# HEPATITIS B VIRUS

## 1. Exposure Data

### 1.1 Structure and biology of hepatitis B virus (HBV)

#### 1.1.1 *Structure of the virus*

The structure of hepatitis B virus (HBV) has been characterized in great detail (Tiollais & Buendia, 1991). HBV belongs to a group of hepatotropic DNA viruses (hepadnaviruses) that includes the hepatitis viruses of the woodchuck (Summers *et al.*, 1978), ground squirrel (Marion *et al.*, 1980), Pekin duck (Mason *et al.*, 1980) and heron (Sprengel *et al.*, 1988).

HBV is a small virus, about 42 nm in diameter ('Dane particle'), composed of a lipid-bilayer envelope containing hepatitis B surface antigen (HBsAg) and an internal nucleocapsid structure (core). The nucleocapsid consists of the core protein and the viral DNA genome, which is about 3200 base pairs (about 2100 kDa) in length, with an associated DNA polymerase/reverse transcriptase (Tiollais *et al.*, 1985; Blum *et al.*, 1989a).

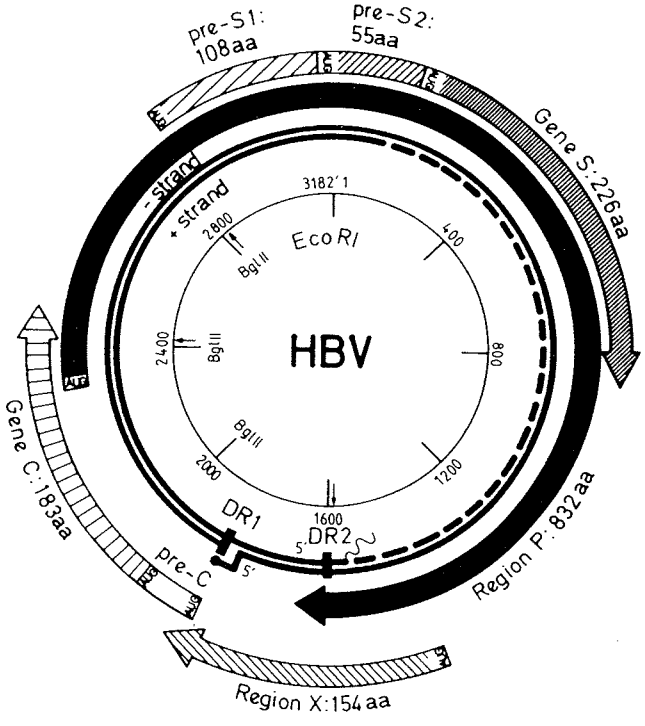
#### 1.1.2 *Structure of HBV genome and gene products*

The viral genome is a partially double-stranded, circular DNA molecule. The genome has four open reading frames, three of which encode for viral proteins whose structures and functions have been well characterized (Fig. 1) (Blum *et al.*, 1989a).

*(a) HBsAg*

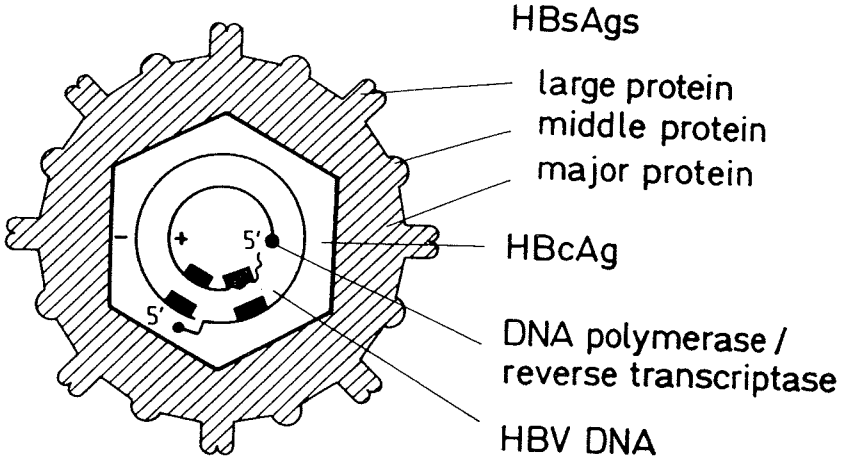
Hepatitis B surface antigen, formerly termed 'Australia antigen' (Le Bouvier, 1971), serum hepatitis antigen, hepatitis antigen and hepatitis associated antigen, is encoded by the pre-surface and surface genes; with lipid, it makes up the envelope of the virus. Excess HBsAg occurs abundantly in serum as small (22 nm) spherical or filamentous, non-infectious particles. In natural infection, the ratio of non-infectious HBsAg particles to virions is about 1000 to 1. Three different HBsAgs are synthesized: the large HBsAg (encoded by pre-S1, pre-S2 and S genes), the middle HBsAg (encoded by pre-S2 and S genes) and the major HBsAg (encoded by the S gene) (Tiollais *et al.*, 1985; Blum *et al.*, 1989a). The large HBsAg presumably mediates binding of the virus to the cell (Neurath *et al.*, 1986, 1990). The function of the middle HBsAg is unknown. The major HBsAg represents the predominant structural protein of the viral envelope (Tiollais *et al.*, 1985) (Fig. 2).

**Fig. 1. The hepatitis B viral DNA genome consists of a complete minus strand and an incomplete plus strand, with a cohesive overlap of their 5′ regions. In the cohesive region, there are two direct repeat sequences (DR1 and DR2), which are important in viral replication. The four open reading frames are indicated as arrows.**




Adapted from Tiollais *et al.* (1985); Blum *et al.* (1989a)

**Fig. 2. Structure of hepatitis B virus, showing surface (HBsAgs) and core (HBcAg) antigens**




From Blum *et al.* (1989a)

HBsAg carries a group-specific determinant, *a*, common to all subtypes of this antigen, and two additional subtypic determinants, *d* or *y* and *w* or *r*. As a result, four major subtypes of HBsAg exist: adw, adr, ayw and ayr (Le Bouvier, 1971; Bancroft *et al.*, 1972). They have distinct distributions worldwide and may therefore be useful for tracing the source of infection, e.g. *w* is commoner than *r* in the USA, but *r* is commonest in Thailand (Bancroft *et al.*, 1972).

The group-specific determinant *a* is encoded by the S genic region, encompassing roughly codons 124 to 147. This epitope is highly immunogenic, resulting in an anti-HBs response after natural infection (Carman *et al.*, 1993) or vaccination (Halliday *et al.*, 1992).

(*b*) *HBsAg and HBeAg*

The hepatitis B core antigen (HBcAg) and its antigenically distinct processed product, hepatitis B envelope antigen (HBeAg), are encoded by the pre-core/core (HBe-C/C) gene. The core (C) gene is transcribed into a core protein which packages the pre-genomic RNA to yield 'core particles'. The pre-C/C gene is transcribed into a pre-C/C fusion protein (Blum *et al.*, 1989a). The core antigen represents the major structural component of the nuclear capsid (Fig. 2). After truncation at its amino and carboxy termini, this protein is detectable in serum as HBeAg, usually indicating a high level of viral replication in the liver.

(*c*) *Viral DNA polymerase/reverse transcriptase*

The hepadnaviral polymerase gene encodes for a protein with a calculated molecular weight of 93.2 kDa. Genetic analysis has demonstrated that the enzyme consists of several functional domains arranged in order from the amino to the carboxy terminus: (i) terminal protein, which presumably serves as a protein primer for reverse transcription of the RNA pre-genome into minus-strand DNA; (ii) a spacer region, which can be deleted without loss of enzyme activity; (iii) DNA polymerase/reverse transcriptase activity; and (iv) RNase H activity. HBV and duck hepatitis B virus polymerases have been expressed *in vitro* (Bartenschlager *et al.*, 1992; Howe *et al.*, 1992; Wang & Seeger, 1992).

(*d*) *HBxAg*

The fourth open reading frame, which is conserved in all mammalian but not avian hepadnaviruses, encodes for a small protein, X. The biological functions of this protein for the viral life cycle and for the pathobiology of HBV have not been firmly established. The X gene product (HBxAg) has been shown to activate transcription of HBV, other viral sequences and a variety of cellular genes (Kekulé *et al.*, 1993). HBxAg does not seem to be required for HBV replication or gene expression *in vitro* (Blum *et al.*, 1992). Data in the woodchuck model suggest, however, that a woodchuck hepatitis virus X-minus mutant is not infectious *in vivo* (Chen *et al.*, 1993).

### 1.1.3 *Replication of HBV*

The hepadnaviral genomes are of similar size and structure and replicate asymmetrically via reverse transcription of an RNA intermediate (Summers & Mason, 1982). The replication strategy of HBV has been analysed in great detail both biochemically and genetically (Seeger *et al.*, 1986; Will *et al.*, 1987). Although hepadnaviruses are similar to retroviruses,

their mode of replication is unique (Miller & Robinson, 1986), with homologies only to the cauliflower mosaic DNA virus (Toh *et al.*, 1983).

### 1.1.4 *HBV and related animal viruses*

Similarities and differences between HBV and the related mammalian viruses of the woodchuck (WHV) and ground squirrel (GSHV) and the related avian viruses of Pekin duck (DHBV) and heron (HHBV) have been reviewed (Wain-Hobson, 1984; Mason & Taylor, 1989; Schödel *et al.*, 1989, 1991). All hepadnaviruses have a similar sized, partially double-stranded genome of about 3000 base pairs, which replicates asymmetrically by reverse transcription of an intermediate RNA template, the pre-genome (Ganem & Varmus, 1987; Feitelson, 1992). Further, the genetic organization of these genomes is identical, except that the avian viruses (DHBV and HHBV) lack the X open reading frame. While hepadnaviruses have a high species specificity of infection, the DNA sequences of HBV and WHV are highly homologous, which results in cross-reactivity between HBsAg and WHsAg (Wain-Hobson, 1984). Further, GSHV has been shown to infect woodchucks (Seeger *et al.*, 1991). Avian hepadnaviruses are more divergent in genomic structure and sequence. Calculations show DNA homologies of about 82% between GSHV and WHV and about 55% between GSHV and HBV, while only scattered homologies were apparent between DHBV and the other hepadnaviruses (reviewed by Sherker & Marion, 1991).

### 1.1.5 *HBV mutants*

The existence of HBV mutants was suspected for many years on the basis of the finding of HBV DNA in liver and serum from HBsAg-seronegative patients with or without antibodies to HBV. Conventional cloning techniques or polymerase chain reaction (PCR) amplification were used to clone and sequence viral DNA from sera and liver biopsy specimens, and naturally occurring mutations were identified in all viral genes. PCR allows amplification and detection of viral DNA at a sensitivity equal to that of tests of transmission in chimpanzees *in vivo* (Ulrich *et al.*, 1989). The PCR product can be sequenced directly or after cloning into an appropriate vector.

In the woodchuck model, the mutation rate has been estimated to be less than or equal to $2 \times 10^{-4}$ base substitutions per genome and year of replication (Girones & Miller, 1989). The hepadnaviral genome therefore appears to be relatively stable during replication in its natural host. The mutation rate of hepadnaviruses is 100–1000 times lower than that of RNA viruses but about 100 times higher than that of other DNA viruses. Since HBV infection frequently persists in humans for many years or decades, base changes can accumulate over time and may eventually result in a significant number of mutations. In addition, defective viral genomes containing major deletions occur frequently in individuals chronically infected with HBV (Takeda *et al.*, 1990). These defective viruses probably arise during active viral replication, but their contribution to the pathogenesis of HBV-related liver disease remains unclear.

#### (*a*) *Pre-S and S gene mutants* (Carman *et al.*, 1993)

While various naturally occurring mutations in the pre-S and S genes have been described, including deletions and point mutations leading to subtypic changes (Le Bouvier,

1971; Okamoto *et al.*, 1987; Lai *et al.*, 1991), a potentially important naturally occurring mutation affects the group-specific determinant *a*. A child from southern Italy, for example, who was infected by HBV despite passive–active immunization at birth and development of an anti-HBs response was found to carry a virus with a mutation in codon 145 of the S gene, resulting in a glycine to arginine substitution. This substitution results in loss of the *a* determinant against which the vaccine-induced anti-HBs response is mainly directed (Carman *et al.*, 1990; Harrison *et al.*, 1991; Waters *et al.*, 1992). Similar findings have been reported from Japan (Fujii *et al.*, 1992; Okamoto *et al.*, 1992), where not only the mutation in codon 145 but a further mutation in codon 126 of the S gene was detected (an asparagine to threonine or isoleucine substitution), which also resulted in loss of the *a* determinant (Okamoto *et al.*, 1992). In liver transplant recipients with chronic hepatitis B treated with a human monoclonal anti-HBs antibody, mutations similar to those described above have been identified in the S gene (McMahon, G. *et al.*, 1992). The transmission efficiency of these mutants has not been established.

Other mutations in pre-S and S genes may affect the sensitivity of antigen–antibody tests routinely used to detect HBsAg in serum. This may be especially relevant for assays based on antigen capture or detection using monoclonal anti-HBs antibodies that may not bind to the mutant HBsAg.

(*b*) *Pre-C and C gene mutants*

Attention has recently focused on mutations identified in the pre-core (pre-C) or core (C) gene region of the viral genome in patients who seroconverted from HBeAg to anti-HBe without loss of viral replication. The mutations in the pre-C gene identified to date most frequently induce a stop codon at the end of the pre-C region (Carman *et al.*, 1989; Akahane *et al.*, 1990; Brunetto *et al.*, 1990; Santantonio *et al.*, 1991a), resulting in an inability to produce HBeAg. While a pre-C stop codon mutation does not interfere with viral replication (Tong *et al.*, 1990), these mutations were found in clinically asymptomatic individuals, in patients with severe and active liver disease (Carman *et al.*, 1989; Akahane *et al.*, 1990; Brunetto *et al.*, 1990; Tong *et al.*, 1990; Naoumov *et al.*, 1992) and in patients with a fulminant course of HBV infection (Terazawa *et al.*, 1990; Carman *et al.*, 1991a,b; Hasegawa *et al.*, 1991; Kojima *et al.*, 1991; Kosaka *et al.*, 1991; Liang *et al.*, 1991a). Given the different clinical presentations of patients with pre-C stop codon mutants and the fact that in any patient with chronic HBV infection many mutants may coexist ('quasispecies') and multiple mutations may be found in a single viral genome, the causal relationship between the pre-C stop codon mutation and a particular course of the disease is unclear. A study in the woodchuck model suggests, however, that the pre-C stop codon may prevent persistence of WHV infection without affecting acute pathogenicity (Chen, H.-S. *et al.*, 1992).

Relatively few mutations have so far been identified in the C gene. In a patient seropositive for human immunodeficiency virus, cytomegalovirus, HBsAg and HBeAg and seronegative for anti-HBc, Bhat *et al.* (1990) identified a viral mutant with two point mutations in the pre-C and C genes as well as an in-frame 36-base pair insertion in the pre-C region. In contrast, in a study of children with HBV infection seronegative for anti-HBc who were undergoing chemotherapy for malignancies, no pre-C or C mutation was found (Melegari *et al.*, 1991). Recent evidence suggests, however, that mutations in the core gene (codons

84–101) are correlated with the severity of liver disease, possibly by altering recognition of infected cells by cytotoxic T cells (Ehata *et al.*, 1992).

(*c*) *X gene mutants*

Three naturally occurring mutations in the X gene have been described: a replication-competent HBV genome with a pre-X open reading frame (Loncarevic *et al.*, 1990); an HBV variant with an 8-base pair deletion at the 3′ end of the X gene (Repp *et al.*, 1992); and a replication-competent HBV variant with a fused X–C reading frame, resulting from a single nucleotide insertion in the X–C overlapping region (Kim *et al.*, 1992). The functional significance of X gene mutations is unclear.

(*d*) *Polymerase gene mutants*

In a patient serologically immune to HBV infection, a viral genome was identified with a point mutation in the protein region of the polymerase gene which terminated HBV replication through loss of RNA encapsidation function; this defect could be transcomplemented by a normal polymerase (Blum *et al.*, 1991). A further naturally occurring polymerase-defective variant was detected in the DHBV system, as a point mutation in the region of the gene that encodes for RNase H activity (Chen, Y. *et al.*, 1992). The functional significance of this mutation is unclear.

1.1.6 *Host range and target cells of HBV infection*

The host range of HBV and the related viruses in woodchuck, ground squirrel, Pekin duck and heron is very narrow. HBV, for example, infects only humans and chimpanzees. This narrow host range is believed to reflect the specificity of the liver-cell receptor for HBV, which interacts with an epitope in the pre-S1 region, 21–47, and which is also found on cells of extrahepatic origin (Neurath *et al.*, 1986, 1990).

In permissive hosts, viral antigens and nucleic acids are found primarily in liver cells. By use of molecular techniques, hepadnaviruses have also been detected in cells other than hepatocytes (Blum *et al.*, 1989b), e.g. bile-duct epithelial cells, endothelial cells in liver (Blum *et al.*, 1983), pancreas, adrenal cortex, kidney, skin, spleen and bone-marrow cells (Halpern *et al.*, 1983, 1984; Tagawa *et al.*, 1985; Tiollais *et al.*, 1985; Freiman *et al.*, 1988; Yoffe *et al.*, 1990; Mason *et al.*, 1992) and various peripheral white blood cells. The biological significance of HBV in cells other than hepatocytes remains largely undefined (Omata, 1990). Activation of HBV has been observed in peripheral blood mononuclear cells (Bouffard *et al.*, 1992), and persistent HBV infection of mononuclear blood cells without concomitant liver infection has been demonstrated (Féray *et al.*, 1990).

## 1.2 Methods of detection

Infection is detected on the basis of assays for viral antigens, antibodies and nucleic acids.

1.2.1 *In serum and plasma*

(*a*) *HBsAg and anti-HBs*

Tests for HBsAg developed in 1965 (Sherker & Marion, 1991) have since been improved significantly with regard to sensitivity and specificity. The early, less sensitive methods

identified only patients with high titres of surface antigen. More recent methods, such as reverse passive haemagglutination and enzyme immunoassay/radioimmunoassay (EIA/RIA), are highly sensitive and specific and allow detection of HbsAg at 100–200 pg/ml serum, that is, about $3 \times 10^7$ HBsAg particles/ml (Dusheiko *et al.*, 1992), and these are the assays used most commonly for HBsAg in serum. In reverse passive haemagglutination, fixed erythrocytes coated with anti-HBs are added to test samples, and haemagglutination patterns are read. In EIA/RIA, a sandwich method, with anti-HBs as both absorbed reagent and label or conjugate, has been employed. Anti-HBs is measured by passive haemagglutination of fixed erythrocytes coated with HBsAg or by an EIA/RIA sandwich method with HBsAg as the adsorbed reagent and label or conjugate.

(*b*) *Pre-S antigens and antibodies*

Pre-S antigens and antibodies are measured by research procedures (Itoh *et al.*, 1986; Coursaget *et al.*, 1990). The significance of these markers in natural infection or protection is not known.

(*c*) *HBcAg and anti-HBc*

HBcAg is not routinely detected in serum; in contrast, anti-HBc is a useful serological marker for current or past HBV infection. Total anti-HBc is measured by the haemagglutination inhibition method (Iizuka *et al.*, 1992) or by competitive binding EIA/RIA. Anti-HBc tests have limited specificity, especially at low titres. Commercial tests for both immunoglobulin (Ig) M and total anti-HBc are available; high titres of IgM class anti-HBc are typically present in acute HBV infection. As IgG class anti-HBc appears and is predominant in the course of chronic infection, IgM-anti-HBc may be a useful marker to differentiate between acute and chronic infection.

(*d*) *HBeAg and anti-HBe*

HBeAg can be measured by sandwich EIA/RIA using anti-HBe as the capture antibody. Early tests for HBeAg, such as gel diffusion, had little sensitivity, and the results of studies based on such tests must be interpreted with caution. Anti-HBe can be measured by competitive binding.

(*e*) *HBxAg and anti-HBx*

HBxAg and anti-HBx are determined by an enzyme-linked immunoabsorbent assay (ELISA) (Horiike *et al.*, 1991), which is not available commercially.

(*f*) *HBV DNA*

HBV DNA in serum can be detected by hybridization analysis (filter hybridization or liquid-phase hybridization) or PCR amplification followed by hybridization.

*Hybridization assays*: In filter hybridization, a test sample is denatured by the addition of sodium hydroxide and filtered through a nitrocellulose membrane to bind DNA. The membrane is then incubated with cloned labelled HBV DNA. If the test sample contains HBV DNA, the labelled probe is annealed to the membrane-bound viral DNA and can be detected by autoradiography. The sensitivity of this assay is 0.1–1 pg HBV DNA, or about

$10^3$–$10^5$ virions. In liquid-phase hybridization, HBV DNA exposed by virion lysis is mixed with a labelled HBV probe. This test system is available commercially, is better standardized than filter hybridization and has the same sensitivity; it is, however, costly and time-consuming (Dusheiko *et al.*, 1992).

*Polymerase chain reaction (PCR)*: The amplification of HBV DNA by PCR is an extremely sensitive test: theoretically, one genome equivalent per sample, at least 10 000 times more sensitive than dot-blot hybridization or RIA of HBsAg; it also facilitates analysis of the sequence of the amplified genomes. Contamination remains the major difficulty of this method, and extreme care must be taken at each step to avoid it. Negative and positive control samples, including reaction mixtures without DNA, should be analysed in each test (Dusheiko *et al.*, 1992; Seelig *et al.*, 1992). PCR followed by sequencing has also been used for subtyping HBV and for characterizing and identifying HBV mutants.

Table 1 gives information on the relative sensitivities and specificities of the tests for HBV markers.

**Table 1. Relative sensitivities and specificities of tests for hepatitis B viral markers in serum**

| Marker[a] | Test | Relative sensitivity | Relative specificity |
|---|---|---|---|
| HBsAg | Immunodiffusion | Low | High |
| | Counterimmunoelectrophoresis | Low | High |
| | Complement fixation | Medium | High |
| | Immune adherence | Medium | High |
| | Reverse passive haemagglutination | Medium | High |
| | Radioimmunoassay | High | High |
| | Enzyme immunoassay | High | High |
| Anti-HBs | Passive haemagglutination | Medium | High |
| | Radioimmunoassay/enzyme immunoassay | High | High |
| Anti-HBc | Haemagglutination inhibition | Medium | Medium |
| | Radioimmunoassay/enzyme immunoassay | High | Medium |
| HBeAg/anti-HBe | Immunodiffusion | Low | High |
| | Radioimmunoassay/enzyme immunoassay | High | High |
| HBV DNA | Hybridization analysis | Medium | High |
| | Polymerase chain reaction | High | High |

[a]HBsAg, hepatitis B surface antigen; anti-HBs, antibody to hepatitis B surface antigen; anti-HBc, antibody to hepatitis B core antigen; HBeAg, hepatitis B envelope antigen; anti-HBe, antibody to hepatitis B envelope antigen; HBV DNA, hepatitis B viral DNA

### 1.2.2 *In liver tissues*

#### (*a*) *HBsAg and HBcAg*

Both HBsAg and HBcAg can be detected by a direct immunofluorescence method in formalin-fixed, paraffin-embedded liver specimens (Yoshizawa *et al.*, 1977). [The sensitivity is limited, however, as shown by the fact that 35–40% of individuals seropositive for HBV markers have no detectable level of antigen in tissues.] HBsAg can also be detected in

infected liver cells by histochemical staining, such as with orcein and other reagents for staining elastic fibres (Shikata *et al.*, 1974). These methods were used to locate HBsAg and HBcAg in liver cells.

(*b*) *HBV DNA*

HBV DNA can be detected in liver tissue by Southern blot hybridization of extracted DNA or by in-situ hybridization. The major contribution of Southern blot analysis is physical characterization of HBV DNA and especially the distinction between extrachromosomal viral replication and integration of viral sequences into the cellular genome (Tiollais *et al.*, 1985). HBV-specific antigen and DNA can be detected simultaneously in paraffin-embedded liver tissue by immunohistochemistry and in-situ hybridization using a digoxigenin-label probe, without significant reduction in the sensitivity of either assay (Han *et al.*, 1992). HBV DNA can also be detected at high sensitivity in formalin-fixed, paraffin-embedded liver tissue by PCR, at a level correlated with serological and immunohistochemical markers (Lampertico *et al.*, 1990; Diamantis *et al.*, 1992).

### 1.2.3 *Interpretation of serological markers of HBV infection*

Typical patterns of serological markers in HBV infection are summarized in Table 2. Further information and correlations with the clinical course of disease are given in section 1.4.

**Table 2. Typical serological patterns in HBV infection**

| Infection status | HBsAg | Anti-HBc | | HBeAg | Anti-HBe | Anti-HBs |
|---|---|---|---|---|---|---|
| | | IgM | Total | | | |
| Acute infection[a] | + | + | + | + | – | – |
| Chronic infection with high levels of viral replication | + | – | + | + | – | – |
| Chronic infection with low levels of viral replication[b] | + | – | + | – | + | – |
| Recovery from acute infection before development of anti-HBs | – | + | + | – | + | – |
| Low titre; possible false positive | – | – | + | – | – | – |
| High titre; possible 'low level carrier' | – | – | + | – | + | – |
| Recovery from acute infection, indicating immunity | – | – | + | – | + | + |
| Vaccine response[c] | – | – | – | – | – | + |
| Susceptible to HBV infection | – | – | – | – | – | – |

For abbreviations, see footnote to Table 1.

[a]Reactivated chronic disease may have this pattern with sensitive anti-HBc IgM assays.

[b]Some patients may be seronegative for HBeAg and anti-HBe.

[c]In unvaccinated individuals, a high titre may represent immunity or be nonspecific; low titres are often nonspecific.

## 1.3 Epidemiology of infection

### 1.3.1 *Transmission*

Hepatitis B virus is transmitted from a person who has circulating virus and is HBsAg seropositive. The person may have an acute infection or be a carrier, a carrier being defined as a person who is seropositive for HBsAg on at least two occasions six months apart. Individuals who are HBeAg seropositive are particularly infectious, since the presence of this antigen is correlated with the level of serum HBV DNA.

The mode of transmission of virus to a susceptible individual varies with age. Transmission occurs at three important times of life: at birth (Mitsuda *et al.*, 1989), in early childhood and in adult life. Neonates born to HBeAg-seropositive carriers have an approximately 85% chance of becoming infected, whereas children of HBeAg-seronegative carriers have only a 31% probability of infection (Beasley *et al.*, 1977). The precise mode of perinatal transmission is unclear.

Infection in childhood is associated with living in households in which there is one or more infected sibling; the risk of infection increases with their number (Whittle *et al.*, 1990). The mode of transmission in childhood is unclear. Traditional practices, such as scarification, ear piercing, circumcision and tatooing, have been proposed (Struve, 1992), but controlled studies have failed to confirm them as risk factors (Fox *et al.*, 1988). HBV transmission through the use of contaminated needles, syringes and acupuncture equipment has been well documented (Kent *et al.*, 1988). Skin lesions, in particular tropical ulcers, have been proposed as a source of infection (Foster *et al.*, 1984), but, again, the evidence is not strong. Arthropods have been suggested as a means of transmission on the basis of studies of mosquitoes (Prince *et al.*, 1972), bedbugs (Wills *et al.*, 1977) and tampans (Joubert *et al.*, 1985). One study found a significant association between infection of HBeAg-seropositive people and infestation of beds with bugs (Vall Mayans *et al.*, 1990). Actual arthropod transmission has not been confirmed.

In adult life, parenteral and sexual transmission are the most important routes. The use of contaminated needles by intravenous drug users[1] is a very well documented form of transmission. For example, in a study of drug users in Sweden, 74% of men and 80% of women had markers of past infection, whereas only 1% and 5%, respectively, in the general population did so (Struve, 1992). In surveillance programmes, it was estimated that 27% of patients with acute hepatitis B in the USA in 1988 (Alter *et al.*, 1990) and 24% in the United Kingdom in 1985–88 (Polakoff, 1990) were intravenous drug users. Blood transfusion and administration of blood products for bleeding disorders were important sources of parenteral exposure, but the risk has now been virtually eliminated by screening blood sources and by treatment of blood products.

Szmuness *et al.* (1975a) indicated the importance of sexual intercourse in transmission of HBV. They compared the prevalence of past infection in spouses of 280 people with and 238 without persistent infection and the cumulative prevalence in women, homosexual men and

[1]The term 'intravenous drug use' refers to the practice of self-injecting drugs for recreational purposes. It is assumed to cover intravenous injection as well as intramuscular and other forms of injection.

attendees at sexually transmitted disease clinics. The results clearly showed that the virus could be transmitted sexually. The prevalence of past infection was 10% in spouses of non-carriers and 27% in spouses of carriers. Homosexual men had a prevalence of 48%, but no increase was seen in homosexual women. Subsequent studies of sexual activity showed that the number of sexual partners, duration of sexual activity and a history of sexually transmitted disease were all risk factors for HBV infection (Alter *et al.*, 1989; Rosenblum *et al.*, 1992; Osmond *et al.*, 1993). In surveillance studies of patients with acute hepatitis B, a history of multiple sexual partners was also found to be an important risk factor; 7% of all such patients in the USA and the United Kingdom had a history of homosexuality (Alter *et al.*, 1990; Kingsley *et al.*, 1990; Polakoff, 1990), and 26% in the USA were heterosexual (Alter *et al.*, 1990). The largest proportion of patients in these surveillance studies reported no known risk factor. Some infection may be intra-familial (Szmuness *et al.*, 1975b), from a household carrier. This form of transmission was associated with skin lesions in one case–control study (Bernier *et al.*, 1982). In both developed (Szmuness *et al.*, 1978a) and developing (Toukan, 1987) countries, infection is associated with low socio-economic status.

### 1.3.2 *Determinants of chronic infection*

Age at infection is the major determinant of whether a person becomes a carrier. Perinatal transmission confers the highest probability of becoming a carrier, with 80 to about 100% of infected children becoming carriers (Beasley *et al.*, 1977; Wong *et al.*, 1984). In children aged 1–10, the risk is 20–40% and appears to decline across this interval (McMahon *et al.*, 1985; Coursaget *et al.*, 1987). In adolescence and adult life, the probability of chronic infection following infection is in the range 0–10% (Nielsen *et al.*, 1971; McMahon *et al.*, 1985). The relationship has been reviewed recently (Edmunds *et al.*, 1993), and there is no evidence of geographical heterogeneity.

This profile of risk for chronic infection contrasts with the risk for acute clinical hepatitis. Symptomatic infection is unusual in childhood but affects 30–60% of individuals in adolescence and adult life (McMahon *et al.*, 1985).

The risk of becoming a carrier after infection is greater in males than in females in a ratio of about 1.6:1 (London, 1979). The sex ratio of prevalent carriers in the population increases with age because of longer chronic infection in males (Coursaget *et al.*, 1987).

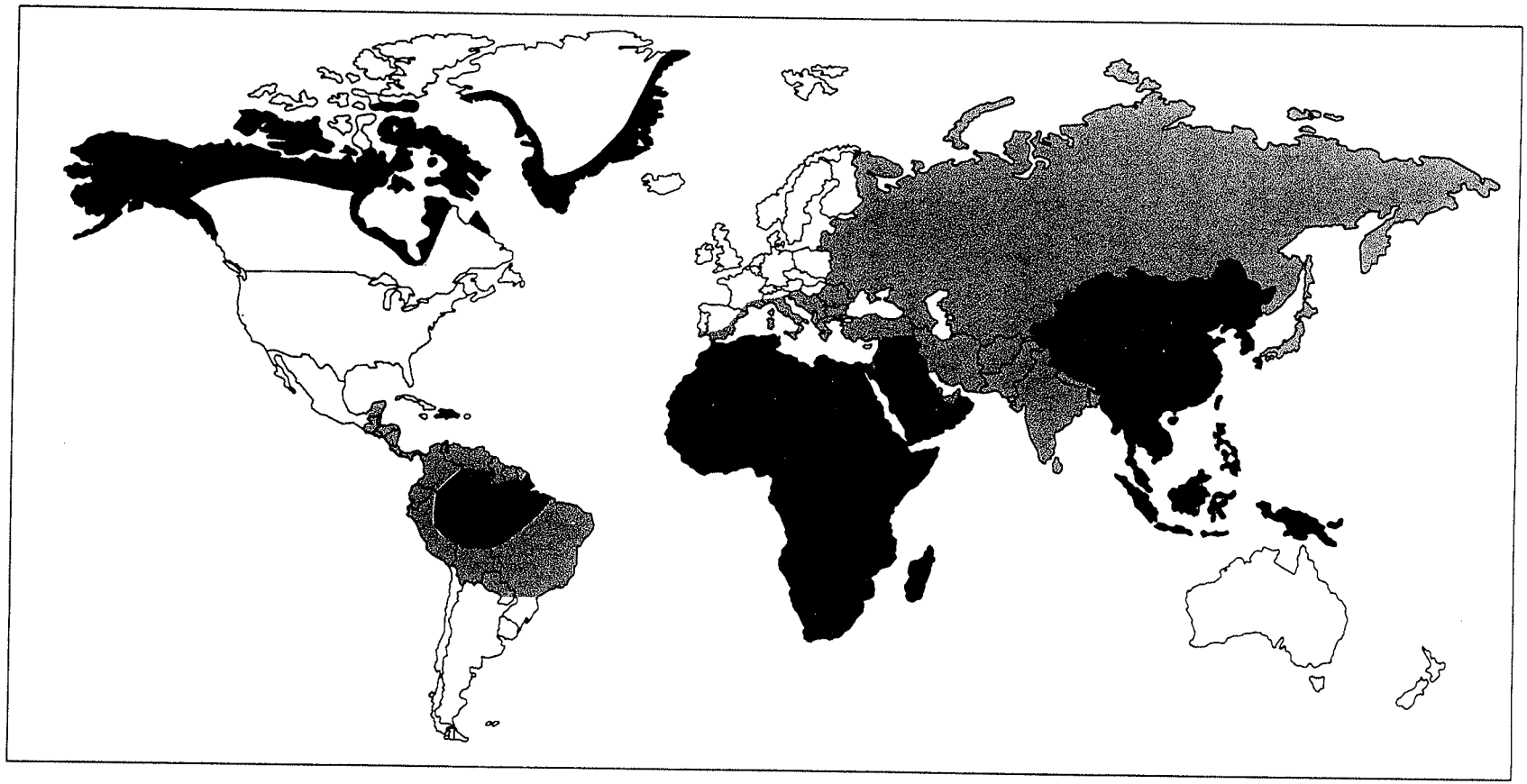
People whose immune system is suppressed, for example by cytotoxic drugs or the human immunodeficiency virus, appear to have a higher risk of chronic infection. They may also convert from apparent immunity to active infection.

### 1.3.3 *Global patterns of chronic infection*

WHO classifies hepatitis B endemicity by the proportion of the adult population who are hepatitis B carriers. Populations with 0–2% carriers are regarded as having low endemicity, 2–7% as intermediate and 8% or greater as high endemicity (Fig. 3).

The prevalence of infection is low in North America, western Europe, Australia and South America, with the exception of the Amazon basin. In these populations, a steady increase in prevalence of viral markers is seen with age. In the USA, blacks have a higher prevalence of infection than whites, particularly at older ages (McQuillan *et al.*, 1989).

**Fig. 3. Geographic pattern of the prevalence of hepatitis B**



Black: ≥ 8% - high; grey: 2-7.9% - intermediate; white: < 2% - low
From WHO (undated)

Intermediate levels of prevalence are found in eastern and southern Europe, in the Middle East, Japan and South Asia; high prevalences are found in China, Southeast Asia and sub-Saharan Africa (Prince, 1970; Szmuness, 1975).

High endemicity is associated with infection in childhood, as shown by the results of population-based surveys of hepatitis B markers before vaccination became widespread (Table 3). In Asia, perinatal transmission plays an important role in childhood infection: 30–40% of carriers are infected around the time of birth; in contrast, only 5–10% of carriers in Africa had perinatal infection. The proportion of mothers who are carriers is similar in Asia and sub-Saharan Africa (15–20%); however, 50% of carrier mothers in Asia are highly infectious (HBeAg seropositive), compared with only 10% in Africa. The reasons for this difference are not known.

Childhood infection plays an important role in countries of both high and intermediate endemicity, whereas adult transmission is predominant in areas of low endemicity (Table 4). Perinatal transmission does occur in countries of low endemicity and is particularly important among migrants from highly endemic regions and their descendants. Adoption of carrier children from highly endemic countries can lead to intra-familial transmission (Christenson, 1986; Friede *et al.*, 1988).

Variation may be seen within countries, as in Nigeria, with high prevalences in the north of the country but intermediate levels in the south (Fakunle *et al.*, 1980; Nasidi *et al.*, 1986); in Italy, with a low prevalence of infection in the north but an intermediate prevalence in the south (D'Amelio *et al.*, 1992); and in China, with a markedly higher prevalence of persistent infection in adults in the southeast of the country than in the northern inland areas (Beasley *et al.*, 1982). Variation in infection is also seen at the village level in Africa (Whittle *et al.*, 1983, 1990) and the Middle East (Toukan *et al.*, 1990): adults in adjacent villages have significantly different prevalences of persistent infection which are associated with the age at infection of children. Urban–rural differences in infection vary, some countries having higher urban rates and some higher rural rates (Soběslavský, 1980). In some countries, minority groups have significantly different risks of infection from the general population. For example, Maoris in New Zealand have higher rates than Caucasians (Milne *et al.*, 1985), and Aborigines in Australia have intermediate to high rates of infection in comparison with the low rate in non-aboriginal Australians (Holman *et al.*, 1987).

### 1.4 Clinical diseases (other than cancer)

The natural history and clinical manifestations of HBV infection are highly variable. In industrialized societies, about 45% of all HBV infections result in acute disease, and 1% have a fatal outcome. Chronic infections develop in 5% of infected people, and the remaining 50% of cases follow an asymptomatic course. There are multiple subtypes of the virus, but there is no known difference between the subtypes with respect to pathogenesis. In contrast, recently described HBV mutants may play a role in the clinical manifestations and natural history of HBV infection.

Whereas HBV replication and gene expression in infected individuals do not appear to be directly cytopathic, hepatic injury appears to be immune-mediated. Cytotoxic T cells are directed against HBcAg (Mondelli *et al.*, 1982; Ferrari *et al.*, 1987; Milich *et al.*, 1989) and

**Table 3. Hepatitis B seroprevalence in children and adults in selected countries**

| Country | Children[a] | | | | Adults | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | HBsAg-positive | | Any HBV marker-positive | | HBsAg-positive | | Any HBV marker-positive | | |
| | % | No. tested | % | No. tested | % | No. tested | % | No. tested | |
| Senegal | 9.0 | 2212 | 52.6 | 2212 | 13.3 | 765 | 89.3 | 683 | Barin *et al.* (1981) |
| Zambia | 7.6 | 264 | 36.0 | 264 | 5.1 | 356 | 68.8 | 356 | Tabor *et al.* (1985) |
| Argentina | 0 | 104 | – | | 0.7 | 922 | – | | Soběslavský 1980 |
| Brazil (Amazon) | 6.7 | 210 | 51.0 | 210 | 7.1 | 238 | 68.5 | 238 | Bensabath *et al.* (1987) |
| Canada[b] | 0.6 | 322 | 0.4 | 452 | 0.7 | 1788 | 4.6 | 1855 | Sobělavský (1980) |
| USA | 0 | 150 | – | | 0.2 | 570 | – | | Soběslavský (1980) |
| USA | – | | 0.8 | 3304 | – | | 6.6 | 10 971 | McQuillan *et al.* (1989) |
| India | 4.9 | 144 | 12.9 | 179 | 6.5 | 556 | 32.2 | 661 | Soběslavský (1980) |
| Japan | 2.2 | 552 | 5.3 | 552 | 2.2 | 1357 | 21.0 | 1357 | Soběslavský (1980) |
| Former Czechoslovakia | 0.3 | 324 | 6.2 | 324 | 2.1 | 668 | 14.4 | 667 | Soběslavský (1980) |
| Germany (eastern) | 0.7 | 294 | 12.7 | 157 | 1.6 | 626 | 17.3 | 458 | Soběslavský (1980) |
| Greece | 7.2 | 470 | 10.2 | 609 | 9.9 | 2150 | 41.3 | 2672 | Soběslavksý (1980) |
| Romania | 13.3 | 218 | 18.0 | 206 | 9.7 | 484 | 53.6 | 491 | Soběslavský (1980) |
| United Kingdom | – | | – | | 0.1 | 871 | 9.7 | 871 | Soběslavský (1980) |
| Former USSR | 3.8 | 131 | 40.5 | 131 | 3.7 | 347 | 46.4 | 347 | Soběslavský (1980) |
| Jordan | 9.5 | 505 | [18.0] | 505 | 10.2 | 610 | 51 | 610 | Toukan *et al.* (1990) |

[a]Under 15 years of age
[b]Urban population only

**Table 4. Endemicity of chronic infection with HBV by area of the world and predominant mode of transmission**

| Endemicity | Geographical area | Predominant time of infection |
|---|---|---|
| High, ≥ 8% | China, Southeast Asia, Pacific Basin, sub-Saharan Africa, Amazon Basin | Perinatal, childhood |
| Intermediate, 2–7% | East, central and southern Europe, Middle East, South Asia, Japan | Perinatal, childhood, adulthood |
| Low, < 2% | North America, western Europe, Australia, southern Latin America | Adulthood |

HBeAg (Ferrari *et al.*, 1992; Tsai *et al.*, 1992) but not against HBsAg (Mondelli *et al.*, 1982), and the exact nature of the target antigens for cytotoxic immune reactions is unknown (Ferrari *et al.*, 1987; Vento & Eddleston, 1987; Ferrari *et al.*, 1992). Conversely, immune suppression by co-infection with human immune deficiency virus or by treatment for organ transplantation reduces inflammatory reactions in the liver and frequently results in normalization of biochemical parameters, with no resolution of liver disease (Davis, 1989; Todo *et al.*, 1991; Martin *et al.*, 1992; McNair *et al.*, 1992).

### 1.4.1 *Acute infection*

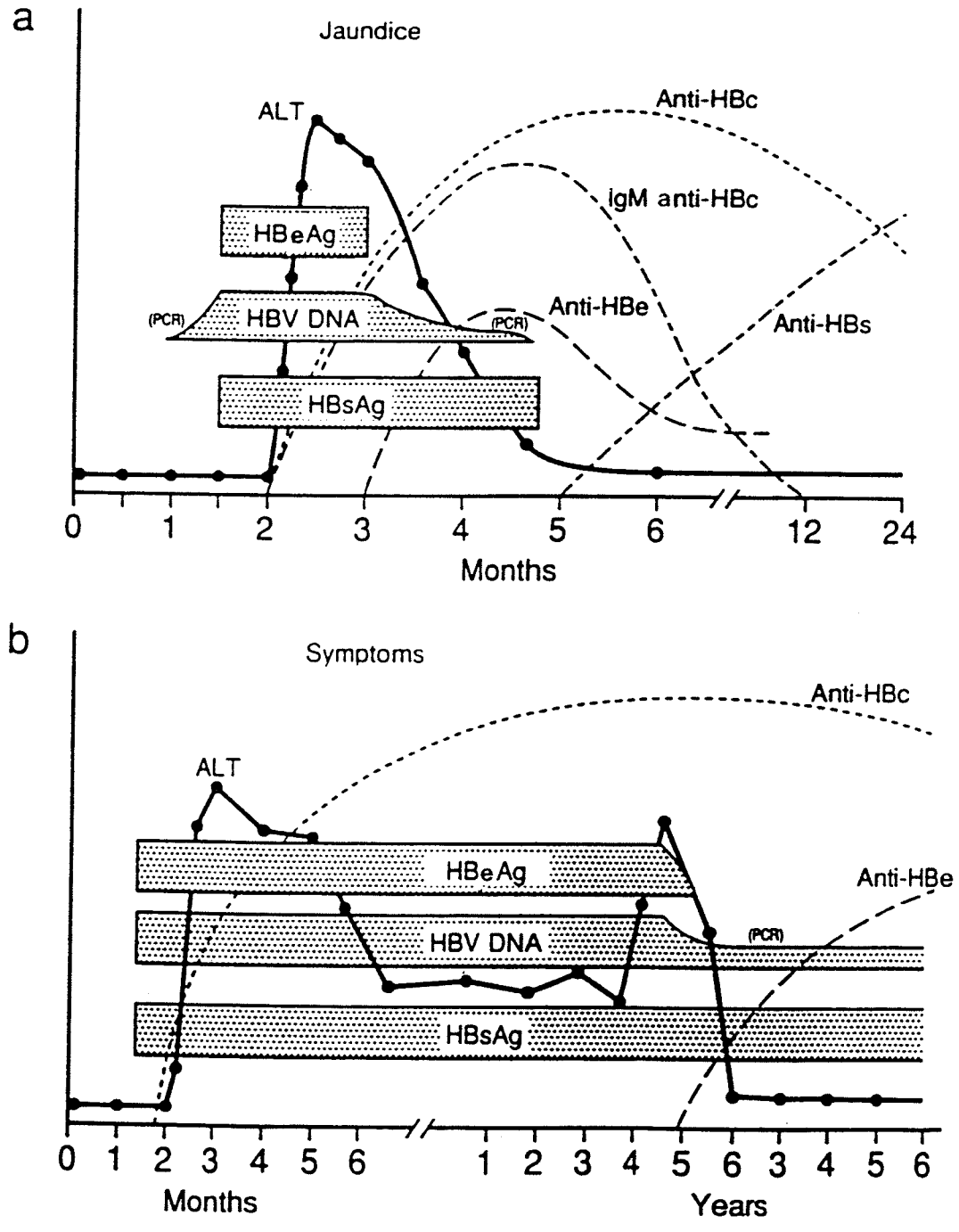
The lag between exposure to HBV and onset of hepatitis B is 2–26 weeks, and the clinical expression of this infection is heterogeneous. Subclinical episodes of acute hepatitis are common, as indicated by the large number of chronically infected patients with no history of acute hepatitis B (Redeker, 1975). The usual clinical attack of hepatitis caused by HBV in adults is more severe than that in either children or patients with hepatitis caused by hepatitis A or C virus. The self-limited bout of icteric hepatitis usually lasts less than three months and only occasionally has a prolonged or cholestatic course. A minority of patients become jaundiced and have symptoms and signs of hepatitis, such as fever, fatigue, hepatosplenomegaly and dark urine. Fulminant hepatic failure is an occasional result of acute hepatitis (Junge & Deinhardt, 1985; Krogsgaard *et al.*, 1985).

In uncomplicated hepatitis, HBV DNA is the first serum marker to appear, during the first four weeks of exposure, followed by HBsAg (Fig. 4a) (Krogsgaard *et al.*, 1985). In acute self-limited hepatitis, HBsAg persists for several weeks. After a variable time (window period), anti-HBs appears. Detection of IgM antibodies to anti-HBc, another early marker of HBV infection, is useful in differentiating acute hepatitis B from other forms of acute liver damage which could also be present in healthy carriers of HBsAg (Chau *et al.*, 1983; Perrillo *et al.*, 1983). HBeAg appears concurrently with HBsAg as a fourth marker in the serum (Krugman *et al.*, 1979). Recovery from acute hepatitis is heralded by clearance of serum HBV DNA, HBsAg and HBeAg and sequential appearance of anti-HBe and anti-HBs (Fig. 4).

### 1.4.2 *Chronic infection*

Chronic HBV infection may follow acute symptomatic or asymptomatic infection but is more frequent after asymptomatic infection. It occurs more frequently in men than in

**Fig. 4. Serological patterns of acute infection (a) and chronic infection (b) with HBV**




Modified from Dusheiko *et al.* (1992)
(PCR), period in which HBV DNA can be detected by the polymerase chain reaction; ALT, alanine amino-transferase

women, in children than in adults and in immunocompromised patients than in immunocompetent patients (Taylor *et al.*, 1988) (see section 1.3.2). The risk of chronicity declines from 80–90% following perinatal infection to 0–10% in older children and immunocompetent adults (McMahon *et al.*, 1985; Edmunds *et al.*, 1993).

Persistence of HBV infection is associated with variable degrees of hepatic inflammation; seroconversion to anti-HBe is paralleled by exacerbation of hepatitis caused by immune-mediated liver-cell necrosis and progressive clearance of infected hepatocytes and serum HBV DNA (Fig. 4). After seroconversion to anti-HBe, many patients show long-term, non-replicating, latent HBV infection. An important molecular process in HBsAg carriers is integration of HBV DNA into the liver-cell genome, and the majority of such carriers show no evidence of replication (De Franchis *et al.*, 1993). Patients with replicating HBV display various degrees of liver damage—from no histological change to benign forms of chronic lobular hepatitis to more severe forms of active hepatitis and cirrhosis. Chronic active hepatitis is the result of host immune attacks on infected liver cells.

The annual rate of HBeAg/anti-HBe seroconversion and disease remission was 10–15% in adult Italian patients (Fattovich *et al.*, 1986), but the rate varies by geographical location and age at infection. Children do not show reactivation of replication of HBV after anti-HBe seroconversion (Bortolotti *et al.*, 1990). Although HBeAg to anti-HBe seroconversion in adults was accompanied by clinical, biochemical and histological remission of disease, in a study of 88 patients followed-up for a mean of five years, 10 (22%) had transient spontaneous reactivation of HBV infection and exacerbation of disease (Fattovich *et al.*, 1990). Thus, seroconversion from HBeAg to anti-HBe during adulthood is not always stable, in contrast to infantile HBV infections (Lok *et al.*, 1987). HBV reactivation may lead to deterioration of the underlying liver disease, from quiescent chronic hepatitis to active cirrhosis (Fattovich *et al.*, 1990). Reactivation of a latent HBV infection occurs more frequently in immunocompromised patients infected with human immunodeficiency virus (Vento *et al.*, 1989) and in patients receiving cytotoxic therapy (Lok *et al.*, 1991).

HBV replication is instrumental in progression of the disease to cirrhosis. In 105 Italian patients with chronic hepatitis B (Fattovich *et al.*, 1991) who were followed prospectively for a mean of 5.5 years, cirrhosis was documented in 34% of patients with persistent serum HBV DNA but in only 15% of those without serum HBV DNA. In these patients, bridging hepatic necrosis was another important predictor of cirrhosis. In 43 Dutch patients with compensated cirrhosis, five-year survival was 72%, but the risk of death was decreased by a factor of 2.2 when HBeAg seroconversion occurred during follow-up (De Jongh *et al.*, 1992).

### 1.4.3 *Extrahepatic manifestations*

Extrahepatic clinical disease is infrequent during hepatitis B and has often been associated with circulating immune complexes containing viral antigens. Some patients in the prodromal phase of acute hepatitis have symptoms indicating immune complex disease, such as serum sickness-like syndrome, fever, urticarial skin lesions and symmetrical arthropathy. Systemic necrotizing vasculitis (polyarteritis) affecting the gastrointestinal tract (Shusterman & London, 1984), peripheral or central nervous system has been reported (Tabor, 1987). The presence of circulating complexes correlated well with disease activity. Membranous or membrano-proliferative glomerulonephritis due to HBeAg immunocomplexes has been

found either alone or as part of a generalized vasculitis (Shusterman & London, 1984). The Guillain–Barré syndrome was reported, with HBsAg-containing immunocomplexes in serum and cerebrospinal fluid (Penner *et al.*, 1982). Aplastic anaemia complicating hepatitis B is extremely uncommon and shows typical features of refractory marrow failure or hepatitis-dependent pancytopenia (McSweeney *et al.*, 1988).

### 1.5 Therapy and immunoprophylaxis

#### 1.5.1 *Therapy*

*(a) Acute or fulminant HBV infection*

Most cases of acute HBV infection are asymptomatic and do not require medical attention. In case of malaise and fatigue, bed rest is advised. In symptomatic acute or fulminant HBV infection, therapy is given to relieve the signs and symptoms associated with the acute phase of the disease. Therapy includes parenteral nutrition in cases of dehydration and inanition due to nausea and vomiting, replacement of coagulation factors in cases of bleeding due to impaired synthetic liver function and liver transplantation in cases of advanced liver failure and hepatic coma (Maddrey & Van Thiel, 1988). Except for anecdoctal reports (Halevy *et al.*, 1990), no trial of antiviral agents in acute HBV infection has been published.

*(b) Chronic HBV infection*

Because of the severe natural course of HBV infection, several therapeutic strategies have been explored in patients: plant extracts (Thyagarajan *et al.*, 1988; Blumberg *et al.*, 1990), the immunomodulator AM3 (Villarrubia *et al.*, 1992), steroids (Tygstrup *et al.*, 1986), thymosin (Mutchnick *et al.*, 1991), adenine arabinoside monophosphate (Garcia *et al.*, 1987), dideoxyinosine (Catterall *et al.*, 1992; Fried *et al.*, 1992) and interferon-α, interferon-β and interferon-γ. Interferon-α or prednisone followed by interferon-α and interferon-β are the only regimens of some value for treating chronic active hepatitis B (Alexander *et al.*, 1987; Hoofnagle *et al.*, 1988; Perrillo *et al.*, 1990; Janssen *et al.*, 1992; Lok *et al.*, 1992). Response is usually defined as seroconversion from HBeAg to anti-HBe, decrease or loss of HBV DNA and normalization of serum transaminase. Long-term follow-up of HBV carriers who responded to interferon therapy indicated that the improvement is sustained over a long time (Carreño *et al.*, 1992).

The parameters that predict a response to interferon-α therapy in HBsAg- and HBeAg-seropositive patients are: high levels of transaminases, low level of HBV DNA, short duration of disease, female sex and seronegativity for human immunodeficiency virus (Brook *et al.*, 1989). The ethnic origin of patients may have some influence on the efficacy of interferon-α therapy, in that Chinese patients appear to respond less well than patients of Caucasian extraction (Lok *et al.*, 1986). Any differences may be attributable in part to the age at infection. Like people infected with human immunodeficiency viruses, patients under immunosuppression after organ transplantation respond poorly to interferon-α therapy (Davis, 1989; Degos & Degott, 1989; Wright *et al.*, 1992).

Interferon treatment seemed to favour the emergence of pre-C stop codon mutants in some studies (Takeda *et al.*, 1990; Santantonio *et al.*, 1991b; Günther *et al.*, 1992) but not in

others (Xu *et al.*, 1992); this phenomenon does not seem to affect virus elimination and thereby the efficacy of interferon. The persistence of HBV in peripheral blood mononuclear cells of patients with chronic hepatitis B after HBsAg clearance may, however, pose a real clinical problem and set the stage for reinfection of the liver (Mason *et al.*, 1992).

Therapeutic trials in HBeAg-seronegative, anti-HBe-seropositive and HBV DNA-seropositive patients have yielded conflicting results (Fattovich *et al.*, 1992; Pastore *et al.*, 1992). Further, combined therapy of chronic active hepatitis B with interferon-β and interferon-γ seems to hold some promise (Caselmann *et al.*, 1989); the usefulness of this regimen has not been confirmed, however. In contrast, interferon-γ therapy alone is clearly ineffective (Ruiz-Moreno *et al.*, 1992). The only effective therapy for chronic active hepatitis B is thus administration of interferon-α, which gives a long-term response rate of 30–50% in selected individuals.

Interferon therapy has, however, several limitations. It must be given by injection over long periods, has very significant side-effects in many patients and is expensive, reducing its availability to patients in developing countries.

### 1.5.2 *Immunoprophylaxis*

Krugman *et al.* (1970) first demonstrated that serum containing HBsAg could be inactivated by heat but retain its immunogenic properties. This finding led to the development of hepatitis B vaccines by purification and inactivation of HBsAg from the plasma of HBV carriers (Hilleman *et al.*, 1975; Maupas *et al.*, 1981). These vaccines are administered intramuscularly, but gluteal injection is less effective than into other muscles (McLean *et al.*, 1985). Although reduced doses can be given intradermally (Whittle *et al.*, 1987), the results are variable. Three doses are generally required: during the first month of age, then at two, four and nine months of age; however, these intervals are not crucial (Inskip *et al.*, 1991). The vaccine is immunogenic in newborns, and the immunogenicity is not affected by maternally derived passive antibody. Immunogenic HBsAg can also be produced by yeast and mammalian cells using recombinant technology. Both plasma-derived and recombinant vaccines are now widely available and licensed.

Local reactions at the injection site occur in about 10% of vaccinees; long-term sequelae are very rare (Whittle *et al.*, 1991). In addition to active vaccination, a passive immunoprophylaxis is available which can be used in children born to infectious mothers and after accidental occupational exposures. As immunoglobulin does not affect the response to vaccine, a combination of the two will protect in both the short and long term (Mitsui *et al.*, 1989). Many studies (for example, Beasley *et al.*, 1983; Wong *et al.*, 1984) have demonstrated the protective effect of a combination of vaccine and immunoglobulin or vaccine alone in preventing perinatal transmission of hepatitis B in the short term.

Nine trials of immunoprophylaxis involved sufficient follow-up to assess protection against acute hepatitis and persistent infection. Three of these (one in China, two in the USA) were designed to prevent perinatal infection, three (one in Senegal, two in the Gambia) to prevent horizontal childhood infection, one to prevent childhood and adult infection (in Inuits) and two to prevent adult, primarily sexual, transmission (in the USA).

Beasley *et al.* (1983) assessed the efficacy of hepatitis B immunoglobulin in neonates of HBeAg-seropositive carrier mothers in Taiwan, China. When immunoprophylaxis was given

at birth and after three and six months, it had a protective efficacy of 71%. The protection persisted until 24 months of age, with no further follow-up available. Because children become susceptible to HBV infection after passive immunoprophylaxis wanes, however, hepatitis B immunoglobulin is no longer used alone.

Children recruited into two studies of perinatal vaccination were followed up for four to nine years to assess long-term protection. In the first study (Stevens *et al.*, 1985), 113 children of HBeAg-seropositive carrier Asian–American women were treated with 0.5 ml hepatitis B immunoglobulin and three intramuscular doses of 20 μg plasma-derived vaccine according to various schedules. In the second study (Stevens *et al.*, 1987), 122 infants of HBeAg-seropositive carrier Asian–American mothers were given plasma- or yeast-derived vaccine according to various schedules after a dose of 0.5 ml hepatitis B immunoglobulin at birth. Only 8.2% of children in these two studies became persistently infected; as the rate expected on the basis of historical controls was 70%, the protective efficacy was approximately 88%. Of 104 children who were seronegative for HBV markers at 9–18 months of age (Stevens *et al.*, 1992), none developed hepatitis, although 6.7% were seropositive for anti-HBc and anti-Hbs, indicating past infection with the virus. Very few lost vaccine-induced antibody.

Three of four studies of vaccination in populations with high rates of 'horizontal' transmission were carried out in West Africa and the fourth among Alaskan Inuits. In Senegal, Coursaget *et al.* (1986) followed up 135 infants who had received four doses of plasma-derived vaccine in the first year of life and 143 who had received no vaccine by the age of seven years, which represented a small fraction of the original children in the vaccination programme. Four children in the vaccine group and 20 in the control group developed HBsAg. As samples were taken at only one point in time, at seven years of age, it is not possible to determine if these children were persistently infected. The protective efficacy against HBsAg-positive events was 85%.

Two studies were conducted in the Gambia: the first was limited to two villages in which hepatitis B was well documented in 1980 and 1984 (Whittle *et al.*, 1983, 1990) and in which trials of intramuscular and intradermal (Whittle *et al.*, 1987) vaccination with plasma-derived vaccine were carried out in 1984. All children under five subsequently born in the villages received intramuscular plasma-derived vaccine in the first year of life, and a complete cross-sectional survey of people under 20 years of age was made in 1989, five years after the initial vaccine trials. Vaccination was 97% effective in preventing chronic infection in comparison with the rate in historical controls, although 5.3% of 264 vaccinees in one village and 19.1% of 94 in the other had evidence of past infection with the virus. The dose, route and schedule of vaccination did not influence protective efficacy in this study. None of the children with 'breakthrough' infection (seroconversion to anti-HBc) had evidence of acute hepatitis (Whittle *et al.*, 1991). The second study was initiated in 1986 to evaluate the protective efficacy of vaccination against chronic liver disease in a 'stepped-wedge' design (The Gambia Hepatitis Study Group, 1987). A cohort of 1041 vaccinees was followed up to four years of age by examining serum samples taken annually. At four years, a cross-section of 816 unvaccinated children was studied as a control group. The efficacy of the vaccine against infection was 84% (95% confidence interval [CI], 78–89%), and that against persistent infection (defined as HBsAg seropositivity on two occasions one year apart) was 94% (95% CI, 84–98%) (Fortuin *et al.*, 1993). In the vaccinated cohort, four children were

found to be chronic carriers; two of three who had had HBsAg-seropositive mothers were also HBeAg seropositive, and both became infected during the first year of life.

The fourth study in populations in which horizontal childhood transmission is common was carried out in Alaska (McMahon *et al.*, 1987; Wainwright *et al.*, 1989). All 1693 susceptible people in a population of 3988 Inuits in 17 villages were vaccinated in 1981–82 with three doses of plasma-derived vaccine. No persistent infection had occurred after five years of follow-up, giving a vaccine efficacy of 100%. Four subjects developed anti-HBc, indicating natural infection, but did not develop acute hepatitis (Wainwright *et al.*, 1989). The annual incidence of acute clinical HBV infection in the entire population declined from 215/100 000 per year before the study to 14/100 000 per year after vaccination (McMahon *et al.*, 1987).

Two large-scale, randomized trials involving adult US homosexual men reported long-term follow-up of vaccinees. In the first (Szmuness *et al.*, 1980), 1083 homosexual men known to be at high risk of HBV infection were recruited and randomized to placebo or plasma-derived vaccine. The protective efficacy against infection at 18 months was 92%. Subgroups of the vaccinees in this trial were followed up for longer periods. Among 138 followed for up to eight years (Taylor & Stevens, 1988), three cases of HBV infection occurred between five and eight years, to give a life-time attack rate of 2.6%.

In a multicentre trial of hepatitis B vaccination among homosexual US men conducted by the Centers for Disease Control, those in the placebo group who remained susceptible at the time of the first analysis were vaccinated (Francis *et al.*, 1982; Hadler *et al.*, 1986). Some of these and the vaccinated group are still being followed up. A total of 733 men were followed for five years after completion of vaccination; 15% of 635 participants with detectable antibody lost it within this time. The duration of antibody persistence was related to the peak antibody response. HBV infection occurred in 55 men; in eight, infection was associated with raised liver enzyme levels and HBsAg seropositivity. The risk for infection was highest in men with the lowest antibody responses. The only two individuals who became persistently infected with HBV did not respond to the vaccine (Hadler *et al.*, 1986). More information is expected from further follow-up of these cohorts. Early indications of the effect of vaccination on chronic liver disease are also expected from studies under way in China (Sun *et al.*, 1986, 1991) and from the mass programme of immunization in Taiwan, China (Chen *et al.*, 1987).

Immunogenicity is reduced in immunosuppressed individuals infected with human immunodeficiency virus (Laukamm-Josten *et al.*, 1987; Collier *et al.*, 1988; Bruguera *et al.*, 1992), in dialysis patients (Jilg *et al.*, 1986) and in individuals immunosuppressed after organ transplantation (Sokal *et al.*, 1992). HBsAg-seropositive individuals (Dienstag *et al.*, 1982) and some patients with anti-HBc as the only marker of HBV infection (McMahon, B.J. *et al.*, 1992) do not respond to vaccination. In such cases, vaccination is not harmful, however. The small proportion of people who do not respond to HBV vaccination, with anti-HBs levels of $< 8$ radioimmunoassay units, may be determined partly genetically (Craven *et al.*, 1986; Kruskall *et al.*, 1992).