample from an Australian aborigine contained an antigen that reacted specifically with an antibody in the serum of an American hemophilia patient. The additional association of the antigen (now designated the hepatitis B surface antigen, HBsAg) with what was then known as serum hepatitis was not recognized fully until 1967. By 1968, Murakami (Murakami, 1968) and Prince (Prince, 1968) had established that the antigen was found specifically in the serum of hepatitis B patients.

The discovery and characterization of HBsAg was a major breakthrough in hepatitis research because it permitted intensive study of the disease and the nature of the infectious agent even though the virus itself had not yet been identified conclusively. In 1970, Dane et al. (Dane et al., 1970) detected the complete hepatitis B virion, a 42 nm double-shelled particle that consisted of and outer envelope composed of HBsAg and an inner core or nucleocapsid containing its own distinct antigen, the hepatitis B core antigen (HBcAg), in addition to a small, circular, partially double-stranded DNA molecule and an endogenous DNA polymerase enzyme (Kaplan et al., 1973) (Robinson, 1975). The third antigen designated hepatitis B e antigen (HBeAg) was described subsequently by Magnius and Espmark (Magnius and Espmark., 1972). This antigen is a reliable marker for the presence of intact virions and thus for enhanced infectivity.

## 2. Biologic Characteristics of Hepatitis B virus

HBV is the prototype virus for a family of DNA viruses called the *Hepadnaviridae*. Related viruses are found in woodchucks, ground squirrels, Peking ducks, and tree squirrels. These viruses share certain features such as virion size and ultrastructure; a distinctive polypeptide and antigenic composition; a comparable DNA size, structure, and genetic organization; and a unique mechanism of viral DNA replication. Separate genera have been proposed for the mammalian and avian hepadnaviruses because of differences that exist in their molecular biology.

Ultrastructural examination of particles observed in the sera of patients with HBV infection reveals three distinct morphologic entities in various proportions. The more numerous forms are the small, pleomorphic, spherical, noninfectious particles measuring 17 to 25 nm in diameter (mean diameter, 20 nm). Tubular or filamentous forms of various lengths, but with diameters similar to those of the small particles, also are observed. A third particle, the hepatitis B virion, is a complex double-shelled particle with a diameter of approximately 42 to 47 nm. Originally designated the Dane particle, it consists of an outer shell or lipid-containing envelope, approximately 7 nm in thickness,

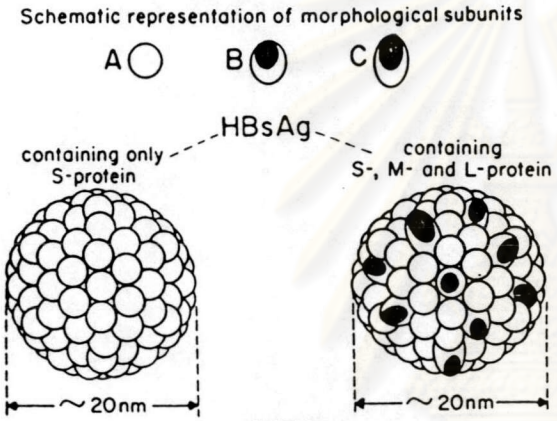
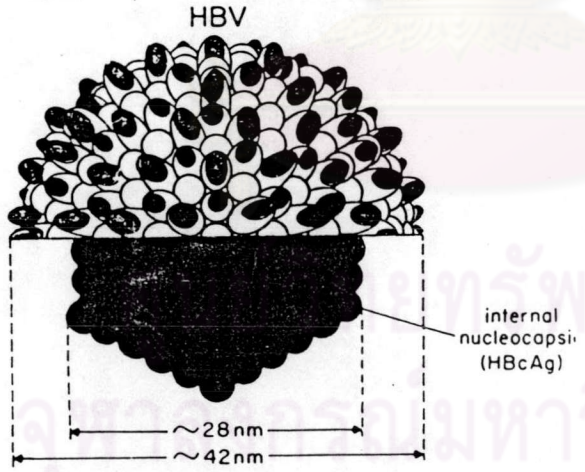



Fig.1



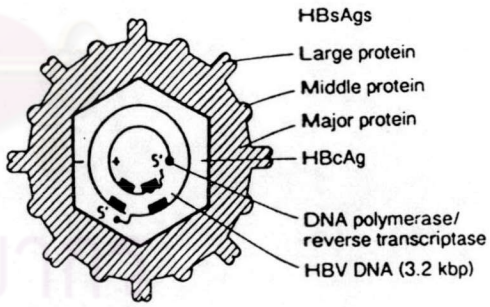




Fig. 2

**Figure 1.** Composition of subunits and assembled HBsAg and virus particles. Individual subunits contain S protein only (a), M protein (b), or L protein (c). The lower half of the virus particle shows the nucleocapsid. (Hans and Laurent, 1995)

**Figure 2.** Hepatitis B virus and its components. (Hans and Laurent, 1995)

that surrounds an electron-dense inner core having a diameter of 25 to 27 nm. The complex antigen found on the surface of HBV and comprising the 20 nm diameter spherical particles and tubular forms is called HBsAg . The envelope or HBsAg proteins are represented by three HBV encoded polypeptides sometimes designated the small, middle, and large proteins of HBsAg. The major of small protein is encoded by the pre-S1, pre-S2, and S regions of the S gene and contains an additional glycosylation site in the pre-S2 region. The large polypeptide consists of 389 to 400 amino acids encoded by the pre-S1, pre-S2, and S regions of the S gene of the HBV genome but generally lacks the pre-S2 glycosylation site.

Fig. 3

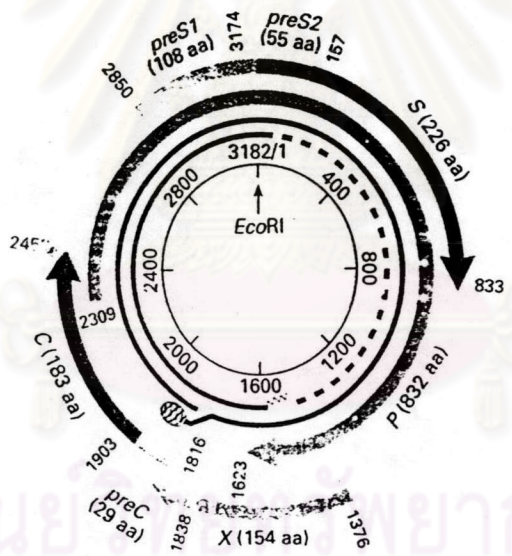



**Figure 3.** Genome of HBV. The viral DNA is partially double-stranded. The long strand of fixed length encodes seven proteins form four overlapping reading frames [surface (*S*), core (*C*), polymerase (*P*), and the *X* gene (*X*)], shown as large arrows, and three upstream regions (*preC*, *preS1*, and *preS2*). The *Eco*RI restriction-enzyme binding site is included as a reference point. (Lee, 1997)

The complete virion has a buoyant density of about 1.22 g/cm$^3$ in CsCl and consists of an envelope of HBsAg, an internal core or nucleocapsid that contains core proteins with hepatitis B core antigen (HBcAg) and HBeAg specificities, and a small (3200 bp), circular, partially double stranded DNA molecule with a molecular weight of $1.6 \times 10^6$ (Takahashi., 1976). The HBV genome is partially double stranded because of incomplete extension of the positive sense strand by the endogenous DNA polymerase enzyme incorporated within the virion. Because some HBV particles may contain no DNA while others contain genomes with single stranded gaps of different lengths, the buoyant densities of the nucleocapsids in CsCl can range from 1.30 to 1.36 g/cm$^3$ (Kaplan et al., 1976). Antibodies to HBsAg (anti-HBs), HBcAg (anti-HBc), and HBeAg (anti-HBe) are produced in response to their respective antigens.

The stability of HBV does not always coincide with that of its envelope protein, HBsAg. Immunogenicity and antigenicity are retained after exposure to ether, acid (pH 2.4 for at least 6 hours), and heat (98 °C for 1 minute, 60 °C for 10 hours); however, inactivation may be incomplete under these conditions if the concentration of virus is excessively high. Exposure of HBsAg to 0.25% sodium hypochlorite for 3 minutes destroys antigenicity (and probably infectivity). Infectivity in serum is lost after direct boiling for 2 minutes, autoclaving at 121 °C for 20 minutes, or dry heat at 160 °C for 1 hour. More recent studies have shown that HBV is inactivated by exposure to sodium hypochlorite (500 mg of free chlorine per liter) for 10 minutes, 0.1% to 2% aqueous glutaraldehyde, Sporicidin (pH 7.9), 70% isopropyl alcohol, 80% ethyl alcohol at 11°C for minutes, Wescodyne (American Sterilizer, Medical Products Division, Erie, PA) diluted 1:213, or combined β-propiolactone and ultraviolet irradiation (Prince et al., 1983) (Bond et al., 1983) (Kobayashi et al., 1984). HBV has been shown to retain infectivity when stored at 30 °C to 32 °C for at least 6 months and when frozen at –20 °C for 15 years.

### 3. Cell Cultures and Animal Models

Despite recent evidence for the growth of HBV in primary cultures of healthy adult or fetal human hepatocytes (Ochiya et al., 1989) (Gripon et al., 1993) (Jumin, 1996), serial propagation over a prolonged period has not been accomplished. Still, the use of stably transfected cell lines provides a model of HBV replication, even though the life cycle is incomplete and only low concentrations of virus are produced. Chimpanzees and other high- order primates are highly susceptible to experimental induction of hepatitis B but are not routinely available (Barker et al., 1975). The pattern of infection is similar to that observed in humans except that the disease is clinically milder. Nevertheless, the chimpanzee model has played an essential role in studies of viral inactivation, vaccine safety and efficacy, disinfection kinetics, infectivity determination and immunopathology, and in seroepidemiologic investigations. Because of its limited role as a model for studying viral replication or evaluating antiviral agents, it is being replaced by other animal systems such as the woodchuck, Peking duck, and transgenic mouse (Marion, 1990).

### 4. Pathology

HBV causes both acute and chronic liver disease. The pattern of liver injury is characterized by hepatocellular destruction, regeneration, and inflammatory infiltrates. although these pathogenic changes typically resolve completely after viral clearance, chronic infection is often accompanied by progressive fibrosis and architectural disarray that culminates in cirrhosis. The pattern of injury accompanying chronic viral hepatitis has distinct features and may be distinguished from other forms of liver disease, such as cholestatic liver disease, autoimmune hepatitis, metabolic liver disease, drug-induced hepatitis, and steatohepatitis. However, the pattern of injury is seen with acute hepatitis B overlaps significantly with other causes of viral hepatitis. Because chronic hepatitis B takes decades to progress to end-stage liver disease, it is important to assess not only the pattern of injury but also the histologic severity and stage of hepatitis B.

**4.1 Acute Hepatitis** The histologic features associated with acute hepatitis B are marked by either ballooning degeneration or acidophilic bodies, which probably reflects two distinct mechanisms of hepatocyte damage. The former is characterized by swollen hepatocytes with fragmenting cell membranes, pale granular cytoplasm, and disintegrating nuclei. The latter alteration represents the histologic manifestation of apoptosis and is marked by a progressively shrinking cell, eosinophilic cytoplasm, and pyknotic nuclei. Focal hepatocellular necrosis, the histologic hallmark of acute viral hepatitis, is characterized by intralobular foci of necrotic of apoptotic hepatocytes surrounded by inflammatory cells including lymphocytes, macrophages, and rarely plasma cells. These necroinflammatory changes are distributed throughout the lobules with more extensive pericentral involvement. Regenerative changes consisting of double-cell-thick hepatic cords, binucleated hepatocytes, and nuclear hyperchromasia often accompany necroinflammatory foci. Other histologic features include lobular inflammatory cell infiltrate, Kupffer cell hypertrophy, and sinusoidal reaction. Bile duct lesions such as ductular proliferation or cholestatic obstruction are rare events.

**4.2 Chronic Hepatitis** The histologic hallmark of chronic hepatitis B is inflammatory destruction of hepatocytes, accompanied by progressive fibrosis. The histologic classification of chronic hepatitis has undergone extensive modification to provide a more quantitative and clinically relevant description of the disease. The classical terms of chronic persistent, chronic lobular, and chronic active hepatitis are being replaced by revised grading and staging systems that consider the degree and extent of necroinflammation and fibrosis (Ludwig, 1993) (Desmet et al., 1994) (Bedossa and Poynard., 1996). These histologic systems describe a continual spectrum of liver injury and disease activity and provide a more accurate assessment of prognosis and therapeutic effect.

The most widely used system is based on the modified Knodell scores (Ishak, 1995) This system has two scores: one based on inflammatory activities (grade) and the other on fibrosis (stage). The grade is indicative of ongoing disease activity, whereas the stage represents the cumulative effect of the disease. The inflammatory component is divided into three compartments: the interface zone between the hepatocytes and the

portal area (limiting plate), the portal area, and the hepatocellular parenchyma. Piecemeal necrosis or interface hepatitis describes the destruction of hepatocytes at the limiting plate, which forms a well-defined structure demarcating the hepatic parenchyma and the portal area. On microscopy, the smooth edge of the limiting plate becomes eroded and indistinct as the junctional surfaces of periportal hepatocytes are invaded by lymphocytes or macrophages (peripolesis). Occasionally, lymphocytes are seen to actively penetrate the hepatocytes (emperipolesis). Piecemeal necrosis may be focal. Involving only one or two hepatocytes with little destruction of the limiting plate, or it may be extensive, involving the entire circumference of the portal area with penetration of the inflammatory cells to a depth of several hepatocytes. In more severe cases, the necroinflammatory process extends into the parenchyma, reaching to the terminal hepatic veins or to other portal areas (bridging necrosis).

The portal area, while contiguous with the periportal compartment, is being considered separately because it delineates a distinct pathologic feature. When bound by the limiting plate, lymphoid infiltrates can fill and expand the portal areas, occasionally forming aggregates of variable density. Like the primary follicles of lymph nodes, the lymphoid aggregates are composed of a mixture of B and T cells with small numbers of macrophages and dendritic cells. Rarely, the lymphoid aggregates resemble reactive germinal centers with a core of B and plasma cells and a mantle of B and T cells. Bile duct lesions (Poulsen lesions) also can be seen accompanying the lymphoid aggregates; they are characterized by reactive epithelial changes and lymphocytic infiltration without true cholestatic features. These histologic changes are more commonly observed in chronic hepatitis C infections, as is steatosis.

Parenchymal inflammation in chronic hepatitis B generally consists of small foci of lymphocytes and macrophages surrounding apoptotic bodies or necrotic ("drop-out") hepatocytes. Plasma cells and eosinophils can sometimes be seen in these foci. The relative abundance of plasma cells serves to distinguish chronic hepatitis from acute hepatitis. The parenchyma often shows areas of ballooning degeneration and focal collapse. There also may be features of regenerative activity such as hepatocellular mitotic figures, binucleated cells and widened liver cell plates. When these foci of

lobular necrosis are the dominant features of the inflammatory pattern, it often is difficult to distinguish chronic from acute hepatitis. Regardless, this pathologic feature can be seen in many patients with chronic hepatitis B, particularly those with HBeAg negative mutant infection who experience periodic flares of symptoms and biochemical abnormalities (Brunetto, 1991). Occasionally, more extensive lobular necroinflammation with confluent, bridging, or panlobular necrosis can be seen. Often, these exacerbations resolve spontaneously with a return of the histologic feature to minimal to mild inflammation. However, frequent and severe attacks can lead to more rapid progression to cirrhosis.

**5. Clinical Features of Hepatitis B**

There are a number of possible features (Fig 4.) (David and Frank, 1994)). Under most circumstances, HBV is not cytopathic. An intact immune system is vital to cell injury and viral clearance (Chisari, and Ferrari, 1995) (Moradpour, and Wands, 1995) (Jung et al., 1994). For practical purposes, the severity of the hepatocyte injury reflects the vigor of the immune response: the most complete immune response is associated with the greatest likelihood of viral clearance and the most severe liver injury. Ninety five percent of infected neonates with immature immune systems become asymptomatic chronic HBV carriers, as compared with 30 percent of children infected after the neonatal period but before six years of age. Only 3 to 5 percent of adults remain chronically infected, the remainder have acute infections resulting in viral clearance. Clinical progress of hepatitis B was divided in four stages (Table 2) (Lee, 1997). The first stage is characterized by immune tolerance. In the healthy adult, this incubation period lasts about two to four weeks. In contrast, with neonatal infection, this period often lasts for decades. In most cases of HBV infection through out the world, active viral replication continues despite little or no elevation in the aminotransferase levels and no symptoms of illness.

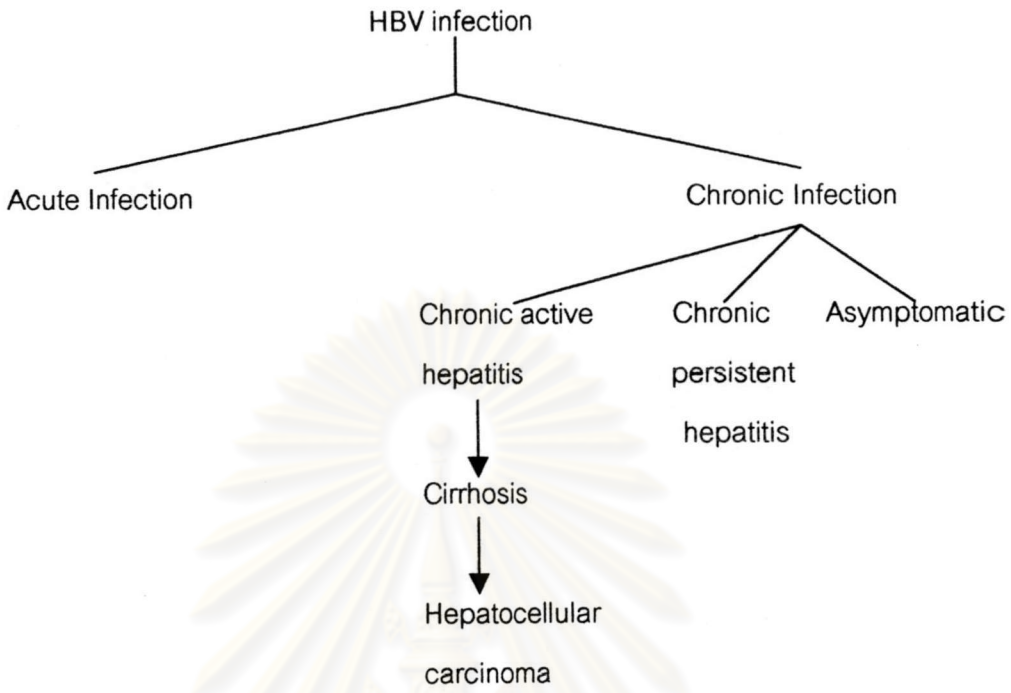



**Figure 4**. Clinical outcomes of hepatitis B virus infection (David and Frank, 1994)

In the second stage, an immunologic response develops or improves, leading to cytokine stimulation and direct cell lysis and the inflammatory process. Secretion of HBeAg still occurs in stage 2, but HBV DNA levels in serum drop as the number of infected cells declines. In patients with acute HBV infection, stage 2 is the period of symptomatic hepatitis and typically lasts three to four weeks. In patients with chronic disease, stage 2 may persist for 10 or more years, leading to cirrhosis and its complications. When the host is able to mount a response that eliminates infected cells or greatly diminishes their number, active viral replication ends and the third stage begins. In this stage, HBeAg is no longer present, and antibody to HBeAg becomes detectable. A marked decrease in viral DNA is observed, although many patients remain

**Table 2.** The four stages of hepatitis B infection (สิริฤกษ์ ทรงศิวไล, 2543)

| Disease marker | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
|---|---|---|---|---|
| HBsAg | + | + | + | - |
| Anti-HBs | - | - | - | + |
| HBV DNA | +++ | + | ± | - |
| HBeAg | + | + | - | - |
| Anti-HBe | - | - | + | + |
| Anti-HBc | + | + | + | + |
| Serum ALT level | Normal | Elevated | Normal | Normal |

positive for HBV DNA as detected by PCR. In stage 3, the infection has cleared, and aminotransferase levels become normal. However, patients remain positive for HBsAg, because of the integration of viral gene into the host's hepatocyte genome.

Most patients eventually become negative for HBsAg and positive for antibody to HBsAg, marking the fourth, or immune, stage in the HBV life cycle. HBV DNA can no longer be detected, and the patient is unlikely to become reinfected or to have a reactivated infection. Factors affecting the evolution through the four stages, in addition to the generic predisposition of the host, noted above, include the presence of other viruses, treatment with immunosuppressive agents, sex, and the appearance of HBV mutants.



### 6. Laboratory Diagnosis

Biochemical tests of liver function distinguish viral hepatitis from the many nonviral, for example, obstructive or toxic, causes of jaundice. Characteristically, levels of serum transaminases (aminotransferases) are elevated markedly (5 to 100 fold) in acute symptomatic viral hepatitis, whether due to hepatitis A, B, C, D, or E. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) rise together late in the incubation period to peak about the time jaundice appears; they gradually revert to normal over the ensuring 2 months in an uncomplicated case. Serum bilirubin may rise

anything up to 25 fold, depending on the severity of the case and may of course be close to normal in anicteric viral hepatitis.

Although many types of immunoassays have been successfully applied to HBV, the most widely used and most sensitive have been radioimmunoassay (RIA) and enzyme immunoassay (EIA). Six markers, all found in serum, are of particular diagnostic important: HBsAg, HBV DNA, HBeAg, anti-HBs, anti- HBe, anti-HBc.

Acute infection with hepatitis B virus is characterized (Fig. 5) (Hoofnagle, 1981) by the appearance of HBsAg in the blood for one or two month coincidence with the rise in transaminase levels. Viral DNA and HBeAg appear at about the same time as HBsAg but disappear abruptly when symptoms and enzyme levels peak. The first antibody detected is anti-HBc; it usually appears before symptoms develop, rises rapidly to high titer, and persists indefinitely. Anti-HBs, on the other hand, does not become detectable until HBsAg has been cleared and recovery is complete (usually within a year); indeed, there is sometimes a window in time during which neither HBsAg nor anti-HBs is demonstrable, and anti-HBc is the only positive marker of infection.

Chronic hepatitis B infection (Fig. 6) (Hoofnagle, 1981) is characterized by the persistence of HBsAg for at least 6 months, but often for years or even for life. As long as HBs antigenemia persists anti-HBs antibody is usually not found free, but in about 10% of carriers it is complexed in low amounts with HBsAg. Anti-HBc rises to very high titer and persist for life in the normal way. This is the picture with the HBsAg carrier state.

Chronic active hepatitis is distinguished from the asymptomatic carrier state by progression of liver damage, as indicated by continuing elevation of serum transaminase levels and histologic evidence on liver biopsy. Persistence of HBV DNA, viral polymerase, HBeAg, and virions implies active viral multiplication, high infectivity, and progressive liver damage, the hallmarks of the high replicate phase. In contrast, anti-HBe, which develops only after HBeAg disappears and enzyme levels have declined, indicated a longer standing carrier state characteristic of the low replicate phase.

Fig. 5

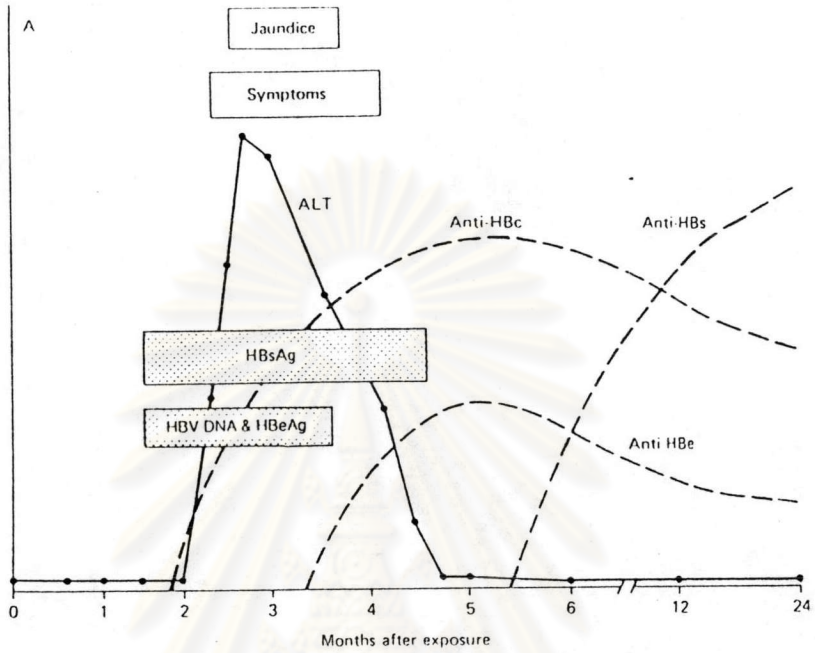



Fig. 6

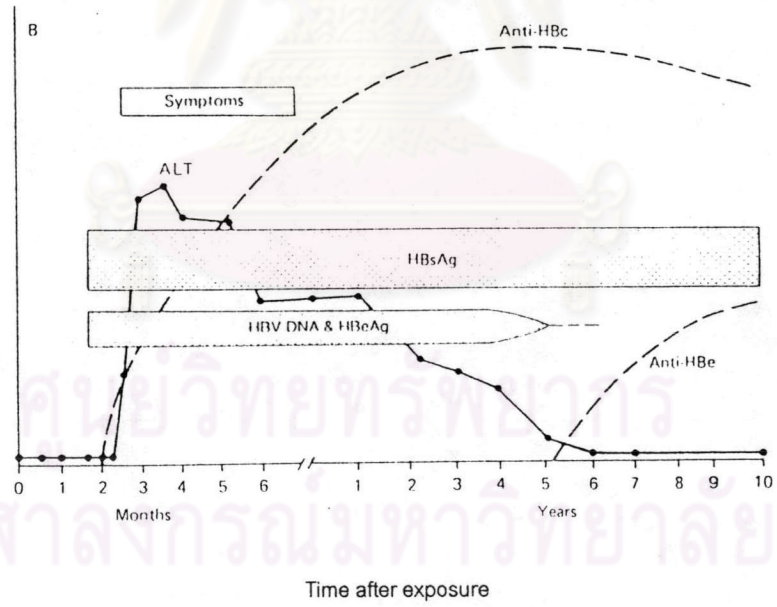



**Figure 5.** Serological events associated with the typical course of acute type B hepatitis (David and Frank, 1994).

**Figure 6.** The development of the chronic hepatitis B virus carrier state (David and Frank, 1994).

Table 3. summarizes the patterns of serological markers that characterize the various outcomes of hepatitis B infection. Note that the key markers are HBsAg, anti-HBs, anti-HBc, and HBV DNA; the pattern of these can distinguish most of the important situations. The most reliable marker of past or present HBV infection is anti-HBc; persistence of HBV DNA in chronic active hepatitis portends an unfavorable outcome in the long run; and anti-HBs, which is the neutralizing antibody, appears only after HBsAg has vanished, hence is a reliable indicator of recovery and of immunity to reinfection.

**Table 3.** Serological markers of hepatitis B infection (David and Frank, 1994)

| Clinical condition | Serological marker[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | HBsAg | Anti-HBs | Total anti-HBc | IgM anti-HBc | [b] HBeAg | Anti-HBe | Viral DNA |
| Acute hepatitis | + | - | + | ++ | + → - | - → + | + |
| Chronic active hepatitis | + | - | + | + | + | - | + |
| Asymptomatic carrier state | +[c] | - | + | -[d] | - | + | -[e] |
| Past infection: immunity | - | + | + | - | - | + → - | - |
| Past immunization | - | + | - | - | - | - | - |

[a] Arrow means transition in due course from one state to the other.

[b] If infected with wild-type HBV, not with pre-core mutant.

[c] Persisting for more than 6 months.

[d] Low titer.

[e] Very low titer

## 7. Epidemiology

HBV is a ubiquitous organism that is globally distributed. Although HBsAg has been found in the serum of certain species of non-human primates, human remain the principal reservoir for HBV. Serological surveys have documented the existence of

hepatitis B throughout the world, including the most isolated and remote areas. The true prevalence of the disease can only be estimated because the collection of accurate data has been hampered by inadequate reporting, lack of laboratory confirmation, insensitive assays, and selection bias. Nevertheless, it is a clear that the HBsAg carrier rate varies from country to country, with higher rates begin reported from developing countries with primitive or limited medical facilities (Fig. 7) (Black et al., 1974) (Blumberg et al., 1965) (Glossman et al.,1975) (Sobeslavsky, 1975) (Szmuness, 1975). Even within

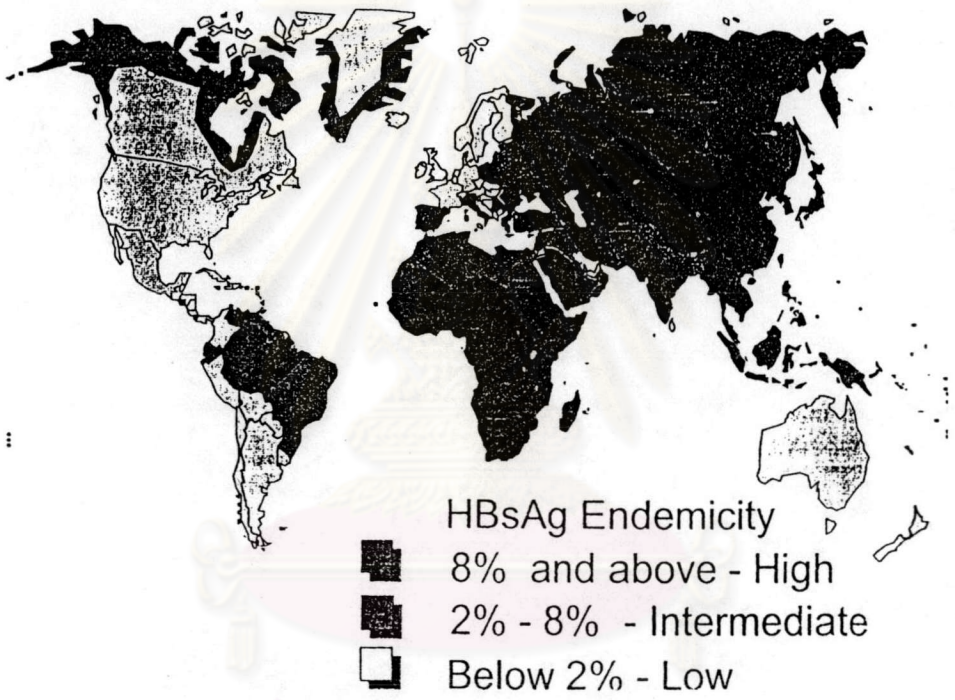



**Figure 7**. Prevalence of HBsAg in the world (David and Peter, 2001)



apparently homogeneous groups in the same country or area and among different ethnic groups. The HBsAg carrier rate may vary substantially (Sobeslavsky, 1975) (Szmuness, 1975) (Szmuness, 1978). There is no seasonal trend for HBV infection, and epidemics are unusual. Cumulative infection rates in the United states increase with age, with an overall age adjusted prevalence of 4.9%, based on the presence of anti-HBc. Among the major racial groups, the rates were 2.6% in non-Hispanic whites, 4.4%

in Mexican-Americans, and 11.9% in non-Hispanic black participants (McQuillan et al., 1999). The male-to-female ratio was 1.5:1. From these data, an estimated 1 to 1.25 million Americans are believed to have contracted chronic hepatitis B. Those with an enhanced likelihood of having acquired this infection include residents and staff of penal institutions and residential facilities for the developmentally disabled; multiply transfused patients (pre-1988); patients and staff oncology or hemodialysis units and organ transplantation patients; male homosexuals and sexually promiscuous groups; users of illicit injectable drugs who share needles; close contacts of chronic carries; sexual partners of patients with acute or chronic hepatitis B; and health care workers (particularly dentists, surgeons, and clinical laboratory staff). However, the availability of an effective vaccine, optimized blood donor screening, and better sterilization procedures for blood derivatives have dramatically altered the risk in many of these groups. In endemic areas of Asia and Africa, the epidemiologic patterns of infection are different from those seen in North America and western Europe (Szmuness et al., 1978). Serologic surveys indicate that most infections in these geographic regions occur in infants and children as a result of maternal-neonatal transmission or early childhood contact, although percutaneous exposure with contaminated needles or following unsafe injections is always a possibility in these countries.

### 8. HBV and Hepatocellular Carcinoma

The incidence of Hepatocellular Carcinoma (HCC) varies with geography, race, sex, and cirrhosis (Okuda and Tabor, 1997). HCC constitutes 90% of the primary malignant tumors of the liver observed in adults. Worldwide, liver cancer ranks eight in frequency. It is the sixth most frequent cancer in men and eleventh most common in women. From 4% to 5% of the global cancer burden is attributed to this condition, and in developing countries it is the third most common cancer in males after the stomach and lungs (Parkin et al., 1985) (Pisani et al., 1985). It is estimated that liver cancer may be responsible for more than 500,000 deaths annually throughout the world, with a male-to-female ratio of 3:1. In the industrialized nations of the world it is rare to relatively uncommon, yielding an age adjusted incidence rate of less than 10 per 100,000

population per year in men from northern Europe, North America (except in migrants from high-risk countries such as China and Korea), Central and South America, and Australia. In the United Stated, HCC accounts for 2% to 3% of new cancer cases of the digestive organs, or approximately 4,000 to 6,000 cases per year (99,689,749). In contrast, in many areas of sub-Saharan Africa, Melanesia, Southeast Asia, China, and Japan, annual age adjusted incidence rates from 23 to 48 per 100,000 among men have been recorded. The mortality-to-incidence ratio is close to or greater than 1 year.

A number of epidemiologic studies have shown a strong relationship between persistent or past infection with HBV and the eventual development of HCC. It is noteworthy that the frequency of HCC follows the same general geographic pattern of distribution as that of persistent HBV infection (Fig. 7) (Maupas and Melnick, 1981). In cohort studies in developing countries, the incidence of HCC ranged from 240 to 281 per 100,000 HBsAg Carrier-years (Larouze, 1976).

### 9. Treatment of Hepatitis B

The need for treatment of hepatitis B depends on the natural history of the Disease. Rates of progression to cirrhosis and hepatocellular carcinoma vary according to the state of the immune system, the age of the patient, the serologic stage of infection, and geographic and genetic factors (Lok, and Lai, 1988) (Liaw et al., 1988) (Villeneuve et al., 1994) (Fattovich et al., 1995). The relative risk of death due to cirrhosis for HBsAg carriers, as compared with normal persons, ranges from 12 to 79 folds, and the relative risk of hepatocellular carcinoma ranges from 148 folds in Alaska to 30 to 98 folds in the Far East (McMahon et al., 1990). In the stage of infection in patients with normal aminotransferase levels, there is virtually no progression to cirrhosis, but the infection will accelerate during stage. In the typical stage 1 infection in patients with normal aminotransferase levels, there is virtually no progression to cirrhosis, but the infection will accelerate during stage 2, with cirrhosis developing in approximately 50 percent of patients in five years (Lok, and Lai, 1988) (Fattovich et al., 1995). Survival of patients with cirrhosis remains about 71 percent at five years (De-Jong et al., 1992).

There is little benefit in treating stage 1 infection with immunostimulants such as interferon (Lok, 1988), nor is there a need to treat stage 3 or 4 infection (Fattovich, 1988) (Carreno, 1992) (Lok, 1993). The goal of treatment is to hasten the progression form stage 2 to 3, with the clearance of hepatocytes replicating virus (Perrillo, 1994). Spontaneous seroconversion occurs at a rate of approximately 5 percent per year (Wong, 1995).

9.1 Interferon Therapy In the early 1980s, Trials using recombinant interferons led to the approval of interferon alfa-2b by the Food and Drug Administration in 1992. Recombinant interferons, resembling the naturally occurring cytokines produced in response to viral infections, have immunomodulatory and antiviral effects, inducing the display of HLA class I molecules on hepatocyte membranes, promoting lysis by CD8+ cytotoxic lymphocytes and directly inhibiting viral-protein synthesis (Greenberg, 1976) (Perrillo, 1990).

Treatment with interferon alfa-2b results in a 36 to 45 percent remission rate after a four month course of treatment in selected patients with stage 2 infection (Carreno, 1992) (Perrillo, 1990). Use of a brief course of corticosteroids with rapid tapering before interferon therapy has had conflicting result (Perrillo, 1990) (Krogsgaard, 1996). Remissions are generally sustained without further treatment (Carreno, 1992) (Niederau, 1996) (Korenman, 1993). Side effects include fever, malaise, neutropenia, and thrombocytopenia.

The criteria for a good response to treatment include elevated aminotransferase levels (>100 IU per milliliter), the presence of HBV DNA but at a level of less than 200 pg per milliliter, and a liver biopsy suggesting moderate or severe inflammatory activity. Patients should be less than 65 years old and in good health, with no complication of cirrhosis, such as variceal hemorrhage, ascites, or encephalopathy. Treatment consists of 5 million units of interferon alfa-2b given subcutaneously each day or 10 million units three times per week for 16 weeks.

A meta-analysis of 15 studies using a variety of interferon regimens demonstrated an overall response rate of 33 percent for treated patients, as compared with 12 percent for untreated controls. Loss of the carrier state (clearance of HBsAg)

was reported in 8 percent of treated patients, as compare with 2% of controls (Wong, 1993). Only 5 to 10 percent of persons in whom seroconversion has occurred will have a reactivation of infection in the next 10 years, and if reactivation dose occur, it may be transient, with subsequent seroconversion (Niederau, 1996) (Korenman, 1991).

Decision analysis shows that interferon treatment for chronic hepatitis B in a 35-year-old man increase life expectancy by 3.1 years, or 3.4 quality-adjusted life-years , and is thus well worth the cost, an average of $6,000 (Dusheiko, 1995).

9.2 Nucleoside Analogues Interferon therapy is successful only in patients with active immune response, but the results are still unpredictable. Patients with stage 1 infection may not need immediate treatment, but they remain infections and are at risk for the development of more active hepatitis, cirrhosis, and hepatocellular carcinoma.

Antiviral agents that directly affect replication are now being tested widely. One previously used agent, fialuridine, was remarkably effective but had delayed toxicity, characterized by profound lactic acidosis, hepatic failure, renal failure, severe coagulopathy, and death (McKenzie, 1995) (Cui, 1995). Several new nucleoside analogues that are active against retroviruses have different modes of action from that of fialuridine and do not have its toxicity. Since HBV replicates through an RNA template, the DNA polymerase resembles retroviral reverse transcriptases.

Lamivudine, an enantiomer of 3′-thiacytidine, inhibits human immunodeficiency virus (HIV) replication and has antiviral activity against HBV (79), with less toxicity than its predecessor, zalcitabine (Tyrrell, 1993) (Benhamou, 1995) (Tyrell, 1993). Studies of lamivudine administered for 4 to 12 weeks in patients with HBV infection demonstrated the clearance of HBV DNA from serum during treatment in virtually 100 percent of the patients, but sustained remissions in only 19 percent (Dienstag, 1995) (Lai, 1997). One-year trials have had similar results, with improvement in inflammation scores in the treated group and the clearance of HBV DNA in up to 96 percent of the patients by the end of treatment (Lai, 1997). With prolonged treatment, escape mutants develop (in approximately 16 percent of patients at one year) because of base-pair substitutions at specific sites within the YMDD locus of the DNA polymerase gene (Lai, 1997) (Honkoop, 1997). Liver transplant recipients appear to benefit from lamivudine. In a small study,

treatment with 100 mg of lamivudine daily resulted in clearance of HBV DNA and HBsAg after one year in 90 percent of patients, when treatment was begun before transplantation (Grellier, 1996). It is likely that treatment with nucleoside analogues will have to be maintained indefinitely, at least in immunosuppressed patients: prolonged treatment is probably feasible, since the side effects appear to be minimal (Honkoop, 1995) (Ling, 1996) (Tipples, 1996) (Bartholomew, 1997) (Aye, 1997).

Famciclovir, another nucleoside analogue with activity against herpesviruses, also inhibits HBV DNA polymerase (Schinazi, 1994) (Main, 1996) (Shaw, 1996). And results in the clearance of HBV DNA from serum. In preliminary trials of famciclovir in HBV positive liver transplant recipients, the drug improved aminotransferase levels, reduced HBV DNA to undetectable levels, and resulted in HBeAg seroconversion in approximately 20 percent of patients (Main, 1996).

**10. Characteristic of PLC/PRF/5 cells (Alexander cells)**

The correlation between hepatitis B surface antigen and serum hepatitis has led to extensive research aimed at finding an *in vitro* system for the study of HBV. A number of reports have appeared on the propagation of the virus in human liver cell and organ cultures (Zuckerman, 1975). After HBV infection, progressive changes in the cells have been noted and supernatant culture fluid could be passaged once or twice, but the short term nature of the experiments and the lack of a constant source of tissue culture material has failed to provide, as yet, a standardized *in vitro* system for the study of this virus.

The tissue culture cell line, PLC/PRF/5, isolated in 1975 from a primary liver carcinoma of an African man with persistent HBV infection, has been shown to continually produce HBsAg in the form of approximately 22 nm in diameter (Macnab et al., 1976). The rate of antigen production by the cells was estimated at 500 ng/day/$10^6$ cells by reference to a purified HBsAg standard. This cell line has an epithelial morphology and secrete proteins characteristic of differentiated liver cells, but do not produce HBV virions (Macnab et al., 1976) (Marion et al., 1977) (Aden et al., 1979). Core antigen (Gerin and Shih, 1978), the HBcAg (Takahashi et al., 1979), viral DNA

polymerase , and free virus (Dane particles) (Edman et al., 1980), are not detected. Genomic analysis shows that Alexander cells contain at least six copies of full or partial HBV genomes (Chakraborty et al., 1980) (Edman et al., 1980) (Marion et al., 1980).

PLC/PRF/5 cell line was used as a tool for many *in vitro* experimetal investigation of HBV. In 1987, Hajnicka and Stancek (Hajnicka and Stancek, 1987) found that HBsAg production from PLC/PRF/5 cells were decreased after the treatment of cells with interferon (IFN). Several randomized controlled trials were shown the efficacy of IFN that was effective to the patients with chronic HBV infection (Shi-Ming et al., 1999) (Emanuel et al., 2001). Glycyrrhizin, a major component of the *Glycyrrhiza uralensis* (licorice), was shown to suppressed the secretion of HBsAg and accumulated it dose-dependently in PLC/PRF/5 cells (Terumi et al., 1994). *Phyllanthus amarus* inhibited the HBsAg secretion of PLC/PRF/5 (Jayaram and Thyagarajan, 1996). The normalization rates of ALT, A/G and SB in the treatment group were significantly higher than that in the control in a comparative study with chronic viral hepatitis patients those were treated with *Phyllanthus amarus* (Xin-Hua et al., 2001). These studies shown that PLC/PRF/5 cell line could be a tool for *in vitro* experimetal for agent that effective with HBV virus.

**11. Medicinal Plants**

*Caesalpinia sappan* (ฝาง) is in family Caesalpiniaceae. Pharmacological activities of this plant are vasorelaxant (Hu, 2003) (Xie, 2000), and anti-convulsant activity (Baek, 2000).

*Derris scandens* (เถาวัลย์เปรียง) is in family Fabaceae. Pharmacological activities of this plant are insecticidal (Wang, 1997), anti-inflammatory (Laupattarakasem, 2003), lymphoproliferation activity (Anchalee and Pranee,1998) (Sriwanthana, 2001).

*Duranta repens* (เทียนหยด) is in family Verbenaceae. Pharmacological activity of this plant is anti-malarial activity (Castro, 1996).

*Gossypium herbaceum* (ฝ้าย) is in family Malvaceae. Pharmacological activities of this plant are anti-HBsAg (Zheng and Zhang, 1990), and antimutagenic activity (Lee and Lin, 1988).

*Homalomena aromatica* (เต่าเกียด) is in family Araceae. Pharmacological activity

of this plant is antifungal (Singh et al., 2000).

*Houttuynia cordata* (พลูคาว) is in family Saururaceae. Pharmacological activities of this plant are anti-herpes simplex (Chiang, 2003), anti-leukemic (Chang, 2001) anti-HIV (Hayashi, 1995), and anti-Influenza virus activity (Hayashi, 1995).

*Litchi chinensis* (ลิ้นจี่) is in family Sapindaceae. Pharmacological activities of this plant are anti-inflammatory (Besra, 1996), and anti-HBsAg activity (Zheng, 1990).

*Loranthus pentandrus* (กาฝากมะม่วง) is in family Loranthaceae. Pharmacological activity of this plant is hypotension effect (คิดคม สเลลานนท์, 1998).

*Santalum album* (แก่นจันทน์) is in family Santalaceae. Pharmacological activities of this plant are anti-Herpes simplex virus type1 and type2 (Benecia and Courreges, 1999).

*Phyllanthus emblica* (มะขามป้อม) is in family Euphorbiaceae. Pharmacological activities of this plant are antioxidant (Bandyopadhyay, 2000), anti-inflammatory (Ihantola-Vormisto, 1997), and hepatoprotective activity (Gulati, 1995).

*Phyllanthus amarus* (ลูกใต้ใบ) is in family Euphorbiaceae. Pharmacological activities of this plant are anticancer (Rajeshkumar, 2002), antimutagenic (Raphael, 2002) (Sripanidkulchai, 2002), anti-diarrhea (odetola, 2000), anti-HBsAg activity (Thyagarajan, 1988) (Mehrotra, 1991) (Yeh, 1994) (Jayaram, 1996), hypoglycemic (Srividya, 1995) (Sabu, 2002), and hypotension effect (Srividya, 1995).

*Rhinacanthus nasutus* (ทองพันชั่ง) is in family Acanthaceae. Pharmacological activities of this plant are antifungal (Kodama, 1993), anti-cytomegalovirus activity (Anna, 1996), and anti-platelet activity (Tian et al., 1998).

*Saussurea lappa* (โกฐกระดูก) is in family Asteraceae. Pharmacological activities of this plant are anti-inflammatory (Cho, 2000) (Gokhale, 2002) (Damre, 2003), anti-arthritic (Gokhale, 2002), and anti-HBsAg activity (Chen et al., 1995).