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).

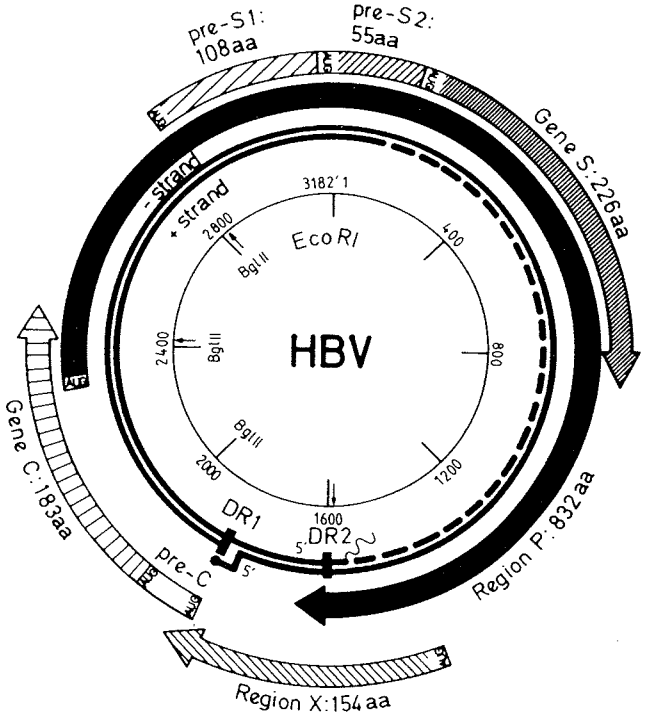
1.1.2 *Structure of HBV genome and gene products*

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

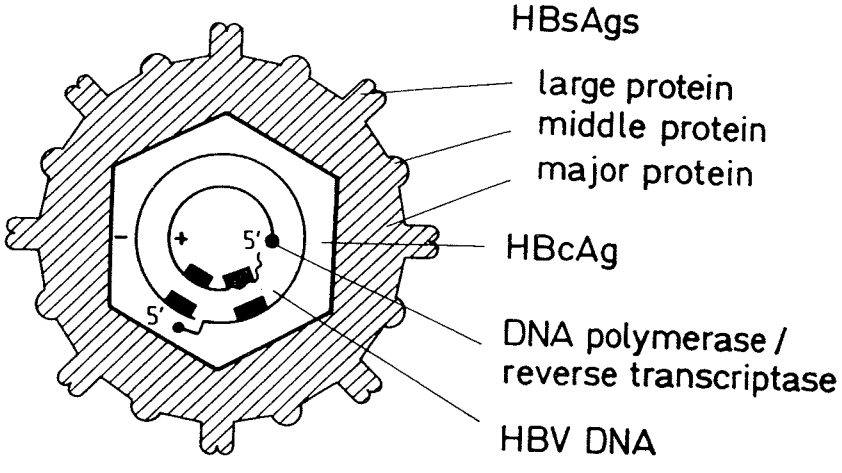
Hepatitis B surface antigen, formerly termed 'Australia antigen' (Le Bouvier, 1971), serum hepatitis antigen, hepatitis antigen and hepatitis associated antigen, is encoded by the pre-surface and surface genes; with lipid, it makes up the envelope of the virus. Excess HBsAg occurs abundantly in serum 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 2×10^{-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 3×10^7 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).

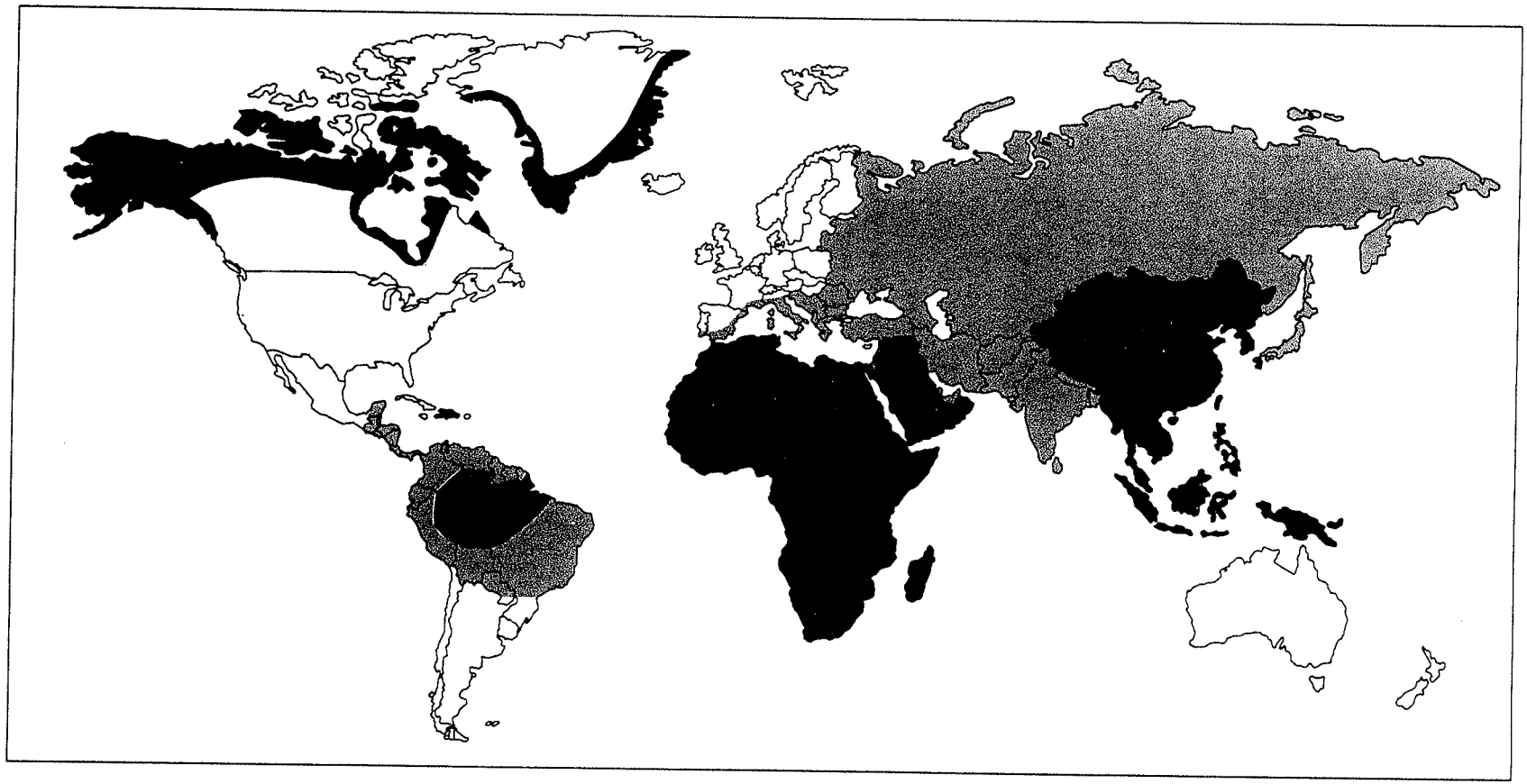
People whose immune system is suppressed, for example by cytotoxic drugs or the human immunodeficiency virus, appear to have a higher risk of chronic infection. They may also convert from apparent immunity to active infection.

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*

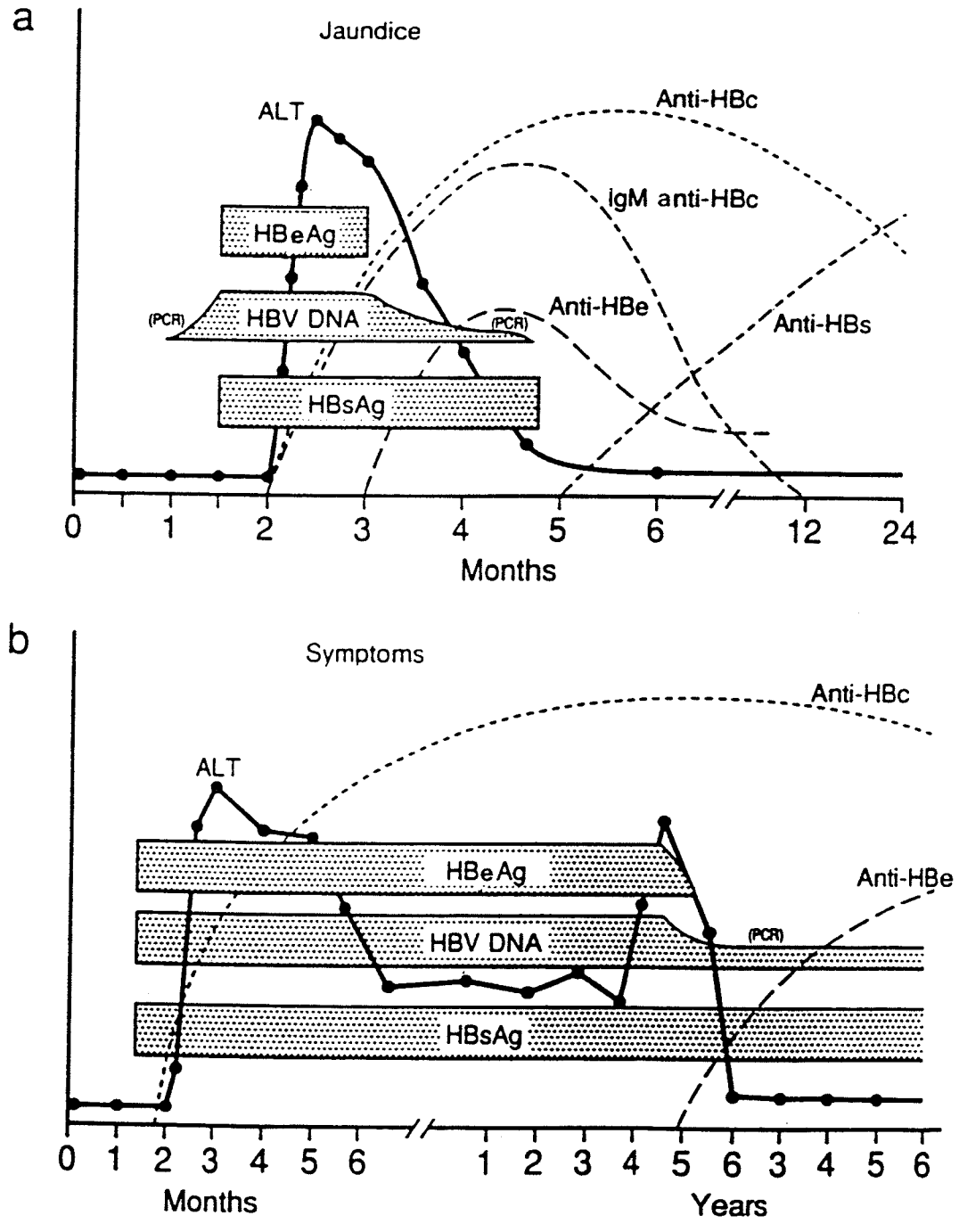
The lag between exposure to HBV and onset of hepatitis B is 2–26 weeks, and the clinical expression of this infection is heterogeneous. Subclinical episodes of acute hepatitis are common, as indicated by the large number of chronically infected patients with no history of acute hepatitis B (Redeker, 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).

2. Studies of Cancer in Humans

Reports of epidemiological studies of liver cancer relevant to these monographs have employed a variety of terms to describe this disease, for example, liver cancer, primary liver cancer, primary hepatocellular carcinoma and hepatocellular carcinoma. We chose to use only the term hepatocellular carcinoma (HCC) in describing these studies. This choice was made because most of the studies have specified primary cancer of the liver or HCC, and the vast majority of primary liver cancers in most areas of the world are HCC (Colombo, 1992). A large number of case–control studies and fewer cohort studies have been conducted on the association between HBV and HCC. The many case reports, case series and descriptive studies are therefore not described in detail.

HBsAg is the marker of infection with HBV most often measured in epidemiological studies of HBV infection and HCC. While it is not often measured twice, six months apart (as required by the strict definition of carrier status), its presence in adults without acute hepatitis has generally been taken to indicate chronic infection with the virus.

The presence of other markers of HBV infection has also been documented in many studies, but most studies in which these markers were measured were not analysed with the intention of estimating their relationship with HCC. In addition, in some countries, the large majority of the population may have been exposed to HBV, and most have some marker of infection. Thus, an appropriate—uninfected—reference population of sufficient size may not be available for comparison with individuals with anti-HBc or anti-HBs alone or in combination. Lastly, in populations in which a suitable reference group may exist, it has usually not been reported separately.

2.1 Case series and case reports

2.1.1 *Hepatocellular carcinoma*

Payet *et al.* (1956) appear to have been the first to have suggested that HCC is a consequence of chronic viral hepatitis. Within five years of the identification of HBsAg (then called Australia antigen) by Blumberg *et al.* (1965), its significance as a marker of chronic viral hepatitis had been appreciated, and case reports and case series had given rise to the suspicion that it was linked to liver cancer. Okochi and Murakami (1968) appear to have been the first to have found HBsAg in a case of liver cancer (one of 19), but they made no specific comment as to its relevance. Wright *et al.* (1969) found no HBsAg-seropositive patients among 11 with liver neoplasms in the United Kingdom. In reporting the presence of HBsAg in sera from 2 of 42 cases of HCC in Hong Kong but not in 11 East African or 12 US cases, Smith and Blumberg (1969) stated the hypothesis that the antigen and its underlying viral infection were linked to HCC. Sherlock *et al.* (1970) reported HBsAg seropositivity in five male patients with HCC, three from Greece, one from the United Kingdom and one from Sierra Leone; Hadziyannis *et al.* (1970) found HBsAg in sera from 4 of 13 Greek patients with HCC superimposed on active cirrhosis and in six other cases of HCC.

With the development and application of more sensitive tests for HBsAg, case series of HCC have consistently shown apparently high proportions seropositive for the antigen

(Blumberg & London, 1982, 1985). The earlier reports were comprehensively reviewed by Szmuness (1978). Reports of HBsAg seropositivity in the rare cases of HCC in children also appeared (Shimoda *et al.*, 1980), often linking the infection in the child to chronic infection in the mother (Ohaki *et al.*, 1983). Instances of multiple HCC have been reported in families, often with an apparently high prevalence of HBsAg in the serum in both the cases and unaffected members of the family (Tong *et al.*, 1979; Tong & Govindarajan, 1988; Alberts *et al.*, 1991).

HbsAg, and sometimes HBV DNA, have also been reported in liver tissue from patients with HCC (see, for example Tanaka & Mori, 1985; Tanaka *et al.*, 1986), usually in non-neoplastic hepatocytes and rarely in the cancer cells (Tanaka & Mori, 1985).

2.1.2 *Other cancers*

In several case series of patients with cholangiocarcinoma, the prevalence of HBsAg (Okuda *et al.*, 1980) or of markers of HBV in liver tissue (Bunyaratvej *et al.*, 1979; Suwangool, 1979) appeared to be lower than that among cases of HCC and similar to that reported in subjects without HCC.

In two early studies of the presence of HBsAg in sera from patients with a range of cancers (e.g. leukaemias, Hodgkin's disease, breast cancer, lymphoma, multiple myeloma), prevalences of 1.1–2.8% were observed (Viola *et al.*, 1972; Al-Sarraf *et al.*, 1973). In a study of pancreatic tissue from patients who had undergone surgery, immunoperoxidase staining revealed HBsAg in five (7%) patients with pancreatic cancer (Hohenberger, 1985). Planes *et al.* (1976) noted that the prevalence of HBsAg in the sera of patients with malignant lymphoma was related to treatment and that infection with HBV may have occurred as a result of chemotherapeutic immunodepression, parenteral injection, transfusion or a prolonged stay in a hospital environment. In several reports of high prevalences of HBV markers in children with cancer, mainly lymphatic and haematopoietic cancers (Mikhailov *et al.*, 1986; Pontisso *et al.*, 1987; Jackowska *et al.*, 1990), it was considered possible that infection had occurred after the cancer developed.

2.2 Descriptive studies

A strong geographical correlation has been found between the incidence of HCC and the prevalence of HBsAg seropositivity (see, for instance, Szmuness, 1978; Maupas & Melnick, 1981; Lin *et al.*, 1986; Hsing *et al.*, 1991). A number of studies have also shown a high prevalence of HBsAg seropositivity in migrants from countries where the risk for HCC is high (see, for example, Szmuness *et al.*, 1978b).

2.3 Cohort studies

Cohort studies of HBV and HCC can be divided into three broad groups: studies of general population groups, studies of blood donors and studies of populations with pre-existing disease.

2.3.1 *Prospective studies of general population groups* (Table 5, p. 73)

Beasley and colleagues recruited 22 707 male Government employees in Taiwan, China, to evaluate their risk for HCC (Beasley & Lin, 1978; Beasley *et al.*, 1981; Beasley & Hwang,

1991). Subjects were recruited at the time of routine physical examinations offered by the Government between March 1976 and June 1978. Study subjects were Chinese men aged from their twenties to over 70; 82% were 40–59 years of age. Follow-up for cancer incidence and mortality from all causes was conducted through medical and life insurance records and also by annual physical examinations of both HBV carriers and a subset of non-carriers. At the time of enrolment into the study, 3454 of the 22 707 men were HBsAg seropositive, 15 570 were anti-HBs seropositive and anti-HBc seropositive, 2411 were only anti-HBc positive and 1272 had no HBV marker. As at 30 June 1989, none of the HBsAg-seronegative men who were re-tested had become HBsAg seropositive. All HBsAg-seropositive men were re-tested annually; about 1% per year became HBsAg seronegative. At 30 June 1989, 194 cases of HCC had occurred, 184 (95%) in the HBsAg seropositive men, conferring a relative risk (RR) of 103 (95% CI, 57–205) as compared with HBsAg-seronegative subjects. The remaining 10 cases of HCC all occurred among the 17 981 anti-HBc-seropositive men. Among HBsAg-seronegative men, the difference in risk between those who were anti-HBc seropositive and those with no marker of HBV infection was not significant. The occurrence of HCC in the entire population was related to age, evidence of cirrhosis, HBeAg seropositivity and IgM anti-HBe seropositivity. The only causes of marked excess mortality found in relation to HBV markers were HCC and cirrhosis.

Tu *et al.* (1985) studied all men over the age of 40 from four communes on Chongming Island, China, for development of HCC; 98% (12 222 men) of those eligible participated and were followed for three years. Of the 12 222 men, 1971 (16.1%) were classified as HBV carriers (defined as being either HBsAg seropositive or anti-HBs seronegative and anti-HBc seropositive at the time of the survey). Thirty-seven of the 70 deaths from HCC occurred among the HBV carriers and 33 occurred among the non-carriers, giving an RR of 6.7 [95% CI, 4.2–10.7]. The authors also studied the effects of water source, cigarette smoking, maize consumption (as a measure of exposure to aflatoxin B_1) and alcohol consumption on the risk for HCC. A significantly higher rate of HCC was seen among carriers who smoked 20 cigarettes or more per day. None of the other factors had a significant effect.

Yeh *et al.* (1989) conducted a prospective study of the effects of HBV and aflatoxins on risk for HCC in the southern Guangxi Autonomous Region, China. The entire cohort consisted of 7917 men who were between the ages of 25 and 64 at enrolment, lived in one of five communities and did not have HCC at the initiation of the study. The cohort was assembled between July 1982 and June 1983, at which time demographic information was collected and a blood sample was drawn; the sera were banked. In 1987, the sera of 2072 men were tested for HBsAg. The 2072 men included 149 men who had died as of 31 July 1986, of whom 76 had died of HCC, and a 25% random sample of the men (1923) still alive. Four live controls were matched to each death from HCC. Of the 76 cases, 69 had occurred in HBsAg-seropositive men (90.7%), whereas 68 of the 304 matched controls were HBsAg seropositive (22.4%) (RR, 39; 95% CI, 16–117). In a geographical analysis of these data, mortality from HCC was not correlated with the mean prevalence of HBsAg seropositivity by commune, but the range of HBsAg seropositivity was narrow (19.5–24.8%). A positive correlation was found between the estimated mean level of aflatoxin B_1 consumed in food and the rate of HCC by commune, and the range of aflatoxin B_1 levels was broad (0.3–51.8 mg/person per year).

Ding *et al.* (1988) carried out a prospective investigation of residents of the Guangxi Autonomous Region, China. A total of 22 830 people [sex distribution unspecified] over the age of 20 years were stratified on the basis of HBsAg status, hepatic enlargement, alanine aminotransferase levels and residence in an area with a high or low rate of HCC. The average period of follow-up was 6.8 years. The highest RR for HCC occurred in HBsAg-seropositive people with evidence of both liver enlargement and abnormal liver function, regardless of place of residence. In the group from the high-rate area, 30.6% who were HBsAg seropositive and 2.7% who were HBsAg seronegative developed HCC. In the group from the low-rate area, 10% of the HBsAg-seropositive and 2.4% of the HBsAg-seronegative people developed HCC. In the total population, the RR for HCC, adjusted for age, was 8.2 [95% CI, 4.5–15] in HBsAg-seropositive as compared with HBsAg-seronegative subjects.

In a study in Shanghai, China, an association between HBsAg seropositivity and HCC was reported among men participating in a prospective study of diet and cancer (Ross *et al.*, 1992). Between January 1986 and September 1989, 18 244 male subjects aged 45–64 were enrolled in the cohort; 22 cases of HCC had occurred by 1 March 1990. A nested case–control study was conducted with 140 controls matched by age (within one year), sample date (within one month) and residence, who had no history of liver cancer when the case was diagnosed. Five cases have been confirmed by biopsy. Conditional logistic regression, controlling for education, urinary aflatoxins, smoking and alcohol use, yielded an RR of 8.5 (95% CI, 2.8–26) for HBsAg seropositivity as compared with HBsAg seronegativity. The RR associated with detectable urinary aflatoxins was 3.8 (1.2–12), adjusted for educational level, HBsAg seropositivity, smoking and alcohol use; the RR associated with both HBsAg seropositivity and detectable urinary aflatoxins was 60 (95% CI, 6.4–562).

In the Japan–Hawaii Cancer Study of 7498 men of Japanese ancestry born between 1900 and 1919 and living in Hawaii, sera were collected in 1967–70 and stored. From 1967 through 1980, 18 histologically confirmed incident cases of HCC were identified; serum was available for 16. In a case–control analysis (Nomura *et al.*, 1982), each of the 16 cases was matched to three controls from the study cohort by age and serum collection date. Ten of the cases were seropositive for HBsAg as compared with none of the controls ($p < 0.0001$). Another indicator of persistent infection, anti-HBc without anti-HBs, was detected in 7 of the 16 patients and in two of the 48 controls.

Iijima *et al.* (1984) and Sakuma *et al.* (1988) studied prospectively two overlapping cohorts of male Japanese employees of Japan National Railways. The first cohort was identified in 1973 and 1978. A total of 6918 men, 126 of whom were found to be HBsAg seropositive at the time of the study (1.8%) were followed to March 1985. Average follow-up was 8.5 years (range, 6.5–11.5 years) and was conducted using annual health examinations, self-reported disease (mandatory for any illness lasting more than six days), follow-up of all men who failed to report for an annual examination, and death certificates. Four HCCs developed in the HBsAg-seropositive group and six in the HBsAg-seronegative group, giving an RR of 30 [95% CI, 8.1–77]. The second cohort consisted of 25 547 men who were identified in 1977–79; the purpose of this study was to determine whether HBeAg status among HBsAg-seropositive men ($n = 513$) was associated with the risk for developing HCC. Average follow-up to 1985 was 7.3 years. RRs were calculated in relation to the risks for HBsAg-seronegative men. HCC was observed in 21 HBsAg-seronegative and 9 HBsAg-

seropositive individuals. The RR was 50 [95% CI, 0.66–280] for the 30 HBsAg-seropositive, HBeAg-seropositive men (one HCC), 9.5 [1.1–34] for the 238 HBsAg-seropositive, anti-HBe-seropositive carriers (two HCCs) and 29 [11–63] for the 245 HBsAg-seropositive, HBeAg- and anti-HBe-seronegative carriers (six HCCs).

A population-based study of risk for HCC was conducted among Alaskan natives (Alward *et al.*, 1985; Heyward *et al.*, 1985; McMahon *et al.*, 1990a,b). About two-thirds of the Alaskan native population was classified according to HBV infection status in a statewide screening programme begun in 1983 with the establishment of a HBV carrier registry. The authors identified 1400 HBV carriers (824 men, 576 women) and followed them up until 1 July 1987 (when 1292 were left) for a total of 7815 person-years. The study cohort comprised people of all three Alaskan native groups (85% Inuit, 7.5% Indian, 7.5% Aleut). Review of HBV sequelae from January 1975 to July 1987 showed that 20 cases of HCC had occurred, 19 of which were histologically confirmed. The annual incidence among men was 387/100 000, and that among women was 63/100 000. The incidence of HCC in HBsAg-seronegative people was estimated from the prevalence of HBsAg seronegativity in the Alaskan native population (97%) and the seven HCCs diagnosed in HBsAg-seronegative Alaskan natives between 1975 and 1987, to give an RR of 148 [95% CI, 59–305] for carriers *versus* non-carriers (McMahon *et al.*, 1990a).

2.3.2 *Prospective studies of blood donors* (Table 5)

Oshima *et al.* (1984) followed a cohort of 8646 HBsAg-seropositive male Japanese blood donors in the Osaka Red Cross Blood Center, who were found to be HBsAg seropositive in 1972–75. Follow-up was conducted by examining data in the Osaka Cancer Registry and the death certificate files of Osaka Prefecture through the end of 1980. The mean length of follow-up was 6.2 years (range, 5–8.5 years). The expected number of cases was based on the age-specific incidence rates among the general population of Osaka. The study cohort developed 20 HCCs during the follow-up period, with 3.0 expected, giving a significant RR of 6.6 [95% CI, 4.0–10]. In a nested case–control analysis, alcohol drinking was significantly related to the risk of developing HCC, with RRs of 5.5 (95% CI, 1.2–26) for moderate drinkers and 8.0 (95% CI, 1.3–50) for heavy drinkers in comparison with non-drinkers.

Fukao (1985) identified 1000 HBsAg-seropositive and 10 000 HBsAg-seronegative blood donors from blood donation records in Miyagi Prefecture, Japan. All cohort members were men over the age of 30 years who had donated blood between 1971 and 1977. The HBsAg-seronegative group was matched 10:1 to the HBsAg-seropositive group by age, district of residence and month of blood donation. Cancer incidence was determined from the records of the Migayi Prefectural Cancer Registry for the years 1971–80. Three HCCs were detected in the HBsAg-seropositive group and one in the HBsAg-seronegative group, giving an RR of 30 [95% CI, 6.0–88] in carriers as compared with non-carriers.

Tokudome *et al.* (1987, 1988) estimated the risk for HCC among HBV carriers in Japan. A cohort of 3769 HBsAg-seropositive women was identified from among blood donors at the Fukuoka Red Cross Blood Center by examining Red Cross records of donations between the years 1977 and 1982. Mortality follow-up was completed until 1985; vital status was determined by checking against each donor's home residence card. Death certificates were

obtained for each donor known to be dead. The 220 women (5.8%) who were lost to follow-up were assumed in the analysis to be alive at the end of the study. The average period of follow-up was five years. Seventeen deaths occurred during the study period, four of which were due to HCC. In comparison with the age-adjusted mortality rates among all women in Fukuoka Prefecture in 1980, the RR for HCC was 5.6 [95% CI, 1.5–14] (Tokudome *et al.*, 1987). In a cohort of 2595 HBsAg-seropositive male blood donors identified during 1977–79 and followed up to 1983 (9.2% lost to follow-up; average length of follow-up, six years), 15 HCCs developed as compared with 2.1 expected on the basis of age-specific mortality rates for Fukuoka Prefecture in 1980. The RR in HBsAg-seropositive men in relation to the general male population was 7.3 [95% CI, 4.1–12] (Tokudome *et al.*, 1988).

Prince and Alcabes (1982) identified a cohort of HBsAg-seropositive men from four sources in the USA: the health departments of New Jersey, New York State and New York City and blood donors in the Greater New York Blood Program. The three health departments maintain lists of HBsAg-seropositive people, most of whom are blood donors who were found to be HBsAg seropositive during routine testing. Men identified as being HBsAg seropositive between 1971 and 1979 and who were residents of either New York City (n = 5353) or New York State (n = 1497) were included. Causes of death were determined by record matching with the New York State and New York City health departments. Four deaths from HCC occurred (three in New York City), as compared with 0.40 expected on the basis of mortality rates for the general populations of New York City and New York State [RR, 10; 95% CI, 2.7–25].

Dodd and Nath (1987) conducted a mortality study of people who had given blood during 1971–80 in the national Red Cross Donor Deferral Registry and identified 15 166 HBsAg-seropositive and 18 144 HBsAg-seronegative people. Vital status was determined by matching records with those of the Social Security Administration, and cause of death was determined from death certificates. The mean length of follow-up for the HBsAg-seropositive group was 3.7 years and that for the HBsAg-seronegative group, 3.3 years. Men comprised 70% of the HBsAg-seropositive group and 63% of the HBsAg-seronegative group. Six HCCs occurred in the HBsAg-seropositive group and none in the HBsAg-seronegative group. The authors give a standardized mortality ratio (SMR) for HCC of 27 [95% CI, 10–39] on the basis of mortality rates in the general population.

A prospective mortality study of HBsAg-seropositive people who donated blood in England and Wales between 1971 and 1981 (Hall *et al.*, 1985) comprised 2880 men and 1054 women, of whom more than 92% could be followed for death to the end of 1983. Seventy-seven cohort members died during the period. Five deaths from HCC were reported among men in contrast to the 0.1 expected, giving a RR of 42 (95% CI, 13–98). No death from HCC occurred among the female cohort members (0.02 expected).

2.3.3 *Prospective studies of populations with pre-existing disease*

The incidence of HCC in HBsAg-seropositive and HBsAg-seronegative subjects has been studied among patients with pre-existing liver disease, as indicated variously by abnormal liver function, 'chronic hepatitis' and cirrhosis. The RRs for HCC associated with HBsAg seropositivity in these studies were generally small, e.g. [RR, 2.1 (95% CI, 0.3–17)] (Liaw *et al.*, 1986). Their interpretation is complicated by the fact that causes of liver disease

other than HBV may also be associated with an increased risk for HCC (Liaw *et al.*, 1986; Dodd & Nath, 1987; Colombo *et al.*, 1991; Johnson, 1991; Kato *et al.*, 1992).

2.4 Case–control studies

2.4.1 *Hepatocellular carcinoma*

Many case–control studies have been published on the relationship between HCC and HBV infection. The prevalences of seropositivity are summarized in Table 6 (p. 90). In all studies, tests for HBV markers were performed on one occasion only, and 'carriers' were taken to be subjects in whom HBsAg was detected at that time. Unless otherwise noted in the Table, testing for HBV markers was done by radioimmunoassay. RRs, as measured by the odds ratio (OR) and 95% Cornfield confidence intervals (CIs), were calculated by the Working Group wherever the data reported in the original papers allowed it. In general, only ORs and *p* values are reported in the text, while ORs and CIs are reported in Table 6. Studies of clinical series (typically, patients with liver disease) in which cases of HCC were a subgroup but in which there was no specifically defined control group were not included.

(*a*) *Africa*

Prince *et al.* (1970) reported a comparison of patients with HCC and other chronic liver diseases with various control groups in relation to the prevalence of HBsAg seropositivity. They tested sera from subjects from Senegal, Uganda and the USA using two serological assays—agar-gel diffusion and high-voltage immunoelectroosmophoresis; the latter was 10 times more sensitive than the former. The prevalences of HBsAg seropositivity among the subjects examined were: in Senegal, 42% of 210 HCC patients, 9% of 201 adult males and 12.7% of 959 army personnel; in Uganda, 12% of 34 HCC patients, 23% of 26 with cirrhosis and 2% of 311 healthy subjects [OR for HCC, 6.8; 1.8–25]; in the USA, 4% of 55 HCC patients, 29% of 42 with chronic active hepatitis, 8% of 124 with cirrhosis and 0.1% of 55 956 blood donors. The authors remarked that, although the controls were not matched to the cases by age, sex or place of residence, hepatitis virus appeared to play an important role in at least a proportion of the cases of chronic liver disease.

Vogel *et al.* (1972) reported the results of a study conducted among in-patients at Mulago Hospital, Kampala, Uganda, or the Solid Tumour Centre in Uganda between October 1969 and May 1970 (Vogel *et al.*, 1970) and January 1970 to May 1971. The 90 HCC cases (in 73 men and 17 women) had a significantly higher frequency of HBsAg in their sera (40%) than the 224 (149 non-neoplastic and 75 neoplastic) controls combined (151 men, 73 women) (3%; $p < 0.001$). Control patients with cirrhosis or hepatitis were excluded; histological confirmation was available for 71 of the HCC cases. There was no difference between cases and controls with respect to anti-HBs seropositivity (28–37%). A nonsignificant association was noted between HBsAg seropositivity in HCC patients and the presence of cirrhosis (44% in comparison with 27% without cirrhosis). Serum was tested by complement fixation, counter immunoelectrophoresis and passive haemagglutination.

Kew *et al.* (1974) investigated 75 male Bantu miners in South Africa with HCC confirmed at necropsy. The control group of 18 377 healthy miners was comparable with respect to age and tribal distribution. Testing of sera for HBsAg by complement fixation and

Table 5. Prospective studies of HBV surface antigen (HBsAg)-seropositive people for the development of hepatocellular carcinoma (HCC)

Region, reference, location	Subjects (age)	HBsAg seroprevalence	Mean duration of follow-up (years)	No. of cases of HCC	Annual incidence (cases/ 100 000)	RR (95% CI)
America						
Nomura *et al.* (1982) Hawaii, USA	Men of Japanese ancestry (born 1900–19) Controls	7498 HBsAg+ 16 HCCs (10 HBsAg+) 48 (0 HBsAg+)	11.8	16	[18]	[∞], $p < 0.0001$ Nested case-control analysis
Prince & Alcabes (1982) New York, USA	Blood donors; men (20–> 50 years)	6850 HBsAg+	4.4	4	[13, crude]	[10; 2.7–26] SMR using HCC mortality in New York
Dodd & Nath (1987) USA	Red Cross blood donors (age at death from HCC, 30–64 years)	15 166 HBsAg+ 18 144 HBsAg-	3.68 3.26	6 0	11 0	27 [10–39] SMR using HCC mortality in US population in 1975
McMahon *et al.* (1990a) Alaska, USA	Alaskan native population; men, women; average age, ~ 22 years	1400 HBsAg+; 59% male	5.58	20	Men, 387 Women, 63	148 [59–305] Compared with estimated incidence in general population
Asia						
Oshima *et al.* (1984) Osaka, Japan	Blood donors; men (more than half < 30 years)	8646 HBsAg+	6.2	20	[37]	6.6 (4–10) Compared with incidence in Osaka general population
Fukao (1985) Miyagi, Japan	Blood donors; men (> 30 years)	1000 HBsAg+ 10 000 HBsAg- Matched by age, residence, month of donation	NR	3 1	45	30 [6.0–88]

Table 5 (contd)

Region, reference location	Subjects (age)	HBsAg seroprevalence	Mean duration of follow-up (years)	No. of cases of HCC	Annual incidence (cases/ 100 000)	RR (95% CI)
Asia (contd)						
Tu *et al.* (1985) Chongming Island, China	Men (> 40 years) from 4 communes	1971 HBsAg+ or anti-HBc+ and anti-HBs – 10 251 HBsAg –	3	37 33	651 (carriers) 98.6 (non-carriers)	6.7 [4.2–11]
Tokudome *et al.* (1987) Fukuoka, Japan	Blood donors; women (age, NR)	3769 HBsAg+	5.05	4	[21]	5.6 [1.5–14] Ccompared with adjusted mortality in Fukuoka population
Tokudome *et al.* (1988) Fukuoka, Japan	Blood donors; men (age, NR)	2595 HBsAg+	5.86	15	[98.6]	7.3 [4.1–12] Compared with adjusted mortality in Fukuoka population
Ding *et al.* (1988) Guangxi, China	Men and women (> 20 years)	1839 HBsAg+ 9233 HBsAg –	6.8	[41] [39]	NR	[5.3; 3.8–7.2]
Sakuma *et al.* (1988) Japan	Railway workers; 6918 men	126 HBsAg+ 6792 HBsAg –	8.5 8.5	4 6	[374] [10]	30 [1–77]
Sakuma *et al.* (1988) Japan	Railway workers; men (40.55 years)	513 HBsAg+ 25 034 HBsAg –	7.3 7.3	9 21	[240] [12]	[21; 9.6–40]
Yeh *et al.* (1989) Guangxi, China	Men (25–64 years) from 5 communes	2072 tested for HBsAg 76 HCC (69 HBsAg+) 304 controls (68 HBsAg+)	3.8	 69 HBsAg+ 7 HBsAg –	NR	 39 (16–117) Nested case–control analysis
Beasley & Hwang (1991) Taiwan, China	Government employees; men (82% 40–59 years)	3454 HBsAg+ 19 253 HBsAg –	[11.25] [10.28]	184 10	474 [52]	103 (57–205)

Table 5 (contd)

Region, reference, location	Subjects (age)	HBsAg seroprevalence	Mean duration of follow-up (years)	No. of cases of HCC	Annual incidence (cases/ 100 000)	RR (95% CI)
Asia (contd)						
Ross *et al.* (1992) Shanghai, China	Men (45–64 years)	18 224 men 22 HCCs (12 HBsAg+) 140 controls (15 HBsAg+)	1.9 years	12 HBsAg+ 10 HBsAg–	NR	8.5 (2.8–26) Nested case-control analysis
Europe						
Hall *et al.* (1985) United Kingdom	Blood donors (age, NR)	2880 HBsAg+ men 1054 HBsAg+ women	NR	5 0	NR	42 (13–98)

NR, not reported

counter immunoelectrophoresis revealed a difference in the prevalence of persistent HBV infection: 40% *versus* 7% [$p < 0.05$]. No significant relationship was noted between HBsAg seropositivity and the presence of cirrhosis (46% in comparison with 31% without cirrhosis) among cases. [No information was provided as to how and when cases and controls were identified.]

A study conducted at Le Dantec Hospital, Dakar, Senegal, between October 1972 and July 1974 involved 165 cases of HCC (in 127 men and 38 women), 154 controls with other cancers (102 men, 52 women) and 328 non-cancer controls (226 men, 102 women) (Michon *et al.*, 1975; Prince *et al.*, 1975). A diagnosis of HCC was histologically confirmed for 80 cases. Controls were matched to cases on sex, age (within 10 years) and admission date (within 30 days) and were of similar ethnicity and religion; non-cancer controls were free of liver disease. A higher frequency of HBsAg seropositivity was reported among the cases than in each control group: 61.2% *versus* 11.8 and 11.3% in the two control groups, respectively [$p < 0.05$]. The prevalence of anti-HBs seropositivity (tested by passive haemagglutination) alone was lower among cases (18.2%) than controls (45.4 and 42.1%, respectively) (Michon *et al.*, 1975). In 94 documented cases of HCC, the presence of cirrhosis was not related to HBsAg seropositivity but was related to anti-HBs seropositivity (6% *versus* 20%; $p < 0.05$) (Prince *et al.*, 1975).

Larouzé *et al.* (1976) matched 28 cases of HCC (in 22 men and 6 women) from Le Dantec Hospital, Dakar, Senegal, to healthy individuals from the same urban neighbourhood or rural village, of the same sex, age and ethnic group. Blood was also collected from parents and siblings. Histological confirmation was obtained in 24 cases. Although no significant association was observed between the presence of HBsAg (by radioimmunoassay) and HCC (79% in cases, 57% in controls; $p = 0.15$ [OR, 2.8]), the prevalence of HBsAg among the controls was very high. Cases were markedly less likely to be seropositive for anti-HBs (by passive haemagglutination) (25% *versus* 64%; $p = 0.006$) and more likely to be seropositive for anti-HBc (by counter immunoelectrophoresis and immunodiffusion) (89% *versus* 64%; $p = 0.05$). [Neither the time frame nor the case selection method was described.]

In a study in Addis Ababa, Ethiopia, HBsAg seroprevalence was compared in 46 HCC cases (in 31 men and 15 women) and 90 healthy hospital employees without a history of liver disease, blood transfusion, leprosy, leukaemia or Down's syndrome (Tsega *et al.*, 1976; Tsega, 1977). The cases from whom serum was collected were a subset of 100 consecutive HCC patients admitted to St Paul's or Haile Selassie I hospitals between June 1972 and November 1974. The diagnosis of HCC was confirmed by biopsy in 25 cases. HBsAg seropositivity was significantly associated with the occurrence of HCC: 50% among cases, 7% among controls [OR, 14, $p < 0.001$].

Tabor *et al.* (1977) analysed serum samples from 47 cases of HCC confirmed by biopsy in Uganda (previously studied by Vogel *et al.*, 1972), 19 in Zambia and 27 in the USA; the controls were 50 in-patients with melanoma or Kaposi's sarcoma in Uganda, 40 healthy Zambian villagers (from the same geographic region as the cases) and three US blood donor groups (6726 total), respectively. Evidence of active HBV infection (HBsAg seropositivity with or without anti-HBs seropositivity or anti-HBc seropositivity with anti-HBs seronegativity) was significantly associated with HCC in each comparison: 72% of cases in Uganda, 68% in Zambia and 41% in the USA. Anti-HBs was tested by radioimmunoassay

and passive haemagglutination and anti-HBc by complement fixation and counter immuno-electrophoresis.

In a study involving blood donor controls, 32 histologically confirmed cases of HCC in 19 men and 13 women in Mozambique were studied (Reys *et al.*, 1977). Their 60% HBsAg seropositivity was significantly higher than the 3–15% [9% overall] observed in 231 male African blood donors from the Hôpital Central de Maputo by subgroup ($p < 0.01$). Anti-HBs was measured by radioimmunoprecipitation (12% compared with 33–49% had anti-HBs). [Neither the methods of subject selection nor the time period were further specified in either study.]

Van Den Heever *et al.* (1978) conducted a study at H.F. Verwoerd Hospital in Pretoria, South Africa, during 1973–76 of 92 histologically confirmed cases of HCC from the Pretoria area (in 75 men and 17 women) matched to 92 orthopaedic out-patients from the same area, of the same age and sex with no history of liver disease; all subjects were black. The association between seropositivity for HBsAg and HCC status was significant (34% of cases, 9% of controls; $p < 0.01$) [RR, 5.3].

In another case–control study in South Africa (Kew *et al.*, 1979), a 62% seroprevalence of HBsAg was found among 289 blacks (280 men, nine women) with histologically confirmed HCC over a four-year period; the prevalence in the 213 healthy controls (gold miners matched by age, sex and ethnic group) was significantly lower, 11% ($p < 0.001$). The cases had been referred consecutively to the South African Primary Liver Cancer Unit from mine hospitals or admitted to two teaching hospitals; most were miners, and the majority were not from South Africa. A significant inverse association was observed for anti-HBs reactivity and HCC (17% in cases, 42% in controls; $p < 0.001$) [OR, 0.28]. In a subset of 74 cases and 104 controls tested for anti-HBc, a significant difference was found (89% *versus* 38%; $p < 0.001$). A detailed serological analysis of 131 HBsAg-seropositive (98 cases, 33 controls) and 222 HBsAg-seronegative (50 cases, 172 controls) male miners was performed (Kew *et al.*, 1981), using the more sensitive radioimmunoassay to detect HBeAg, anti-HBe and anti-HBc. Significant positive correlations with HCC were observed for anti-HBc ($p < 0.01$) and anti-HBe ($p < 0.05$) among the HBsAg-seronegative subjects, and significant inverse relationships for anti-HBe ($p < 0.02$) among HBsAg-seropositive individuals and for anti-HBs ($p < 0.05$) among HBsAg-seronegative subjects. No relationship was observed between HBsAg seropositivity and the presence of cirrhosis among HCC cases (Kew *et al.*, 1979). [The case identification period was not further specified. The later study (Kew *et al.*, 1981) may have included additional subjects not in the earlier one.]

Seventy-six cases of HCC (in 60 men and 16 women) and 33 controls matched for age, sex and tribe were studied by Bowry and Shah (1980) in Nairobi, Kenya. The cases attended Kenyatta National Hospital between January 1976 and April 1979; histological or cytological confirmation was obtained for 56. Of the controls, 28 were relatives of hospital patients and five were hospital patients. HBsAg was detected (by passive haemagglutination) in 51% of cases and 6% of controls [$p < 0.05$]. A report published a year later by the same group (Bowry *et al.*, 1981) probably involved a subset of 60 of these HCC cases [subject selection was not described]. Seropositivity only for anti-HBc was found in eight [13%] of the 60 HCC cases, 15% of 20 matched hospital controls and 4% of 104 volunteer blood donors;

the prevalence was higher among the 13 HCC patients with known cirrhosis (31%). [The blood donors were younger than the cases.]

Coursaget *et al.* (1981) conducted a study in Senegal of 134 cases of HCC (in 114 men and 20 women) diagnosed at Le Dantec Hospital and 100 blood donor controls from the National Blood Center of Dakar, in which anti-HBc was measured by counter immunoelectrophoresis. A significant association was found between HCC and active HBV infection [OR, 14] (67% *versus* 13%; $p < 10^{-6}$). A significantly lower frequency of past infection (HBsAg seronegativity and anti-HBs seropositivity or anti-HBc seropositivity by radioimmunoassay) was noted for cases (33%) in comparison with controls (74%; $p < 10^{-6}$). HBsAg was detected in 63% of the HCC patients and in 12% of the 100 blood donors [OR, 12], 14% of 833 rural country dwellers (72 men, 761 women) [OR, 11] and 25% of 560 leprosy patients from the Pavillon de Malte (410 men, 150 women) [OR, 50]. Two earlier reports of this study (Maupas *et al.*, 1977; Coursaget *et al.*, 1980) showed consistent findings. [Little detail could be found as to subject selection.]

Gombe (1984) found a significantly higher prevalence of HBsAg seropositivity among 65 cases of HCC (in 47 men and 18 women) (74%) than among 120 blood donors (115 men, five women) (9% [$p < 0.05$]) or 71 other cancer controls (13 men, 58 women) (3%; $p < 0.05$) in the Congo. The blood donors were tested in two centres by radioimmunoassay and passive haemagglutination, and the HCC cases and cancer controls from the Brazzaville General Hospital by passive haemagglutination. [The process for selecting subjects was not clear.]

Sebti (1984) in Rabat, Morocco, reported a significant but weaker association between HCC and HBsAg seropositivity (17% in 46 cases and 5% in 379 controls) [OR, 4.2]. The HCC patients were a subset of 63 cases hospitalized at Avicenne Hospital between 1976 and 1983; the controls were healthy subjects and people with non-hepatic disease. [The process for selecting subjects was not clear.]

Another study by Kew *et al.* (1986a) focused on southern African blacks living in an urban environment. Markers of HBV infection were assayed in 62 urban-born patients with histologically confirmed HCC (41 men, 21 women) and in pair-matched urban-born hospital controls, matched according to race, sex, age, tribe (when possible), hospital and ward. Subjects were identified from two large general hospitals, Baragwanath in Soweto and Hillbrow in Johannesburg. HBsAg was detected significantly more frequently among the cases (40%) than the controls (3%) ($p < 0.001$). No difference was observed with regard to past infection (HBsAg seronegativity and anti-HBs or anti-HBc seropositivity). [Information about the period of subject selection was not provided.]

Otu (1987) studied 200 consecutive, histologically confirmed cases of HCC (in 180 men and 20 women) at the University of Calabar Teaching Hospital, Nigeria, between January 1978 and December 1982. Two symptomless controls matched for sex and age (within five years) were selected per case from the general out-patient department. HBsAg was detected in 49% of the cases and 8% of the controls ($p < 0.01$). [Little detail was provided about selection of controls.]

Gashau and Mohammed (1991) compared the prevalence of HBV markers in 65 HCC patients (57 men, eight women) and 69 sex- and age-matched healthy controls in Nigeria. The cases of HCC were examined consecutively at the University of Maiduguri Teaching Hospital

between 1986 and 1987; needle biopsy was used for diagnosis in 21. The controls were examined at the hospital during the same period; most were blood donors. The cases had a significantly higher rate of HBsAg seropositivity (65%), measured by ELISA and reverse passive haemagglutination, than the controls (35%) [OR, 3.2]. Seropositivity for anti-HBc, measured by ELISA, was about the same in those tested in the two groups; HBeAg seropositivity was higher and that of anti-HBe lower in the cases. [Few details were provided about the controls.]

Mohamed *et al.* (1992) examined the prevalence of current (HBsAg seropositivity) and past (seronegative for HBsAg and seropositive for anti-HBs or anti-HBc) infection among 101 black South Africans with HCC, matched for ethnic origin, sex, age (within two years) with patients from the wards of Baragwanath Hospital, Johannesburg (77 men, 24 women). Controls were excluded if they had a disease caused by alcohol abuse or were unable to answer questions. Histological confirmation was obtained for 85 cases of HCC. Among men, 35% of cases and 5% of controls were currently HBsAg seropositive; the OR, adjusted for alcohol and smoking, was 7.5 (95% CI, 2.2–25). Among women, HBsAg seropositivity was 25% for cases and 4% for controls, with an adjusted OR of 12 (95% CI, 1.0–154). [The time frame for subject selection was not given.]

At the Parirenyatwa Teaching Hospital in Zimbabwe, Tswana and Moyo (1992) studied 182 HCC cases (in 128 men and 54 women) and 100 non-liver disease patient controls (50 men, 50 women). Pregnant women, cigarette smokers and alcohol consumers were excluded from the study. The diagnosis of HCC was made clinically and confirmed by α-fetoprotein level. Controls were selected randomly and were comparable to the cases with respect to age and sex; subjects were 20–65 years old. A significant correlation ($p < 0.0001$) was reported between HBsAg seroprevalence and HCC [OR, 10] (56% in cases *versus* 11% in controls); anti-HBc was detected in 54% of cases and 4% of controls [OR, 29; $p < 0.05$]. Among HBsAg-seropositive subjects, the seroprevalence of HBeAg was 20% among cases and 9% among controls. HBV markers were determined by ELISA. [The time frame for the study was not given.]

Ryder *et al.* (1992) studied HCC and HBV infection in the Gambia between 1 December 1981 and 30 November 1982. In a community-based surveillance system, they identified 70 cases (in 61 men and nine women); 44 were confirmed histologically. Patients were interviewed within one month of diagnosis in their village, at which time the person living closest to the case, of the same sex and age (within five years) was identified as a control. All potential subjects agreed to participate, and the two groups were not significantly different with respect to length of residence, alcohol consumption, smoking habits or family size. After adjustment for age, the following associations were reported: HBsAg (64 cases and 67 controls tested), OR, 6.9 ($p < 0.01$); anti-HBs (63 cases and 68 controls tested), OR, 0.31 (not significant); HBeAg (63 cases and 68 controls tested), OR, undefined ($p < 0.001$); and anti-HBe (62 cases and 66 controls tested), OR, 2.3 (not significant). The prevalence of HBsAg seropositivity was 63% among cases and 21% among controls; that of HBeAg was 17% among cases and 0 among controls. In all instances, the relationships were strongest among people under 50 years of age.

Serological HBV markers were investigated by the immunoperoxidase procedure in 40 cases of HCC selected in 1985 among 223 cases collected at the Department of Pathology

of the University Hospital and Medical School of Kinshasa, Zaire, and in 68 age- and sex-matched controls selected from among blood donors (Kashala *et al.*, 1992). The proportion of seropositive individuals among cases of HCC was significantly higher for HBsAg (57.6% *vs* 7.35%) and for anti-HBeAg (27.5% *vs* 16.2%) but was significantly lower for anti-HBs (25% *vs* 63%), and no significant difference was observed for anti-HBc, HBe or HBV DNA.

(*b*) *Americas*

Yarrish *et al.* (1980) analysed sera collected from patients attending hospitals in Philadelphia, USA, between 1968 and 1977. The sera from 34 HCC cases (in 28 men and six women) were then matched to those of 38 patients (30 men, eight women) with colon cancer, 45 (36 men, nine women) with lung cancer and 56 blood donors (48 men, eight women) matched for age (within five years) and sex. All but one HCC case was histologically confirmed. Blood donor samples were collected prior to routine screening for HBsAg and were stored on average 39 months longer than the sera from HCC cases; the sera of the colon cancer controls were stored for four months less and those of the lung cancer controls for 17 months less than those of the HCC cases. Five HCC cases (15%) were seropositive for HBsAg (assayed by radioimmunoassay), which was significantly higher than in any control group ($p < 0.05$). Seropositivity for anti-HBs, as assayed by passive haemagglutination, was not significantly different between cases and controls. [The details of subject selection were not provided.]

In the Japan–Hawaii Cancer Study, described in detail on p. 69, Nomura *et al.* (1982) found a significant excess of HBsAg in HCC patients in Hawaii (63%, with none in controls).

Austin *et al.* (1986) performed a multicentre study of 67 HCC patients (45 men, 22 women), aged 18–84, from 12 US hospitals, for whom HBsAg status was known (49 assayed by radioimmunoassay, 18 from medical records). The 18% (all in men) HBsAg prevalence among these cases was significantly higher ($p = 0.0002$) than the 0 prevalence for the 63 controls, who had no liver disease or a condition related to tobacco use and were matched by sex, year of birth (within five years), race and current residence (59 assayed by radioimmunoassay, four from records). Of the people seronegative for HBsAg who were tested, 7/40 (18%) of cases and 5/58 (9%) of controls were seropositive for anti-HBs [not significant]. [Details were not given about the time and method of case selection.]

Yu *et al.* (1990) described a study of black and white residents of Los Angeles County, USA, 18–74 years of age. Histologically confirmed incident cases of HCC were identified between January 1984 and August 1989; 392 cases were eligible, but only 51 (12 blacks, 39 white; 35 men, 16 women) were analysed, either because of death (290), refusal (29), inability to locate (9), incorrect diagnosis (7) or serum sample depletion (6). Controls were selected from among 404 community control subjects used in a case–control study of lymphoma from 1978 to 1982; 128 of 404 controls were randomly selected to be frequency matched by sex and age (10-year intervals) to the cases (1 black, 127 white; 81 men, 47 women). All interviews were performed in the subjects' homes. The age- and sex-adjusted ORs for the various HBV markers, tested by radioimmunoassay with no markers as the reference level, were: HBsAg, infinity ($p = 0.002$); anti-HBc, 7.3 ($p < 0.0005$); anti-HBs, 5.2 ($p < 0.0005$). Neither

adjustment for level of education nor a separate analysis of US-born or white subjects affected the results.

In a study conducted in Baltimore, USA, 99 consecutive histologically confirmed HCC patients at the Johns Hopkins Oncology Center were compared between January 1987 and May 1988 with 98 consecutive patients with other malignancies seen at the same centre between November 1987 and January 1988 (Di Bisceglie *et al.*, 1991). The cases were from the eastern half of the USA and were referred for inclusion in therapeutic radiation trials. No significant difference was reported between the two groups with regard to age, sex or race. HBsAg and IgM anti-HBc were detected only in the cases (7% and 8%, respectively), at significantly higher prevalences than in the controls ($p = 0.009$ and 0.004). Cases and controls were similar with regard to the presence of the other HBV markers measured. Anti-HBc was determined by enzyme immunoassay. [The fact that the patients had advanced disease might have affected HBsAg levels.]

(*c*) *Asia*

In Taiwan, China, Tong *et al.* (1971) examined the prevalence of detectable antigen in 55 cases of HCC (in 52 men and three women) and 943 male personnel at the Tsoying Naval Base. The cases were from the Chinese Veterans' Hospital, and 25 diagnoses were confirmed by needle biopsy or autopsy. No subject had Down's syndrome, leprosy, leukaemia or a recent transfusion (within 12 months); the controls had no past or present liver disease. A significant difference in HBsAg seropositivity was found by a modified immunodiffusion technique: 80% among cases and 15% among controls ($p < 0.001$) [OR, 23]. The cases were older than the controls (mean, 48 *versus* 30 years). [No details were provided concerning the timing or selection of subjects.]

Simons *et al.* (1972) studied HBsAg seroprevalence among 156 Chinese HCC patients in Singapore; 114 (87 men, 27 women) had been reported previously (Simons *et al.*, 1971). The control groups consisted of 1516 male blood donors, 260 women attending antenatal clinics and 207 patients investigated for suspected nasopharyngeal carcinoma at one of the hospitals; all controls were Chinese. HBsAg seropositivity was markedly higher among the HCC cases (35%) than in any of the control groups (8%, 2% and 6%, respectively). [The associations calculated by the Working Group were all statistically significant; the blood donors and women attending antenatal clinics are combined in Table 6 as 'normal controls'. No details were found concerning selection of controls.]

Lee (1975) compared the prevalence of HBsAg seropositivity in 100 cases of HCC (in 85 men and 15 women) and 120 patient controls (98 men, 22 women) in Hong Kong with no history of liver disease or blood transfusion; the diagnosis of HCC was confirmed by biopsy in 81 cases. Testing by immunoelectroosmophoresis and complement fixation showed a significant difference (49% *versus* 9%; $p < 0.001$). [No data were provided on the timing or process of subject selection or on the gender distribution of the cases.]

Chainuvati *et al.* (1975) reported a higher frequency of HBsAg seroprevalence, measured by crossover immunoelectrophoresis, among 49 HCC patients with cirrhosis (16%) than among 87 hospitalized controls (74 men, 13 women) without liver disease (2%) in Thailand ($p < 0.005$). No HBsAg was found in eight HCC patients without cirrhosis. The

cases in this study had been identified between August 1972 and April 1973 and were histologically confirmed.

Kubo *et al.* (1977) studied 124 cases of HCC (in 107 men and 17 women) seen at Kurume University and Chiba University Schools of Medicine, Japan; the diagnosis was histologically confirmed in 108 cases. Healthy employees of the Japan National Railways (290 men, nine women) seen at regular physical check-up were used as controls. The difference in seroprevalence of HBsAg between cases and controls was significant (46% *versus* 4%; $p < 0.01$) [OR, 20], as were smaller differences in anti-HBc seropositivity (73% *versus* 30%; $p < 0.01$) [OR, 6.2] and the presence of any marker (81% *versus* 34%; $p < 0.01$) [OR, 8.1]. No difference between cases and controls was observed for anti-HBs, as tested by passive haemagglutination. Immune adherence haemagglutination was used to detect anti-HBc. [No details were given as to when subjects were diagnosed.]

In Taiwan, China, 127 HCC cases admitted to the National Taiwan University Hospital between May 1974 and December 1976 were compared with 729 healthy controls (Chen & Sung, 1978). The controls comprised 241 40–67-year-old adults from a cancer education programme and 488 18–22-year-old university students receiving a regular check-up in 1975. HCC was histologically confirmed in 63 cases. HBsAg (assayed by reverse passive haemagglutination) was found in 83% of cases and 15% of controls [OR, 28; $p < 0.05$], and anti-HBs (detected by passive haemagglutination) in 14% and 45%, respectively [OR, 0.21; $p < 0.05$]. An analysis of 68 HCC cases with and without cirrhosis revealed no association with HBsAg seropositivity (81% in each group). [The gender breakdown for the subjects was not given.]

Chien *et al.* (1981) conducted a study among Chinese HCC patients seen at the Taiwan Veterans General Hospital (Tong *et al.*, 1971). The 102 cases (in 97 men and five women) were matched by age and sex to 100 healthy controls from the out-patient clinic; histological confirmation was obtained for 36 cases. A larger proportion of cases than of controls were seropositive for HBsAg (71% *versus* 12%) [OR, 18] or anti-HBc (98% *versus* 84%) [OR, 4.7], and a lower proportion for anti-HBs (27% *versus* 54%). HBsAg-seropositive patients had higher levels of HBeAg and lower levels of anti-HBe than controls. All differences except those for HBeAg and anti-HBe were significant [$p < 0.05$].

Lam *et al.* (1982) performed a study in Hong Kong which included 107 Chinese cases (in 95 men and 12 women) of HCC at the Queen Mary Hospital, who were matched by sex and age (within five years) to 107 control (94 men, 13 women) trauma patients in the orthopaedic ward of the same hospital; 106 cases were histologically confirmed. Between March 1977 and September 1980, 149 Chinese HCC patients were admitted to the hospital, 72% of whom were interviewed; controls were interviewed within one month of the index case. After adjustment for age and sex, the OR for HCC associated with the presence of HBsAg was 21 (95% CI, 10–46).

In the Republic of Korea, Sjøgren *et al.* (1984) reported an HBsAg seroprevalence of 82% among 110 histologically confirmed HCC cases (in 90 men and 20 women) and 14% among 63 controls with other cancers matched for sex and age ($p < 0.001$). Subjects were identified between 1973 and 1981 at St Mary's Hospital in Seoul; the control patients had no evidence of liver disease, although five had metastatic liver cancer (Chung *et al.*, 1983). IgM anti-HBc was present in 74 cases (67%) and one control (2%) ($p < 0.001$) [OR, 127]; the

association between HCC and presence of IgM anti-HBc was also seen among the HBsAg-seropositive subjects (81% *versus* 11%; $p < 0.005$). [No details of subject selection methods were given.]

A report from Yamanashi Prefecture, Japan (Inaba *et al.*, 1984), described a matched analysis of 62 cases (in 49 men and 13 women) of HCC from seven hospitals between April 1977 and August 1979. Patient controls with no hepatic disease were selected by sex, age (within five years) and hospital. Confirmation of the diagnosis in liver biopsies was obtained for 36 of the HCC cases. There was a significant, 10-fold increase in risk for liver cancer associated with HBsAg seropositivity, assayed by reverse passive haemagglutination (36% in cases *versus* 3% in controls) ($p < 0.01$), and a weaker association with anti-HBs (27% *versus* 18%) ($p < 0.05$) [crude OR, 1.7].

A matched analysis of cases of HCC in Guangxi Autonomous Region, China, revealed a high OR (17; 95% CI, 4.3–99) for the relationship between HBsAg seropositivity and HCC (Yeh *et al.*, 1985a,b), based on 50 cases (in 47 men and three women) and 49 controls without liver disease matched by sex, age (within five years) and ward/clinic; 86% of the cases were seropositive for HBsAg *versus* 22.45% of the controls. Case identification began on 1 July 1982 and continued until 50 cases were obtained at the College Hospital; only four diagnoses were based on histological examination. In the HBsAg-seropositive subjects assayed, HBeAg reactivity was not significantly greater in HCC patients (31%) than in controls (18%); the seroprevalence of anti-HBe was lower among cases than controls (50% *versus* 64%) (Luo *et al.*, 1988).

In Riyadh, Saudi Arabia, a significant difference ($p < 0.001$) was found between cases of HCC and local population controls for HBsAg seropositivity [60% *versus* 12%] (Arya *et al.*, 1988). The 30 histologically confirmed cases (in 25 men and five women) were a subset of cases in 75 local HCC patients hospitalized at King Fahad Central Hospital from September 1984 to October 1985, from whom serum was available. The control group comprised 326 patients aged 20–> 40 treated for minor ailments in the area. Among the HBsAg-seropositive subjects, a significant inverse association was reported between HCC and anti-HBe seroprevalence [OR, 0.15] (24% in cases *versus* 67% of controls; $p < 0.01$), but no significant association was seen for HBeAg [OR, 2.7]. ELISA was used to assay all HBV markers. [Little detail was available about control selection.]

A matched analysis by Lu, C.Q. *et al.* (1988) of 30 HCC patients and 60 matched controls with other tumours or anorectal diseases in the same hospital in Tianjin, China, showed significant associations with reactivity to HBsAg ($p < 0.001$) (OR, 5) and anti-HBc ($p < 0.05$) (OR, 39). The prevalences among the cases were 57% and 83%, respectively, and those among controls were [17%] and [20%]. HBV markers were assayed by passive haemagglutination and ELISA.

Lingao (1989) conducted a study in the Philippines of 340 HCC cases (in 288 men and 52 women) individually matched by age and sex to asymptomatic population-based controls from five rural areas. About 90% of the diagnoses of HCC were confirmed histologically. The presence of HBsAg was evaluated by reverse passive haemagglutinin, radioimmunoassay and ELISA only in patients with HBV infection and was significantly higher for HCC patients than for the controls [75% *versus* 14%; $p < 0.0001$; OR, 19 (12–29)]. Among the HBsAg-reactive subjects, a significant association was found between HCC and anti-HBe

seropositivity (73% *versus* 52%; $p < 0.01$) [RR, 2.6] but not with HBeAg; these markers were determined by gel diffusion followed by radioimmunoassay and ELISA. Among 99 cirrhosis patients studied, the HBsAg seroprevalence was 58%. In a preliminary study of 104 histopathologically confirmed HCC cases (in 88 men and 16 women), which probably represented a subset of the larger case group (Lingao *et al.*, 1981), the control group consisted of 84 asymptomatic controls (42 men, 42 women). [No details of subject selection were provided.]

In the study of Yeh *et al.* (1990) in southern Guangxi Autonomous Region, China (see p. 68), 91% of 76 HCC cases were HBsAg seropositive compared with 22% of 304 controls (OR, 39; 95% CI, 16–117).

In a study by Tsukuma *et al.* (1990) in Japan, of 229 (192 men, 37 women) newly diagnosed HCC patients admitted to the Center for Adult Diseases in Osaka between November 1983 and June 1987, 221 (96.5%) were interviewed; 87 cases were histologically confirmed. One control per male case and two per female case (266 in all) were selected from among patients admitted for gastroenterology, people admitted for health check-ups and those admitted for gastroenterological endoscopy. People with liver disease, malignancy, smoking- and alcohol-related disease, and lack of HBsAg testing were excluded. All subjects were interviewed at admission or at the time of endoscopic examination. In the analysis, confounding by sex, age, history of blood transfusion, heavy drinking, cigarette index and family history of liver cancer was controlled by unconditional logistic regression; the OR for HBsAg seropositivity was 14 (95% CI, 5.7–36; $p < 0.0001$). HBsAg was determined by reverse passive haemagglutination; the results were abstracted from medical records.

Lin *et al.* (1991) studied cases of HCC and hospital controls at the Chang-Gung Memorial and Kaohsiung Medical College Hospitals in Taiwan, China, and interviewed them between 20 February 1985 and 20 December 1986. Preliminary results were reported previously (Lu, S.N. *et al.*, 1988). The subjects were 243 hospitalized or out-patient cases of HCC (in 218 men and 25 women) and 302 orthopaedic and ophthalmic in-patient controls (260 men, 42 women). Two controls were matched to each case by age (within three years) and sex, but some subsequently refused to have blood drawn; the authors stated that no significant difference was found between cases and controls with regard to age or sex. Adjustment for age, sex and hepatitis markers yielded significantly increased risks in association with seropositivity for HBsAg (OR, 10; 77% in cases *versus* 19% in controls) and HBeAg (OR, 3.2; 18% *versus* 2%) and significantly decreased risks in association with seropositivity for anti-HBs (OR, 0.1; 28% *versus* 78%) and anti-HBc (OR, 0.1; 97% *versus* 100%). [No information was given about how many subjects refused to have blood drawn or how many cases were histologically confirmed.]

Chen *et al.* (1991) examined 200 male HCC patients from the same two hospitals in Taiwan who were recruited consecutively between September 1985 and July 1987. Healthy community controls were matched individually to cases by age (within three years), ethnic group and residence, using a roster from household registration offices. Seventeen female pairs had also been identified but were excluded owing to their small number. Only two cases and three controls were seronegative for markers of HBV infection; thus, the authors focused on detection of HBsAg and HBeAg. In comparison with those seronegative for both antigens, those only seropositive for HBsAg had a 17-fold higher risk for HCC (95% CI, 7.4–38) and those seropositive for both had an OR of 58 (95% CI, 27–124). [Refusal rates

were not provided. The cases of HCC overlapped with those analysed by Lin *et al.* (1991); the number of histologically confirmed diagnoses was not stated.]

Srivatanakul *et al.* (1991) studied subjects from three hospitals in several areas of northeast Thailand as part of a larger study of liver cancer (Parkin *et al.*, 1991). Sixty-five cases (in 47 men and 18 women) were compared with 65 controls matched by sex, age (within five years), residence and hospital. Controls were either in-patients or clinic patients with nonmalignant, nonhepatic diseases and diseases unrelated to tobacco or alcohol. All subjects were under 75 years, were recruited during 1987–88 and were interviewed in hospital; histological confirmation was obtained for 20 HCC cases. Conditional multivariate analysis, controlling for consumption of alcohol, shrimp paste, powdered peanuts and fresh vegetables and for betel-nut chewing gave a significant OR of 15 (95% CI, 2.3–103) for the relationship between HCC and seropositivity for HBsAg ($p < 0.001$); 42% of the cases were HBV carriers *versus* 8% of the controls. HBV markers were determined by ELISA.

A study in Japan involved 204 patients with HCC (31 were not studied 'for logistic reasons') admitted to Kyushu University Hospital between December 1985 and June 1989 (Tanaka, K. *et al.*, 1988, 1992). The cases were diagnosed within one year of identification, were aged 40–69 and were residents of Fukuoka or Saga Prefecture (168 men, 36 women). The diagnosis of HCC was confirmed by histology in 82 cases. The 410 controls selected were residents of Fukuoka City, had undergone a health examination between January 1986 and July 1989 at a nearby public health centre, did not have chronic liver disease and had had a blood specimen taken; they were frequency-matched by sex and age to the cases (291 men, 119 women). Cases and controls were similar with respect to education and occupation and were interviewed in the hospital wards or at the health centre. The OR for HBsAg seropositivity as a risk factor for HCC was 14 (95% CI, 5.9–33) after adjustment for sex, age, history of blood transfusion, family history of liver disease, alcohol consumption and smoking (19% prevalence in cases *versus* 2% in controls). Case sera were tested by radioimmunoassay or reverse passive haemagglutination and control sera by the latter method.

In Kaohsiung Medical College Hospital, Taiwan, China, Chuang *et al.* (1992) studied 128 histologically or cytologically confirmed cases of HCC (in 112 men and 16 women) and 384 community controls (336 men, 48 women) matched for age (within five years) and sex; no significant difference was found in age and sex distributions. HBsAg was detected in 77% of the cases, which was significantly higher than in controls (28%) ($p < 0.001$). Using these data, Leandro and Duca (1993) calculated an OR of 14 for HBsAg seropositivity (95% CI, 7.8–25).

A case–control study of HCC was carried out in Hanoi, Viet Nam, between 1989 and 1992 (Cordier *et al.*, 1993). A total of 152 male cases were recruited from two hospitals and frequency-matched on sex, age, hospital and residence to 241 controls admitted to the abdominal surgical departments of the same hospitals. HBsAg status was investigated using a second-generation ELISA test. One hundred and thirty-eight (93%) cases and 44 (18%) controls were seropositive for HBsAg (OR, 62; 95% CI, 30–128). The effect of alcohol consumption was significant only among HBsAg-seronegative individuals.

(*d*) *Europe*

Trichopoulos *et al.* (1978) reported the findings of a study of 80 HCC patients (69 men, 11 women; 47 histologically confirmed cases) admitted to one of eight large hospitals in

Athens, Greece, between April 1976 and June 1977. Two control patients were matched by sex and age (within five years) to each case, who had diagnoses exclusive of neoplasm and liver disease. After Mantel–Haenszel adjustment for age and sex, the OR for an association between HCC and active HBV infection (HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity) was 10 ($p < 0.001$) by comparison with people with no evidence of active infection. No relationship with any antigen was observed in 40 metastatic liver cancer patients (OR, 1.2). In addition, the presence only of anti-HBs did not confer a greater risk for HCC than the absence of HBV markers (OR, 0.8; 95% CI, 0.3–2.1). Among the HCC cases, the prevalence of active HBV infection was significantly higher in the 45 with cirrhosis (67%) than in the 35 without cirrhosis (26%) ($p < 0.001$).

The prevalence of active HBV infection (HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity) was determined in 34 cases of HCC (22 with cirrhosis) and 100 healthy general population controls of similar age and sex in Barcelona, Spain (Pedreira *et al.*, 1980). Eighteen cases were verified by biopsy. A strong association was observed, with 52% of cases and 5% of controls having this HBV status [OR, 21; $p < 0.05$]. The prevalence was somewhat higher for HCC cases without cirrhotic liver (58%) than for those with (50%); 38% of 139 cirrhotic patients (47 alcoholics, 92 not) had active HBV infection. HBsAg was determined by reverse passive haemagglutination. [No details of subject selection methods were given.]

Goudeau *et al.* (1981) reported the prevalence of HBV markers in 46 histologically confirmed cases of HCC (in 39 men and seven women) and in 10 000 blood donors in Tours, France. All subjects were Caucasian. Significant differences were found for HBsAg seroprevalence (4% *versus* 0.5%; $p < 0.01$) and the seroprevalence of anti-HBc alone (15% *versus* 2%; $p < 10^{-6}$). [No information was given on the timing and method of subject selection.]

De Franchis *et al.* (1982) studied 42 subjects with HCC (33 men, nine women) and two groups of controls matched for age (within five years) and sex, comprising 42 patients with chronic liver disease and 84 patients with diagnoses other than neoplasm or liver disease. Subjects were identified from 1974 at the University Hospital in Milan, Italy, and histological examination was used to diagnose HCC. The cases were significantly different from the other hospital controls with respect to all HBV markers analysed, the greatest differences ($p < 0.0005$) being for the presence of HBsAg (36% *versus* 2%) [OR, 23], of anti-HBc (95% *versus* 51%) [OR, 19], of HBeAg (19% *versus* 0%) and of active infection (seropositivity for HBsAg or a high titre of anti-HBc alone; 44% *versus* 2%) [OR, 32]. The only significant associations ($p < 0.05$) for HCC cases in the comparison with chronic liver disease controls were for HBsAg seropositivity (12% of controls) [OR, 4.1], anti-HBs seropositivity (43%) [OR, 0.14] and active infection (17%) [OR, 3.9]. The direction of the associations between HCC and markers of HBV infection was the same in comparison with both control groups. [No information was given about the timing of subject selection.]

In a multicentre case–control study in Italy (Pagliaro *et al.*, 1982), a significant OR of 14 (1.4–∞) was reported for HBsAg seropositivity in relation to HCC (80% confirmed histologically) in a matched analysis of 50 case–control pairs (37 men, 13 women). Consecutive prevalent cases were collected from 23 hospitals and university medical departments from December 1974 through December 1976; controls were diagnosed with non-surgical

diseases other than liver disease. Matching was by sex, age (within five years), hospital and admission date (within six months). HBsAg seropositivity was assayed by the same method for each case–control pair, by radioimmunoassay, counter electroimmunophoresis or reverse passive haemagglutination.

A study from 17 centres in Italy of patients newly diagnosed with HCC during 1979–80 (Pagliaro *et al.*, 1983) comprised 286 HCC cases with cirrhosis (in 250 men and 36 women), who were compared with 3629 patients with cirrhosis (2340 men, 1289 women), and 64 HCC cases without cirrhosis (in 52 men and 12 women), who were compared with 1545 patients with chronic, non-hepatic disease (1038 men, 507 women). The latter control group represented a random sample of non-surgical disease. Among the cirrhotic subjects, cases were significantly different from controls for seropositivity for all HBV markers except HBeAg and anti-HBe: HBsAg, 30% *versus* 17% ($p < 0.0005$); anti-HBs, 26% *versus* 37% ($p < 0.005$); anti-HBc alone, 26% *versus* 16% ($p < 0.005$). Among those without liver cirrhosis, a significant difference was reported for seropositivity for HBsAg (33% *versus* 2%; $p < 0.0005$). HBsAg was detected by radioimmunoassay or ELISA; anti-HBs and anti-HBc were assayed by radioimmunoassay in a subset of the sample that was similar to the whole group with respect to sex, age and HBsAg reactivity. [The data were incompletely reported.]

In London, United Kingdom, 27 consecutive HCC patients (20 men, seven women) who had undergone liver biopsy between 1979 and 1981 were compared with 112 hospital in-patient and staff controls (60 men, 52 women) (Bassendine *et al.*, 1983); the subjects were Caucasian. The cases had significantly higher frequencies than the controls of HBsAg (15%; controls, 0.9%; $p < 0.005$), anti-HBc (48%; controls, 11%; $p < 0.005$) and anti-HBe (26%; controls, 7%; $p < 0.001$). [Details about the control selection process were not available.]

An association with HBsAg seropositivity was reported by Pirovino *et al.* (1983) in Switzerland: 31% in 65 HCC cases and 17% in 115 liver cirrhosis controls ($p < 0.05$) [RR, 2.3]. The cases were a subset of 75 histologically confirmed HCC cases diagnosed between 1975 and 1982 at the City Hospital Waid in Zurich on whom HBV testing was done. [Although all of the controls had cirrhosis, it is not known what proportion of cases did.]

Filippazzo *et al.* (1985), in Palermo, Italy, enrolled 120 consecutive in-patients with HCC (99 men, 21 women) between December 1980 and December 1983 and three controls from the same hospital matched for sex and age (within five years), who had either cirrhosis, solid tumour or chronic non-neoplastic disease. Biopsy or laparoscopy was used to verify the diagnosis in 62 cases of HCC. The difference in HBsAg prevalence between cases (17%) and controls was greater for the two non-cirrhotic control groups (2–3%) than for the cirrhotic controls (15%). [The prevalences reported in the paper did not correspond exactly to those calculable from the data given. No information was provided on how HBsAg status was determined.]

Colloredo Mels *et al.* (1986) conducted a study in Bergamo, Italy, which included 72 histologically confirmed HCC cases (in 60 men and 12 women), 57 of whom also had cirrhosis. Cases were identified between January 1980 and December 1984, as were two control groups from the same hospital: 199 without liver disease (159 men, 40 women) and 156 with liver cirrhosis (114 men, 42 women). The OR for the relationship between seropositivity for HBsAg and HCC was 11.5 among the non-cirrhotic patients ($p < 0.001$) and 1.0 among the cirrhotic patients: 47% of the HCC cases without cirrhosis, 7% of their

controls and 28% of those with cirrhosis and 26% of their controls were seropositive for HBsAg. The authors calculated an overall OR of 4.6 on the basis of an HBsAg seroprevalence of 9.1% in the general population. Among the subset of HBsAg-seronegative subjects assayed, no significant difference was found for the prevalence of past HBV infection in either comparison. [The sample sizes were small, and the means of subject selection could not be determined.]

A case–control study in Greece (Trichopoulos *et al.*, 1987) involved 194 cases of HCC (in 81 cirrhotic subjects out of 173 men and 21 women) and 456 in-patient controls (400 men, 56 women). Cases admitted to eight hospitals in Athens between April 1976 and October 1984 were interviewed in hospital; 113 were confirmed by histology. Controls with diagnoses other than neoplasm or liver disease were selected from the same hospitals (as well as the hospital for accidents and orthopaedic disorders) and were also interviewed in hospital. All subjects were Caucasians of Greek nationality and were comparable with respect to education and birthplace. Data on about one-third of these subjects were analysed previously (see above: Trichopoulos *et al.*, 1978; 1980a), but all assays were repeated for this study. Multiple logistic regression was used to control for age, sex and anti-HCV status (Kaklamani *et al.*, 1991). A significant, 11-fold association was observed between HBsAg seropositivity (46% in cases; 7% in controls) and HCC on the basis of 185 cases (in 166 men and 19 women; 108 confirmed by histology) and 432 controls (381 men, 51 women). This estimate was slightly lower than the earlier one, in which HCV infection was not controlled for (OR, 14; 95% CI, 8.0–24); the association with HBsAg seropositivity was stronger in comparison with subjects with no HBV marker (OR, 19; 95% CI, 10–38) (Trichopoulos *et al.*, 1987). An additional analysis of these cases according to the presence of cirrhosis (Tzonou *et al.*, 1991) revealed a stronger association with HBsAg seropositivity among 78 cases with cirrhosis (65% positive; OR, 33) than those 107 without (32% positive; OR, 6.7), after control for age, sex, anti-HCV seropositivity and smoking.

Vall Mayans *et al.* (1990) investigated 96 cases of HCC (in 67 men and 29 women; 83 cases with cirrhosis) admitted to the University Hospital in Barcelona, Spain, between October 1986 and March 1988; 74 cases were confirmed histologically or cytologically. Two controls were chosen for each case from the same hospital and matched for sex and age (within five years), within one month after identification of the case; controls with diagnoses related to the risk factors of interest (HBV infection, alcohol consumption, smoking and oral contraceptive use) were not eligible, leaving 199 controls for analysis. Cases and controls were Caucasian and had comparable histories of occupation and blood transfusion. All interviewing took place in hospital. A significant, nearly five-fold association was observed for HBsAg seropositivity after adjustment for age and sex (OR, 4.9, exact 95% CI, 1.3–22); the seroprevalence of HBsAg was low (9% in cases, 2% in controls). The OR for anti-HBc seropositivity (2.3; 95% CI, 1.3–3.9) was also significant, 50% of cases and 31% of controls being seropositive. The authors reported that adjustment for alcohol drinking did not change the association with HBV infection.

Leandro *et al.* (1990) studied 457 patients with liver cirrhosis in Italy: 140 (117 men, 23 women) had confirmed cases of HCC, and 317 without HCC (209 men, 108 women) were used as controls. HCC patients were diagnosed in 1980–88 at a hospital in either Bari or Bergamo; the controls were admitted to the same centres between 1 January 1984 and

3 December 1985. After control for age and sex by logistic regression, the association with HBsAg seropositivity was significant ($p < 0.05$), with an OR of 2.3 [similar to the crude estimated OR of 1.8 (95% CI, 1.1–3.1)]. [The ORs are related to the probability of developing HCC given the presence of pre-existing cirrhosis.]

A study was carried out in four cities in Italy (Stroffolini *et al.*, 1992) to investigate HBsAg seropositivity in 65 incident cases of HCC with underlying cirrhosis (in 47 men and 18 women) admitted to four teaching hospitals during 1990. Patients with chronic nonhepatic disease, matched for age (within five years) and sex and admitted consecutively to the same hospitals in the same year were selected as controls (75 men, 23 women). Multiple logistic regression methods were used to control for age, sex, anti-HCV status and HBV markers. A significant OR of 12 (95% CI, 3.1–41) was found for the association between HBsAg reactivity and HCC; 25% of cases and 6% of controls were seropositive for the antigen. HBV markers were determined by ELISA. [Not all cases were confirmed histologically.]

(*e*) *International collaborative studies*

Not included in Table 6 are the results of two large collaborative studies. In one, patients with liver disease (including HCC) were compared with healthy subjects in Burma, China, Hong Kong, India, Indonesia, Japan, Kenya, Papua New Guinea, the Philippines and Thailand (Nishioka *et al.*, 1975). HBsAg was determined by immune adherence haemagglutination and anti-HBs by passive haemagglutination. The seroprevalence of HBsAg ranged from 33 to 80% among HCC patients and from 3 to 18% among healthy controls; that for anti-HBs was 0–26% among HCC patients and 12–43% among controls. In each country, HBsAg seropositivity was always higher and anti-HBs lower among HCC cases than among controls.

In the other collaborative study, data on blacks in Senegal, Burundi and Mali were combined (Coursaget *et al.*, 1984, 1985) to give a total of 453 HCC patients, 221 cirrhotic patients and 7051 adult controls. HBsAg seropositivity was 58–65% among HCC cases, 4–17% among controls and 63% among cirrhotic patients. HBeAg was detected in 25% of cases and 13–19% of controls, and anti-HBe was detected in 60% of cases and 75% of controls. An analysis of HBsAg-seropositive Senegalese subjects (Coursaget *et al.*, 1986a) revealed an OR of 6.2 (95% CI, 4.1–9.6) for HCC; HBsAg/IgM complexes were detected in 14% of controls, 40% of cirrhotics and 50% of HCC cases. [No details were provided about subject selection.]

Some studies have addressed immunohistochemical identification of HBV antigens in liver tissues and detection of HBV DNA in serum or liver tissue. Their results are important in elucidating pathogenetic mechanisms but cannot provide directly interpretable estimates of effect parameters like the OR, since it is inherently difficult to assess the suitability of the comparison groups. These studies provide strong support for the hypothesis that HBV is an important factor in the etiology of HCC (Nayak *et al.*, 1977; Tan *et al.*, 1977; Turbitt *et al.*, 1977; Omata *et al.*, 1979; Bréchot *et al.*, 1981a, 1985; Röckelein & Hecken-Emmel, 1988; Sjøgren *et al.*, 1988; Guan *et al.*, 1989).

Table 6. Summary of results of case–control studies of hepatocellular carcinoma and presence *versus* absence of hepatitis B surface antigen (HBsAg)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Africa								
Prince *et al.* (1970); Uganda	Sex unspecified	4	12	6	2	[6.8]	[1.8–25]	Blood donor controls
Vogel *et al.* (1972); Uganda	Women and men	90	40	224	3	[19]	[7.6–45]	Adjusted for age and sex; testing by CF, CEP and PHA
Kew *et al.* (1974); South Africa	Men	75	40	18 377	7	[8.7]	[5.3–14]	Mineworkers; testing by CEP and CF
Michon *et al.* (1975); Prince *et al.* (1975); Senegal	Women and men	165	61	154	12	[11]	[5.8–19]	Adjusted for age
	Controls with other cancer							
	Controls without cancer	165	61	328	11	[14]	[8.7–24]	
Larouzé *et al.* (1976); Senegal	Women and men	28	79	28	57	[2.8]	[0.74–10]	
Tsega *et al.* (1976); Tsega (1977); Ethiopia	Women and men	46	50	90	7	[14]	[4.6–44]	
Tabor *et al.* (1977)	Women and men							
Uganda		47	47	50	6	[14]	[3.8–51]	
Zambia		19	63	40	8	[21]	[4.7–96]	
USA		27	30	6726	0.02	[134]	[53–337]	
Reys *et al.* (1977); Mozambique	Women and men	32	60	231	9	[15]	[5.9–37]	Male controls; solid-phase RIA + CEP
Van Den Heever *et al.* (1978); South Africa	Women and men	92	34	92	9	[5.3]	[2.2–14]	Blacks
Kew *et al.* (1979) South Africa	Women and men	289	62	213	11	[13]	[7.6–21]	Blacks; solid-phase RIA
Bowry and Shah (1980); Kenya	Women and men	76	51	33	6	[16]	[3.4–106]	Testing by PHA

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Africa (contd)								
Coursaget *et al.* (1981); Senegal	Women and men	134	63	100	12	[12]	[5.9–26]	
	Blood donor controls							
	Rural controls	134	63	833	14	[11]	[6.9–16]	
	Leprosy patient controls	134	63	560	25	[5.0]	[3.3–7.5]	
Gombe (1984); Congo	Women and men	65	74	120	9	[32]	[11–87]	Adjusted for sex; testing by RIA or PHA
	Blood donor controls							
	Other cancer controls	65	74	71	3	[55]	[12–256]	Adjusted for sex
Sebti (1984); Morocco		46	17	379	5	[4.2]	[1.6–11]	
Kew *et al.* (1986a); South Africa	Women and men	62	40	62	3	[20]	[4.3–132]	Blacks
Otu (1987); Nigeria	Women and men	200	49	400	7.5	[12]	[7.3–19]	
Gashau & Mohammed (1991); Nigeria	Women and men	65	65	69	36	[3.2]	[1.5–7.0]	Testing by ELISA and reverse PHA
Mohamed *et al.* (1992); South Africa	Men	77	35	77	5	7.5	2.2–25	Adjusted for alcohol intake and smoking; blacks
	Women	24	25	24	4	12	1.0–154	
Tswana & Moyo (1992); Zimbabwe	Women and men	182	56	100	11	[10]	[5.0–22]	Testing by ELISA
Ryder *et al.* (1992); Gambia	Women and men	70	63	70	21	6.9		Adjusted for age; $p < 0.01$; 64 cases, 67 controls tested
Kashala *et al.* (1992); Zaire	Women and men	40	57.6	68	7.4	[17]	[5.7–51]	Testing by immunoperoxidase

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Americas								
Yarrish *et al.* (1980); USA	Women and men	34	15	38	0			$p < 0.05$; control sera stored 4 months less than case sera
	Controls with colon cancer							
	Controls with lung cancer	34	15	45	0			$p < 0.05$; control sera stored 17 months less than case sera
	Blood donor controls	34	15	56	0			$p < 0.02$; control sera stored 39 months longer than case sera
Nomura *et al.* (1982); Hawaii, USA	Men	16	63	48	0			$p < 0.0001$; subjects of Japanese ancestry
Austin *et al.* (1986); USA	Women and men	67	18	63	0	-	3.8–∞	$p = 0.0002$
Yu *et al.* (1990); USA	Women and men	51	10	128	0	-	3.8–∞	Adjusted for age and sex
Di Bisceglie *et al.* (1991); USA	Women and men	99	7	98	0			$p = 0.009$
Asia								
Tong *et al.* (1971); China	Women and men	55	80	943	15	[23]	[11–49]	Male controls; testing by modified ID
Simons *et al.* (1972); Singapore	Women and men	156	35	1776	7	[7.6]	[5.1–11]	Controls were male blood donors and female antenatal clinic attendees; testing by immune adherence HA
	Normal controls							
	Suspected cancer controls	156	35	207	6	[8.9]	[4.4–18]	
Lee (1975); Hong Kong	Women and men	100	49	120	9	[9.5]	[4.4–21]	Testing by immunoelectroosmophoresis and CF
Chainuvati *et al.* (1975); Thailand	Women and men	49	16	87	2	[8.3]	[1.5–59]	Testing by cross-over immunoelectrophoresis
	Cases with cirrhosis							
	Cases without cirrhosis	8	0	87	2	Not significant		

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Asia (contd)								
Kubo *et al.* (1977); Japan	Women and men	124	46	299	4	[20]	[9.9–43]	RIA + reverse PHA + CEP
Chen & Sung (1978); China	Women and men	127	83	729	15	[28]	[17–46]	Testing by reverse PHA [$p < 0.05$]
Chien *et al.* (1981); China	Women and men	102	71	100	12	[18]	[8.0–40]	
Lam *et al.* (1982); Hong Kong	Women and men	107	82	107	18	21	10–46	Adjusted for age and sex
Chung *et al.* (1983); Sjøgren *et al.* (1984); Republic of Korea	Women and men	110	82	63	14	[27]	[11–70]	Solid-phase RIA
Inaba *et al.* (1984); Japan	Women and men	62	36	62	3	10		Matched-pairs analysis; $p < 0.01$; 59 controls tested; testing by reverse PHA
Yeh *et al.* (1985a,b); Luo *et al.* (1988); China	Women and men	50	86	49	22	17	4.3–99	Matched analysis
Arya *et al.* (1988); Saudi Arabia	Women and men	30	60	326	12	[11]	[4.6–27]	ELISA
Lu, C.Q. *et al.* (1988); China	NR	30	57	60	17	5		Matched analysis; $p < 0.001$
Lingao (1989); Philippines	Women and men HBV-infected subjects	329	75	238	14	[19]	[12–29]	Adjusted for sex
Yeh *et al.* (1989); China	Men	76	91	304	22	39	16–117	Matched analysis

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Asia (contd)								
Tsukuma *et al.* (1990); Japan	Women and men	229	19	266	2	14	5.7–36	Adjusted for sex, age, history of blood transfusion, heavy drinking, cigarette index and family history of liver cancer; testing by reverse PHA
Lin *et al.* (1991); China	Women and men	243	77	302	19	10		Adjusted for age, sex and other hepatitis markers; $p < 0.05$
Chen *et al.* (1991); China	Men	200	20	200	2	58	27–124	Matched-pair analysis; HBsAg(+) and HBeAg(+)
		200	64	200	19	17	7.4–38	Matched-pair analysis; HBsAg(+) and HBeAg(–)
Srivatanakul *et al.* (1991); Thailand	Women and men	65	42	65	8	15	2.3–103	Adjusted for alcohol, shrimp paste, powdered peanut and fresh vegetable consumption, betel-nut chewing, by conditional multivariate regression; ELISA
Tanaka *et al.* (1992); Japan	Women and men	204	19	410	2	14	5.9–33	Adjusted for sex, age, history of blood transfusion, family history of liver disease, alcohol consumption and smoking amount; testing by RIA or reverse PHA
Chuang *et al.* (1992); China	Women and men	128	77	384	28	[9.9]	[5.9–17]	Adjusted for anti-HCV status
Cordier *et al.* (1993); Viet Nam	Men	152	93	241	18	62	30–128	Testing by second-generation ELISA

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Europe								
Trichopoulos *et al.* (1978); Greece	Women and men	80	49	160	8	10	5.2–21	Adjusted for age and sex; $p < 0.001$; HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity
Pedreira *et al.* (1980); Spain	Women and men	34	52	100	5	[21]	[7–66]	Testing by reverse PHA; $p < 0.005$; HBsAg seropositivity or anti-HBc seropositivity and anti-HBs seronegativity
Goudeau *et al.* (1981); France	Women and men	46	4	10 000	0.5	[10]		$p < 0.01$
De Franchis *et al.* (1982); Italy	Women and men	42	36	42	12	[4.1]	[1.2–15]	
	Controls with chronic liver disease							
	Other hospital controls	42	36	84	2	[23]	[4.5–155]	
Pagliaro *et al.* (1982); Italy	Women and men	50	NR	50	NR	14	1.4–∞	Matched analysis; testing by RIA, CEP or reverse PHA
Pagliaro *et al.* (1983); Italy	Women and men	286	30	3629	17	[2.1]		$p < 0.0005$; testing by RIA or ELISA
	Subjects with cirrhosis							
	Subjects without cirrhosis	64	33	1545	2	[24]		$p < 0.0005$
Bassendine *et al.* (1983); UK	Women and men	27	15	112	0.9	[19]	[1.9–476]	
Pirovino *et al.* (1983); Switzerland	Women and men	65	31	115	17	[2.3]	[1.0–4.9]	
Filipazzo *et al.* (1985); Italy	Women and men	120	18	120	14	[1.4]	[0.68–2.9]	Adjusted for age
	Controls with cirrhosis							
	Controls with solid tumour	120	18	120	3	[6.8]	[2.2–20]	Adjusted for age
	Controls with chronic disease	120	18	120	4	[5.3]	[1.9–15]	Adjusted for age

Table 6 (contd)

Reference and location	Subjects	Seroprevalence of HBsAg				OR	95% CI	Comments[a]
		Cases		Controls				
		No.	%	No.	%			
Europe (contd)								
Colloredo Mels *et al.* (1986); Italy	Women and men	15	47	199	7	12		$p < 0.001$
	Subjects without cirrhosis							
	Subjects with cirrhosis	57	28	156	26	1		NS
Kaklamani *et al.* (1991); Greece	Women and men	185	46	432	7	11	6.7–19	Adjusted for age, gender and anti-HCV status
Trichopoulos *et al.* (1987); Tzonou *et al.* (1991); Greece	Cases with cirrhosis	78	65 of 81	432	7	33	15–70	Adjusted for age, gender, anti-HCV status and smoking (percentages calculated from the data of Trichopoulos *et al.*; no. with cirrhosis from Tzonou *et al.*)
	Cases without cirrhosis	107	32 of 113	432	7	6.7	3.6–12	Adjusted for age, gender, anti-HCV status and smoking
Vall Mayans *et al.* (1990); Spain	Women and men	96	9	190	2	4.9	1.3–22	Adjusted for age and sex
Leandro *et al.* (1990); Italy	Women and men	140	23	317	14	2.3		Adjusted for age and sex; $p < 0.05$
Stroffolini *et al.* (1992); Italy	Women and men	65	25	98	6	11	3.1–41	Adjusted for age, gender, anti-HCV and HBV markers; testing by ELISA

OR, odds ratio; CI, confidence interval; NR, not reported. The estimates in square brackets were calculated by the Working Group and are unadjusted unless otherwise indicated in the comments.

[a]Serological testing for HBV markers was by radioimmunoassay (RIA), unless otherwise specified. ID, immunodiffusion; HA, haemagglutination; CF, complement fixation; CEP, counter immunoelectrophoresis; PHA, passive HA, ELISA, enzyme-linked immunosorbent assay; NS, not significant; HCV, hepatitis C virus; HBeAg, hepatitis B envelope antigen

(f) Factors that modify the risk for HCC associated with HBV

Male HBsAg carriers are more likely to develop HCC than female carriers (Anthony, 1984; Coursaget *et al.*, 1987), and there is some evidence that establishment of the carrier state prenatally or early in life is associated with a higher OR for HCC than establishment of a similar state in adulthood (Larouzé *et al.*, 1976; London, 1981; Hsieh *et al.*, 1992).

Several factors other than HBV have been evaluated as causally associated with HCC. In particular, aflatoxins, drinking of alcoholic beverages and oral contraceptives have been determined to be human carcinogens (IARC, 1987, 1988, 1993). Whether these factors modify the effect of HBV in the causation of HCC is inconclusive. The modifying effect of concurrent infection with HCV on the action of HBV is discussed in the monograph on HCV (p. 165).

2.4.2 *Cholangiocarcinoma*

Two case–control studies found no association between HBsAg seropositivity and the occurrence of cholangiocarcinoma. Parkin *et al.* (1991) conducted a case–control study in north-east Thailand involving 103 cases and 103 hospital controls matched for sex, age and hospital; patients with tobacco- and alcohol-related disease and other liver disease were excluded. No association was found with HBsAg seropositivity (OR, 1.0; 95% CI, 0.4–2.7). In Taiwan, China, Chen and Sung (1978) reported that of seven cases one (14%) was HBsAg seropositive, giving a similar rate to that seen among 729 controls (15%).

2.4.3 *Other cancers*

The relationship between HBsAg seroprevalence (as measured by reverse passive haemagglutination) and the occurrence of oral and uterine cervical cancer was examined in one study (Vijayakumar *et al.*, 1984). The subjects analysed were 350 oral cancer patients (232 men, 118 women), 150 cervical carcinoma patients and 100 healthy controls (50 men, 50 women); all were 40–60 years old and had no history of jaundice. Significant differences ($p < 0.001$) were found for all sex-specific comparisons between cases and controls: the seroprevalence of HBsAg was 11% in male and 12% in female oral cancer cases, 13% in cervical cancer cases and 4% in both male and female controls. [No data were provided on social class, nor was it clear whether the carrier state preceded treatment of the disease.]

3. Studies of Cancer in Experimental Animals

3.1 Primates

3.1.1 *Infection with HBV*

(a) Chimpanzee

Chimpanzees (*Pan troglodytes*) have been used for many years to test for the presence of pathogens in biological products derived from human serum. Chimpanzees inoculated with HBV (Barker *et al.*, 1975) or cloned HBV DNA (Sureau *et al.*, 1988) express HBV antigens in

liver and blood and can develop a carrier state. Chimpanzees chronically infected with HBV can develop a mild chronic hepatitis resembling chronic persistent hepatitis in human patients infected with HBV. The extent of inflammation in chronically infected chimpanzees appears to be milder than that seen in human patients, and chronic active hepatitis (Shouval *et al.*, 1980) and cirrhosis have apparently not been reported in HBV-infected chimpanzees. HCC has not been seen in chimpanzees infected with HBV, except in one brief report of the occurrence of a liver tumour in a 15-year-old male (Muchmore *et al.*, 1990). This animal had been under surveillance since 1978 after developing seropositivity for anti-HBs and anti-HBc (HBsAg seronegativity) two years after inoculation of human serum thought to be infectious for non-A, non-B hepatitis. The chimpanzee's serum did not transmit non-A, non-B hepatitis to another susceptible animal [details not presented]. HCC was found 10 years later during investigation of liver disease associated with elevated serum alanine aminotransferase and gamma glutamyl transpeptidase. The HCC was composed of neoplastic hepatocytes arranged in trabeculae and plates, and the surrounding non-neoplastic liver was infiltrated by amyloid. The authors reported that hybridization showed free HBV genomes in the liver but no HBV sequences in tumour cells [data not presented]. [Limited details were reported, and there was limited evidence that HBV was causally involved.]

[No other report of HCC developing in chimpanzees with HBV in serum was available to the Working Group. The Group noted the limited reporting of studies on chimpanzees observed for many years after infection with HBV. Little published evidence is available to suggest that HBV-infected chimpanzees develop progressive liver disease.]

(*b*) *Monkey*

Five monkeys (three male and one female rhesus and one female cynomolgus), ranging in age from less than one month to 19 months, were inoculated intravenously with a single dose of 2 ml of a pool of five human sera each containing HBsAg titres ranging from 1:640 to 1:2560. Between 22 and 26 months later, three monkeys (two male and one female rhesus) were given a second inoculation of a single human serum with a complement fixing titre of 1:1280, containing 'abundant HBV particles'; all animals were killed three years after the first inoculation. Another group of 12 monkeys (six rhesus and six cynomolgus) [sex and age unspecified] served as uninoculated controls. All monkeys were HBV seronegative before initiation of the study, and all survived up to three years. None of the monkeys was seropositive for HBsAg three to four weeks after inoculation, but HBV core particles were occasionally observed in hepatocytes by electron microscopy. Gross and histological examination of the animals at the end of the study showed no tumour in the livers of those inoculated with HBV, but there was mild persistent hepatitis in the livers of three monkeys. No liver tumour was observed in uninoculated controls (Gyorkey *et al.*, 1977). [There was no evidence that the monkeys were infected with HBV, and the observation period after the second inoculation was brief.]

Seven of 10 monkeys (*Macaca assamensis*; nine males and one female, 6–12 months of age) were inoculated with serum from patients seropositive for HBsAg, anti-HBs, anti-HBc, anti-HBe, HBV DNA or Dane particles [viral titres not stated], and three served as controls. Liver biopsy samples were taken to establish histopathological evidence of hepatitis and hepatic neoplasia and were analysed for the presence of HBsAg, HBcAg, HBeAg, anti-HBs,

anti-HBc, anti-HBe and HBV DNA and also for alanine aminotransferase four to six times over a period of 2.5 years. Alanine aminotransferase levels were elevated after inoculation and were still persistently high in five of seven animals 137 weeks after inoculation. Two animals died of other causes during the course of the study. HBsAg, anti-HBs, anti-HBc and HBV DNA were found in sera of all seven treated monkeys. Histopathological changes consistent with hepatitis, including hepatocellular degeneration, necrosis, inflammatory cell infiltration, bile-duct proliferation and fibroplasia, were seen. Liver lesions in some animals progressed to cirrhosis, and one of them developed a mucin-producing, well-differentiated tumour described as an HCC (Ge *et al.*, 1991). [The reporting was limited, and the statement that the HCC secreted mucin and arose from bile ducts was noted.]

3.1.2 *Infection with HBV with concomitant administration of chemical carcinogens*

Monkey

In a study described in section 3.1.1 (Gyorkey *et al.*, 1977), three groups of animals were used. Nine monkeys in group 1 (four male and two female rhesus, one male and two female cynomolgus), ranging in age from less than one to 20 months, were given intraperitoneal injections of 20 mg/kg bw *N*-nitrosodiethylamine (NDEA) [vehicle unspecified] twice a week for two years. Six of the monkeys (two male and one female rhesus, one male and two female cynomolgus) were given a single intravenous injection of 2 ml of serum containing HBV (see section 3.1.1) 25–33 months after the NDEA injections were started, and three were killed three to six months after inoculation and the remaining three 11 months after inoculation. The three animals that were not inoculated were killed three years after the start of the experiment. A second group of 11 monkeys ranging in age from one to 20 months (four male and five female rhesus, one male and one female cynomolgus) were given a single intravenous injection of 2 ml of the pooled HBV serum (see section 3.1.1), followed one month later by the same NDEA treatment as animals in group 1. Five of the 11 monkeys in group 2 (three male and two female rhesus) received a second inoculation of the individual HBV serum (see section 3.1.1) approximately two years after the initial inoculation and were killed one year later. Animals in group 3 (seven monkeys aged 1–21 months: one male and three female rhesus, two male and one female cynomolgus) were given intraperitoneal injections of 20 mg/kg bw NDEA twice a week for two weeks. One month after the first injection of NDEA, each monkey received a single intravenous injection of 2 ml of the pooled HBV serum, followed two weeks later by re-institution of the twice-weekly NDEA treatment, which was continued for two years. Three of the seven animals received a further inoculation with the individual HBV serum about 21 months after the first HBV inoculation. All surviving animals were killed three years after the start of the experiment. The livers of all animals in group 1 had large invasive HCCs with central haemorrhagic necrosis; some animals also had cirrhosis. Six had metastases to the lung. The incidence and time of onset of liver tumours in monkeys given injections of NDEA in combination with HBV were not different from those in animals that received NDEA alone. All monkeys in group 2 that survived to the end of the experiment developed cirrhosis and invasive multifocal HCC; six developed metastases to the lung. Reinoculation with HBV of monkeys in group 3 failed to affect tumour outcome. [No evidence of viral infection was observed, and the study was

inadequately designed to allow demonstration of an enhancing effect of HBV on hepatocarcinogenicity.]

3.2 Transgenic mice

3.2.1 *With no concomitant administration of chemical carcinogens*

The expression of various HBV gene sequences in livers of transgenic mice has been examined in several studies (for reviews, see Chisari, 1991; Slagle *et al.*, 1992). Only those transgenic models in which hepatic neoplasia was the end-point are included in this section (for a discussion of gene expression and mechanisms of tumour induction, see also section 4.3.2).

Male mice of three transgenic lineages producing the HBV large surface antigen were back-crossed with normal C57Bl/6J females (see Table 7). Mice from the first generation (36 animals from lineage 50-4, 12 from lineage 45-2, eight from lineage 45-3 and four non-transgenic controls) were observed for two years for the development of liver injury (as indicated by increased levels of serum glutamic and pyruvic transaminases) and neoplasms (determined by abdominal palpation and serum levels of α-fetoprotein). Variable expression of large surface antigen (as measured by western blot of total liver protein) was correlated with lineage, being highest in lineage 50-4, medium in lineage 45-2 and lowest in lineage 45-3. Lineages that expressed the highest levels of large surface antigen and filamentous protein in hepatocytes had the highest level of liver injury, and liver tumours developed in animals that had hepatocellular injury. Tumours were first detectable in lineages 50-4 and 45-2 at 9–12 months after the onset of injury, and virtually all mice of the 50-4 lineage with pre-existing chronic liver-cell injury developed HCC by 18–21 months of age. One or two large tumours (1.0–2.0 cm) usually predominated, and numerous smaller tumours were scattered throughout the livers. Thirty-eight animals (25 males and 13 females) [lineage unspecified] with palpable abdominal masses were examined histologically at necropsy. Males developed more palpable liver tumours and displayed more HCCs (18/25) than did females (4/13). Adenomas predominated (5/7) in younger males (10–15 months) and carcinomas (13/18) in older males (16–21 months). All tumours occurred concurrently with hepatocellular injury, characterized by ground-glass hepatocytes, necrosis and inflammation. Neither metastases nor fibrosis or cirrhosis were observed (Chisari *et al.*, 1989). [The Working Group noted that the terms 'hepatoma' and 'hepatocellular carcinoma' appear to have been used interchangeably.]

A group of 59 male and female transgenic mice (lineage 50-4; see Table 7) were examined for abdominal masses every four months for 24 months. Selected animals were killed at monthly intervals from 1 to 23 months, and nine control nontransgenic animals were killed at 3, 11, 18 and 24 months [exact number of animals killed at each time point not specified]. Liver sections were examined histologically for the presence of hepatocellular adenomas and carcinomas. Livers of nontransgenic mice were normal histologically at all time points. Starting at two months, mice progressively developed liver injury and inflammation, including hepatocellular necrosis, Kupffer-cell hyperplasia and mononuclear-cell infiltration, with concurrent preneoplastic lesions which appeared by seven months of age. Preneoplastic lesions consisted of hepatocellular dysplasia and foci of altered

hepatocytes, which progressively developed into larger compressive nodular masses. Seventy-five adenomas, characterized by masses of neoplastic hepatocytes which compressed adjacent parenchyma, occurred in 18 mice from eight months and peaked in incidence around the 17th month of the study. HCC (29 in all) had occurred in all surviving transgenic mice by 20 months of age (Dunsford *et al.*, 1990).

Table 7. Transgenic mice that express hepatitis B surface antigen as the major product

Founder strain	Crossing strain	Lineage	Promoter	HBV sequence					Reference
				Pre-S	S	X	C	P	
C57Bl/6J × SJL/J	C57Bl/6J	50–4[a]	Albumin	+	+	+			Dunsford *et al.* (1990); Chisari *et al.* (1989)
C57Bl/6J × SJL/J	C57Bl/6J	45–2[b]	Albumin						Chisari *et al.* (1989)
C57Bl/6J × SJL/J	C57Bl/6J	45–3[c]	Albumin						Chisari *et al.* (1989)
C57Bl/6 × SJL/J	C3H/He	E36	HBV	+	+	+		+	Babinet *et al.* (1985); Dragani *et al.* (1990)
CD1	CD1	C11 H9 E1	X			+			Kim *et al.* (1991)

[a]Current designation: Tg (Alb–1 HBV)Bri 44
[b]Current designation: Tg (Alb–1 HBV)Bri 43
[c]Current designation: Tg (Alb–1 HBV)Bri 141

Transgenic mice containing the entire coding region of the HBx gene, including the X promoter, the principal RNA start sites, transcriptional enhancer and polyadenylation site, were created by microinjecting embryos from outbred CD_1 mice (see Table 7). Six transgenic mice, each with at least one intact, stably expressed copy of the X gene, were identified by Southern blot analysis, and three animals with a high level of expression were bred into permanent lines (lineages C11, H9 and E1) [strain and sex of crosses and total numbers of transgenic and nontransgenic offspring from all three lineages unspecified]. Livers were examined histologically at various times. At four months, preneoplastic lesions consisting of multifocal areas of altered hepatocytes were observed in progeny from all three lines of transgenic mice but not in nontransgenic littermates. Neoplastic nodules [sizes unspecified], which occurred by 8–10 months of age, compressed surrounding hepatocytes and accumulated high levels of HBx protein. The authors reported that fewer than 10% of control male CD_1 mice develop hepatic neoplasms during an average lifespan of 24 months [no data shown]. HCCs were observed in 19/21 males and 12/20 females of line C11, in 8/10 males and 4/6 females of line H9 and in the E_1 line [details not given]. Most males of the C11 line died with HCC between 11 and 15 months of age, and most females between 17 and 21 months of age. There was no difference in the incidence of liver tumours in male and female mice. Liver damage, determined by concentration of serum alanine aminotransferase, was not observed, and the levels were consistently within normal range (Kim *et al.*, 1991). [Detailed data on liver lesions in nontransgenic littermates were not provided.]

In another study of a transgenic lineage expressing the X gene driven by the α-1-antitrypsin promoter, mice did not exhibit liver disease or tumour development. This lineage exhibited an early but transient expression of the HBx protein (Lee *et al.*, 1990).

3.2.2 *With concomitant administration of known chemical carcinogens*

The transgenic mouse strain E36 was derived from founder (C57Bl/6 × SJL/J)F_1 mice containing all of the HBV genome except for the core gene, allowing expression of HBsAg under control of the HBV promoter and enhancer sequences (see Table 7). Two hundred and four transgenic and nontransgenic mice (F_1 hybrids resulting from crosses of males of the transgenic strain E36 with C3H/He females) were allocated to three treatment groups. Animals of group 1 (23 HBV-seropositive males, 21 HBV-seropositive females, 19 HBV-seronegative males and 22 HBV-seronegative females still alive at 30 weeks) were treated at seven days of age by oral gavage with a single dose of 10 mg/kg bw NDEA in 0.9% saline solution. Animals of group 2 (12 HBV-seropositive and 22 HBV-seronegative males still alive at 30 weeks) were treated with a single dose of 150 mg/kg bw *para*-dimethylaminoazobenzene (DAB) in corn oil by gavage at seven days of age. Group 3 consisted of 52 untreated controls (15 HBV-seropositive males, 11 HBV-seropositive females, 14 HBV-seronegative males and 12 HBV-seronegative females still alive at 30 weeks). Survivors at 30 weeks were 92% of those treated with NDEA and 98% of those given DAB. Animals were killed at 30 weeks of age and examined both grossly and microscopically for the presence of liver tumours. No tumour was observed in the 52 control animals. Liver nodules > 220 μm in diameter were counted, and those 5 mm in diameter were classified as either adenomas or carcinomas by histological criteria. In NDEA-treated male groups, the total number of nodules per cubic centimetre of liver was about the same for HBV-seropositive and HBV-seronegative animals, but larger nodules (> 330 μm diameter) occurred at about twice the frequency in HBV-seropositive mice as compared with HBV-seronegative mice ($p < 0.05$, Wilcoxon test). The frequency of nodules in the DAB-treated group was much lower than that in NDEA-treated animals. The frequency of nodules per cubic centimetre of liver was 1.5–2 times higher in transgenic than in nontransgenic animals, but the increase was significant only for nodules ≤ 110 μm. The incidence of hepatocellular adenomas and carcinomas was higher in HBV-seropositive (18/56) than in HBV-seronegative (14/63) animals treated with either NDEA or DAB; this difference was not significant (Dragani *et al.*, 1990).

Six groups of 10 female transgenic mice that produce the HBV large surface antigen (lineage 50-4; see Table 7) and of 10 nontransgenic littermates were treated as follows. Group 1 served as untreated controls; group 2 received five monthly intraperitoneal injections of 0.25 μg/g bw aflatoxin B_1 as a suspension in tricaprylin beginning at three or four months of age; group 3 received a single intraperitoneal injection of 0.25 μg/g bw aflatoxin B_1 suspended in tricaprylin at three or four months of age; group 4 received three weekly intraperitoneal injections of 2.0 μg/g bw aflatoxin B_1 suspended in tricaprylin at four months of age; group 5 received a single intraperitoneal injection of 50 μg/g bw NDEA dissolved in sterile saline at four or five months of age; and group 6 received 0.1% phenobarbital in powdered diet beginning at six months of age for one year. The study was terminated when the animals were 15 months of age. Survival rates were approximately 90%, except for

group 6 in which survival was about 50%. Liver nodules and tumour masses were observed grossly *post mortem* and by histological examination. Nodules were classified by size into three categories: 0.1–1.9 mm, 2–4.9 mm and > 4.9 mm in diameter. Adenomas and HCCs were distinguished histologically. No gross or histological lesions were seen in the livers of nontransgenic control mice, whereas the livers of control transgenic mice contained multiple nodules of different sizes. Livers of transgenic mice treated with aflatoxin B_1 had 15–23 nodules (0.1–1.9 mm in diameter) per liver, as compared with 0.1–0.2 nodules of the same size per liver in nontransgenic aflatoxin B_1-treated mice and five nodules per liver in transgenic control mice not treated with aflatoxin B_1. Similar results were obtained for the incidence of larger nodules. Aflatoxin B_1-treated transgenic mice had 6.2–8.8 nodules (2.0–4.9 mm in diameter) per liver, whereas untreated transgenic mice had an average of 3.7 nodules per liver and aflatoxin B_1-treated nontransgenic mice had 0–0.1. Adenomas and HCCs were seen only in transgenic mice treated with aflatoxin B_1 or NDEA. In the three aflatoxin-treated groups (2, 3 and 4), a total of 20 adenomas and two HCCs were observed in 26 transgenic mice and none in 27 nontransgenic mice. In the NDEA-treated group (5), nine adenomas and two HCCs were seen in eight transgenic mice and none in nine nontransgenic mice examined. Livers of transgenic mice fed phenobarbital showed increased nodularity but no adenoma or HCC; however, survival was poor (Sell *et al.*, 1991).

3.3 Woodchucks (*Marmota monax*)

3.3.1 *Hepatocellular carcinoma in woodchucks naturally infected with woodchuck hepatitis virus*

The first non-human hepadnavirus was identified in woodchucks (*Marmota monax*) in a series of studies that began at the Philadelphia (USA) Zoo (for a review, see Paronetto & Tennant, 1990).

In the initial report, which appeared as an abstract (Snyder, 1968), a group of 50 woodchucks (42 males and eight females), trapped in the wild in the vicinity of Philadelphia when about five months of age, were held in captivity one or two per cage on tap-water and a standard feed. After about 72 months in captivity, 30 animals had died. HCC were observed in nine (six males and three females). In one of the nine animals, metastatic nodules were found in retroperitoneal fat. The author concluded that dietary carcinogens were probably not responsible, since other captive animals in the Philadephia Zoo fed on the same diet had not developed liver tumours; he proposed that a viral agent was involved in the etiology of liver cancer in woodchucks.

Ten years later, Summers *et al.* (1978) reported that post-mortem examination of 102 woodchucks that had been caught in the wild and kept at the Philadelphia Zoo for 18 years had revealed 23 HCCs (22.5%), which appeared at a mean age of 59 months. Three animals had acute hepatitis. About 15% of serum samples taken from captive woodchucks were found to contain DNA polymerase-containing particles in amounts comparable with those found in some human sera positive for HBsAg. Detailed investigations were carried out on three animals, two of which had died with HCC and one of which had died with a normal liver: Sera from the two animals with HCC, but not that from the control animal, had detectable levels of DNA polymerase-containing particles. When the particles were

characterized and compared with particles from an HBV-infected human by caesium chloride equilibrium sedimentation, electron microscopy and electrophoresis, the particles from the woodchucks were found to be similar, but not identical. DNA of similar size and physical structure was found in sera and liver samples from the two animals with HCC. The authors concluded that the particles represented a distinct virus, which they called 'woodchuck hepatitis virus', which is phylogenetically related to HBV.

In a review, Summers (1981) reported that all 16 woodchucks in the colony at the Philadelphia Zoo that developed HCC also had chronic active hepatitis of varying severity and had been persistently infected with WHV from an early age, when they were obtained from the wild. No HCC had developed in groups of animals with anti-WHs and no marker of viral infection.

Seventy-three woodchucks from Pennsylvania and Delaware which had been trapped as yearlings or as adults and observed for at least one month in a colony established at the National Institute of Allergy and Infectious Diseases (NIAID) (Mitamura *et al.*, 1982) were studied by Popper *et al.* (1981). Thirty-three selected animals, including all six animals that had developed HCC [criteria for selection of the remaining 27 animals not described], were studied in detail. The six animals with HCC were all seropositive for WHsAg, WHV DNA and WHV DNA polymerase. Of the remaining 27 animals, four were seropositive for all three markers and four for anti-WHs, three were seronegative for all markers and 16 were seropositive for one marker only or gave inconsistent or discrepant results. The authors pointed out that cirrhosis did not occur in animals with HCC. Furthermore, inflammation was generally characterized as mild, and chronic active hepatitis was seen in only two animals with HCC. The authors also noted the direct transition to HCC from neoplastic nodules in these woodchucks.

Mitamura *et al.* (1982) extended the observations on the NIAID colony of woodchucks and analysed markers of WHV infection among 62 animals that had died of various causes. Death from HCC occurred in 11 of 13 (85%) chronic carriers of WHV, in two of 33 (6%) animals with anti-WHs and no evidence of viral replication, and in none of 16 animals with no viral marker.

Of 113 woodchucks that had been trapped in different areas of Pennsylvania, Maryland and Delaware and kept in a colony at the New Bolton Center at the University of Pennsylvania, eight developed HCC between 44 and 88 weeks of captivity (Millman *et al.*, 1984). Seven of the animals were seropositive for WHsAg at the time of capture; one animal that was seronegative at that time converted to WHsAg seropositivity after 33 weeks of captivity.

Nineteen WHsAg-seropositive woodchucks that had been trapped in Pennsylvania and Maryland were kept for up to two years at Cornell University, New York (Roth *et al.*, 1985), and the livers of 16 animals were examined. HCC was found in 13, all of which had chronic active or persistent hepatitis. Metastases to the lung were observed in one animal. Among 149 WHsAg-seronegative woodchucks trapped in New York State and kept in captivity for four weeks or more, a single case of HCC was observed, although five had acute hepatitis.

3.3.2 *Hepatocellular carcinoma in woodchucks experimentally infected with woodchuck hepatitis virus*

(*a*) *Infection with woodchuck hepatitis virus*

A breeding colony of woodchucks consisting of the offspring of female woodchucks trapped in New York State and shown to be free of present or past WHV infection was established at Cornell University, New York (Popper *et al.*, 1987). Newborn animals were inoculated with $10^{5.5}$–$10^{6.5}$ 50% infectious doses of WHV one day after birth. Adult woodchucks with no evidence of active or past WHV infection, maintained in the NIAID woodchuck colony, were inoculated with $10^{5.8}$ 50% infectious doses. Animals were kept on tap-water and aflatoxin-free laboratory chow. A total of eight woodchucks, six infected at birth and two as adults, developed chronic infection, as indicated by the presence of WHsAg for one year or longer. All eight animals subsequently developed HCC 17–36 months after infection; no HCC was observed in 19 animals with virological markers of past infection or in 15 uninfected controls followed for 18–57 months. Mild hepatitis, characterized by lymphocytic infiltrates, was seen in the portal tracts of woodchucks infected as adults or newborns. In animals infected as adults, the portal inflammation regressed with time and the liver assumed the appearance of control livers. In woodchucks with HCC, the portal tract inflammation was more extensive, occasionally resembling that seen in human chronic active hepatitis. Furthermore, inflammation appeared to be most severe in the immediate vicinity of the HCC. Cirrhosis was not seen.

Two groups of 43 woodchucks were inoculated with infectious serum at birth or at eight weeks of age. Thirteen of those inoculated at birth (32%) became chronic carriers, 28 animals cleared the infection and two died within six months after birth. After three years, 11 of the chronic carriers and two of the animals with past infection had developed HCC. Of those inoculated at eight weeks of age, 23 developed acute WHV infection; three became chronic carriers (13%), while 20 animals recovered from the infection. Two of the three chronic carriers and eight of the 20 animals with past infection were followed for three years. Both chronic carriers but none of the eight woodchucks with past infection developed HCC. None of 46 uninfected, laboratory-born woodchucks followed for three years or more developed HCC (Tennant *et al.*, 1988).

Gerin *et al.* (1989) extended the analysis of HCC occurrence in experimentally infected woodchucks maintained at Cornell University: HCC developed in 61/63 chronic carriers (97%), 11/63 (17%) animals with past infection and in none of 108 concurrent, uninfected controls. Follow-up was for at least three years; the sex of the animal did not influence the occurrence of HCC. All three pair-wise comparisons between the three groups were significant at $p < 0.001$ by Fisher's exact test.

(*b*) *Infection with woodchuck hepatitis virus in combination with aflatoxin B_1*

In a study described in an extended abstract (Tennant *et al.*, 1990), 52 woodchucks [sex unspecified] were inoculated subcutaneously with WHV (5×10^6 50% infectious doses) at 1–3 days of age. A group of 27 of these animals received no further treatment; 25 inoculated and 29 uninoculated animals subsequently received aflatoxin B_1 in the diet (0.25–1.0 μg/kg) from three months of age for six months or comparable cumulative doses of aflatoxin B_1 in

dimethyl sulfoxide solution by intraperitoneal injection (125 μg/kg bw, three times weekly) beginning at 1–4 months of age for 3–4 months. Twenty-three animals served as untreated controls. WHV-specific serological tests [unspecified] indicated that the rate of chronic infection (73%) at one year of age in the group given aflatoxin B_1 and WHV was similar to that of those infected with WHV alone (70%). Survival rates were 60% for woodchucks infected with WHV and given aflatoxin B_1 and 72% for those that received aflatoxin B_1 alone; no death occurred among animals infected with WHV alone. Histological analysis of livers from aflatoxin B_1-treated woodchucks revealed lesions consistent with hepatotoxicity due to that compound. Thirty-six months after initiation of the study, 6 of the 15 surviving animals (40%) given aflatoxin B_1 and WHV had HCC, in contrast to 21 of the 27 animals (78%) inoculated only with WHV. During the same period, 2 of 21 woodchucks that were treated with aflatoxin B_1 alone and survived more than one year developed HCC, while none of 22 untreated controls had hepatic tumours. [The high dose of aflatoxin B_1 compromised the interpretation of the results of this study by reducing the survival of the animals.]

3.3.3 *Hepatocellular carcinoma in woodchucks experimentally infected with ground squirrel hepatitis virus*

Seeger *et al.* (1991) reported experiments in which woodchucks were infected with Beechey ground squirrel (*Spermophilus beecheyi*) hepatitis virus (GSHV) or WHV. Three-day-old woodchucks from the breeding colony at Cornell University were inoculated subcutaneously with serum from infected woodchucks or from infected ground squirrels. Of 29 woodchucks infected with GSHV, 17 (59%) became chronic carriers; of 36 woodchucks inoculated with WHV, 27 (75%) became chronic carriers. Sixteen of these were selected for comparison with the 17 chronic carriers of GSHV. Two years after experimental infection, 7 of the 16 WHV-infected but none of the 17 GSHV-infected woodchucks had liver masses (detected by ultrasound imaging), all of which were verified histologically as HCC. Histological examination of all animals after 26 months revealed neoplastic lesions in two GSHV-infected woodchucks. At 51 months after infection, all 16 WHV carriers had developed one to five HCCs each (total, 41), and 6 of the 14 GSHV carriers that were tumour-free at 26 months and that survived laparotomy at that time developed one to four HCCs (total, 14). The median time to diagnosis of HCC in WHV-infected woodchucks was 32 months; the projected median time to diagnosis of HCC in GSHV-infected woodchucks was 55 months. The extent of non-neoplastic liver disease and chronic inflammation did not differ according to the virus inoculated.

3.4 Ground squirrels, ducks and other species

3.4.1 *Beechey ground squirrels*

GSHV infecting Beechey ground squirrels was discovered in 1979 in northern California, USA, as a result of a search for a virus similar to HBV in animals related to woodchucks (Marion *et al.*, 1980). The biology, genetic structure, gene products and viral replication of GSHV have been reviewed recently (Marion, 1991). The virus was originally detected in sera taken from apparently healthy animals. To date, the only known location of this virus is on the San Francisco Peninsula, although the virus that putatively infects

Richardson ground squirrels (*Spermophilus richardsonii*) (see section 3.4.2) (and viruses that possibly infect other ground squirrel species) may be a variant of the Beechey squirrel virus.

In an experiment described in a series of reports (Marion *et al.*, 1983, 1986, 1987), Beechey ground squirrels, estimated to be one to two years of age, were trapped live at various locations on the San Francisco Peninsula between 1980 and 1984. Animals were held individually in quarantine for one month, during which time their serum was tested for (i) surface antigen (GSHsAg), by a commercial solid-phase radioimmunoassay for cross-reacting HBsAg; (ii) anti-GSHs, by a virus-specific solid-phase radioimmunoassay; and (iii) virion-associated DNA polymerase activity, as a measure of virus load (Marion *et al.*, 1983). Animals with serum GSHsAg and DNA polymerase activity were housed in a room separate from GSHsAg-seronegative animals (Marion *et al.*, 1986). Marion *et al.* (1987) reported that 24/103 ground squirrels examined at necropsy had tumours at various sites; all tumour-bearing squirrels were 4.5–8 years of age and had been in captivity for a minimum of 2.4 years. Among animals under 4.5 years, no tumour of any kind was observed in 19 persistent carriers of GSHV, 22 seropositive for anti-GSHs or 19 with no serological marker of GSHV. Of the tumours observed in older squirrels, 11 were found in 17 GSHV carriers, eight in 11 squirrels seropositive for anti-GSHs and five in 15 GSHV marker-free squirrels. The predominant type of tumour observed in squirrels over 4.5 years of age was HCC, which was detected in 10/17 persistent GSHV carriers and in 3/11 squirrels seropositive for anti-GSHs, but in none of 15 GSHV marker-free squirrels in the same age range, resulting in a highly significant association between HCC and the GSHV carrier state ($p = 0.0005$, Fisher's exact test) and a weaker association with seropositivity for anti-GSHs. Development of HCC in carrier squirrels may be related either to age or to the length of the carrier state, as all animals appeared to have become carriers before 1.5 years of age. HCC was seen at necropsy in six of nine carrier squirrels (67%) over six years of age but in only three of nine carriers aged four to six years and none of 17 carrier squirrels less than four years of age. All HCCs except one were of the same histological type: a trabecular, highly differentiated liver carcinoma; the only non-trabecular HCC was seen in one of the three squirrels seropositive for anti-GSHs and was of the medullary type and less differentiated. The diameters of the major tumours were generally larger in squirrels that were older when the HCC was detected. Single nodules of HCC were commoner in the younger squirrels, while older squirrels usually had more than one nodule. Four of the five oldest squirrels with HCC also had metastases to or adhesions of the tumour in the spleen. While viral DNA was integrated into the host DNA of some of the HCCs examined, the majority of those from squirrels with GSHV markers did not have detectable integrated viral DNA. Chronic active hepatitis and cirrhosis were not seen (Marion *et al.*, 1986, 1987).

In a further assessment of the development of HCC in the squirrel colonies after nine years of observation (Marion & Cullen, 1992), 18 cases (45% of all neoplasms) were observed in the study population of 24 GSHV-infected, 20 anti-GSHs-seropositive and 26 GSHV marker-free ground squirrels over four years of age. Eleven of the liver tumours were seen in carrier animals, five in anti-GSHs-seropositive squirrels and two in GSHV marker-free animals. The association of HCC with the GSHV carrier state was significant ($p = 0.0016$). As in WHV-infected woodchucks, the incidence of HCC in animals that had recovered from infection was relatively high (20%). Anti-GSHs-seropositive squirrels that

developed tumours experienced only a brief period of viraemia. No sex difference was noted. The average age of carrier squirrels at the time of detection of HCC was 6.5 years.

3.4.2 *Richardson ground squirrels*

HCC has been observed in Richardson ground squirrels from the southern half of the Canadian province of Alberta. The hepadnavirus thought to be associated with these tumours has not been characterized genetically or biologically, nor has it been transmitted experimentally to other animals. In a study by Minuk *et al.* (1986), animals were trapped and kept in captivity for less than one month. Two of 25 adult squirrels but none of 15 juveniles had HCC at necropsy (Table 8). Anti-GSHs was found in 7 of the 25 adult animals, and the serum of one animal reacted positively when tested with a commercial radioimmunoassay for HBsAg known to detect GSHsAg. Serum was not tested for the presence of virions. Anti-GSHs seropositivity was assayed with a commercial radioimmunoassay for anti-HBs. Of the animals in which HCC was found, one had GSHsAg reactivity in the serum, while the other was seropositive for anti-GSHs. No viral DNA was detectable in the HCC of the seropositive animal or in the DNA of adjacent liver tissue. [The assay to detect anti-GSHs was unspecific and insensitive.]

Table 8. Studies of hepatocellular carcinoma (HCC) in Richardson ground squirrels trapped or born in captivity in Alberta, Canada, according to age at necropsy

Age at necropsy	No. of animals	Location	GSHsAg-seropositive	Anti-GSHs-seropositive	Liver tumours	Reference
Adult	25	South of Calgary	1	7	2 HCC	Minuk *et al.* (1986)
Juvenile	15	South of Calgary	1	4	0	
1– ≥3 years	562	Picture Butte	ND	ND	0	Tennant *et al.* (1991)
3–4 months	56[a]	Picture Butte	ND	ND	0	
14–17 months	54[a]	Picture Butte	ND	ND	31 with nodules	
15 months	36	Cochrane	ND	ND	1 HCC, 4 with nodules	
≥3 years	5	Edmonton	0	10/12	2 HCC, 2 with nodules	
≥3 years	7[b]	Picture Butte	0		4 HCC	

GSHsAg, ground squirrel hepatitis surface antigen; ND, not determined
[a]Born in captivity
[b]Dams of 54 born in captivity

In a study by Tennant *et al.* (1991), several groups of Richardson ground squirrels were examined for the presence of masses in the liver at necropsy. The majority, collected at Picture Butte, Alberta, Canada, and not maintained in captivity, were not tested for hepadnavirus markers or examined histologically. None of 618 squirrels ranging in age from three to four months to three years or more had evidence of liver cancer (Table 8). Squirrels held in captivity for 14 months or longer for various experiments were also examined for HCC at necropsy; nodules or histological evidence of HCC were detected in some animals (Table 8).

HCCs were found in squirrels trapped at Picture Butte only after the animals had been maintained in captivity. Hepadnavirus markers were assayed in the sera of the five squirrels trapped near Edmonton and the seven from Picture Butte. None cross-reacted with HBsAg, but most had evidence of anti-GSHs. Viral DNA was detected in two of four HCCs; no anti-GSHc was detectable in any sample using an assay which readily detects this antibody. Non-neoplastic lesions in the livers of animals kept for three years in captivity included mild to moderate portal inflammation, with somewhat more severe inflammation adjacent to tumours. In the livers of six of seven animals with moderate portal inflammation, focal hepatocellular necrosis and inflammation were seen. [Limited data are available to support hepadnavirus infection *per se*.]

3.4.3 *Ducks*

Observations of liver tumours in domestic ducks (*Anas domesticus*) were first described in China by Wang *et al.* (1980), which led to the discovery of duck hepatitis B virus (DHBV) (Mason *et al.*, 1980). The biology, genetic structure, gene products and viral replication of DHBV have been reviewed (Schödel *et al.*, 1991).

Studies of the oncogenic potential of DHBV are of three types: (i) assessment of liver tumours and markers of DHBV in ducks collected on farms or free-ranging in communities; (ii) prospective studies of the development of HCC in ducks of known DHBV status and history; and (iii) experimental studies of the joint effects of DHBV infection and aflatoxin B_1 exposure in the development of HCC in ducks.

(*a*) *Liver tumours and markers of duck hepatitis B virus in ducks collected on farms and in free-ranging flocks*

After the initial discovery of DHBV in ducks with hepatitis and liver tumours in the Chinese Province of Qidong, several studies were carried out to determine whether the presence of HCC and hepatitis in domestic ducks was linked to current or past replication of DHBV in the same animals (see Table 9).

DHBV was found in 70/195 ducks from three of five locations in China but in none of 17 ducks from Chiba, Japan (Table 9); HCC was found only in four ducks from Qidong, and evidence of present or past DHBV infection was seen in three of them. Moderate to severe hepatitis was observed in both DHBV-seropositive and -seronegative ducks from Qidong, where there are known to be relatively high levels of aflatoxin B_1, a known cause of liver disease in ducks (IARC, 1993). Moderate hepatitis consisted of mild portal inflammation, with rare necrosis of hepatocytes. Severe hepatitis was associated with dense chronic inflammation of portal tracts, which extended into adjacent parenchyma and was accompanied by focal necrosis of hepatocytes. Severe hepatitis was sometimes accompanied by septal fibrosis, focal areas of parenchymal collapse and regenerative nodules; cirrhosis was seen in one duck with HCC (Marion *et al.*, 1984). Three of the four HCCs were observed in Chinese ducks and none in white Pekin ducks. Overall, while there was concomitant presence of DHBV and HCC in some ducks from Qidong, the two have not been firmly linked, nor has the simultaneous presence of DHBV replication and liver inflammation been associated in these ducks.

Table 9. Presence of hepatocellular carcinoma (HCC) and duck hepatitis B virus (DHBV) infection in populations of ducks on farms and free-ranging

Age at necropsy (years)	Provenance	Breed	No. of ducks	No. with serum DHBV particles or serum or liver DHBV DNA	No. with HCC	Viral DNA in liver of ducks with carcinoma	Reference
1–2	Qidong, China	White Pekin	24	12	1[a]	ND	Omata *et al.* (1983)
1–2	Changchun, China	White Pekin	20	0	0		
1–2	Chiba, Japan	White Pekin	17	0	0		
3–5	Qidong, China	Chinese	14	7	2	1+, 1–	Marion *et al.* (1984)
1–3	Qidong, China	White Pekin	4	0	0		Yokosuka *et al.* (1985)
1–3	Qidong, China	Chinese	19	13	1	+	
1–3	Shanghai, China	White Pekin	17	1	0		Omata *et al.* (1987)
1–3	Shanghai, China	Chinese	10	7	0		
1–3	Xiamen, China	Chinese	28	14	0		
1–3	Qidong, China	White Pekin	4	1	0[b]		
1–3	Qidong, China	Chinese	19	15	0[b]		
1–3	Funan, China	White Pekin	36	0	0		

ND, not determined

[a]Cirrhosis was also seen.

[b]Seems to overlap with Yokosuka *et al.* (1985)

(*b*) *Prospective studies of hepatocellular carcinoma in ducks of known duck hepatitis B viral status*

Ducks infected either congenitally or by injection with DHBV as hatchlings were monitored for development of HCC in four studies (Table 10). HCC was seen in only 1/37 experimentally infected ducks aged 0.3–1.8 years and in none of eight congenitally infected and none of 26 uninfected ducks of similar ages. The single HCC observed was in a white Pekin duck similar to those used in all of the studies (Cullen *et al.*, 1991).

Table 10. Presence of hepatocellular carcinoma (HCC) in ducks of known duck hepatitis B virus (DHBV) status

Age at necropsy (years)	Breed	Type of infection	No. of ducks	No. with DHBV markers in serum or liver	No. with HCC	Reference
0.6–1.0	Japanese	Experimental	20	17	0	Omata *et al.* (1984)
Not reported	Japanese		10	0	0	
0.8	Japanese	Experimental	2	2	0	Uchida *et al.* (1988)
0.3–1.8	White Pekin	Experimental	15	12	1	Cullen *et al.* (1989)
	White Pekin		16	0	0	
2.3	White Pekin	Congenital	8	8	0	Cullen *et al.* (1989, 1990)
2.3	White Pekin		16	0	0	

Inflammation of the liver was much less severe in two breeds of domestic ducks from California inoculated with known amounts of DHBV than in the free-range Chinese ducks (above). The majority of domestic birds (25/25 nonviraemic and 17/28 viraemic) showed only insignificant or mild inflammation; seven viraemic birds exhibited moderate inflammation (Marion *et al.*, 1984).

[The relative absence of both inflammation and cancer in experimentally infected ducks is noteworthy. Further, the prospective experimental studies have been of limited duration relative to the lifespan of ducks.]

(*c*) *Synergy between infection with duck hepatitis B virus and treatment with aflatoxin B_1 in inducing hepatocellular carcinoma in ducks*

Ducks have been reported to be sensitive to the effects of aflatoxin B_1 and to the development of HCC as a consequence of treatment with this mycotoxin (IARC, 1993). Studies of the combined effect of DHBV infection and exposure to aflatoxin B_1 in the development of liver cancer all involved white Pekin ducks and a variety of dosing schedules (Table 11). Aflatoxin B_1 was highly toxic, increasing the mortality rate in treated over that in untreated ducks. The rate of appearance of HCC was not significantly different in DHBV-infected aflatoxin B_1-treated ducks from that in DHBV marker-free aflatoxin B_1-treated ducks, suggesting a lack of synergy between current viral infection and exposure to aflatoxin B_1. Integrated viral DNA was found in three of the eight DHBV-associated HCCs examined.

Table 11. Development of hepatocellular carcinoma (HCC) in ducks with and without duck hepatitis B virus (DHBV) infection treated with aflatoxin B_1 (AFB_1)

Treatment	Age at necropsy	No. of ducks	Effective number	No. with DHBV markers in serum	No. with HCC	Integrated viral DNA in carcinoma	Reference
Experimentally infected at hatch; AFB_1 soon after inoculation							Uchida *et al.* (1988)
0.1 mg/kg, 2 ×/week, oral, 54 weeks	54 weeks	22	22	22	0/8 surviving		
0.1 mg/kg, 2 ×/week, oral, 54 weeks	54 weeks	16	16	0	2/8 surviving		
0.1 mg/kg, 2 ×/week, oral, first 5 weeks only	54 weeks	5	5	4	1	–	
0.1 mg/kg, 2 ×/week, oral, first 5 weeks only	54 weeks	3	3	0	0		
0.1 mg/kg, 2 ×/week, oral, last 25 weeks only	41 weeks	5	5	5	0		
Solvent only	41 weeks	2	2	2	0		
Congenitally infected; AFB_1 started at three months of age							Cova *et al.* (1990)
0.08 mg/kg, 1 ×/week, i.p., 27 months	2.3 years	15	6	6	3	–	
0.08 mg/kg, 1 ×/week,, i.p., 27 months	2.3 years	13	10	0	3	–	
0.02 mg/kg, 1 ×/week, i.p., 27 months	2.3 years	15	13	13	0		
0.02 mg/kg, 1 ×/week, i.p., 27 months	2.3 years	13	10	0	2	–	
None	2.3 years	16	15	15	0		
None	2.3 years	15	12	0	0		
Congenitally infected; AFB_1 started three days after hatch							Cullen *et al.* (1990)
0.2 mg/kg, 60 days, oral	28 months	12	8	8	4	3+	
0.2 mg/kg, 60 days, oral	28 months	10	4	0	3		
Solvent only	28 months	8	6	6	0		
Solvent only	28 months	9	6	0	0	NA	

[The existence of species-specific hepadnaviruses closely related to HBV, which produce HCC in two species (woodchuck and Beechey ground squirrels), strengthens the plausibility of the conclusion that HBV is carcinogenic.]

3.4.4 *Other species*

The DNA of a hepadnavirus that infects herons (HHBV) has been cloned and characterized genetically, but it has not been characterized biologically nor has infection with the virus been associated with the development of liver cancer (Sprengel *et al.*, 1988).

Evidence that a hepadnavirus infects tree squirrels (*Sciurus carolinensis pennsylvanicus*) was reported from studies of their livers, but viraemia has never been described in tree squirrels, and the virus remains uncharacterized both genetically and biologically (Feitelson *et al.*, 1986a,b). Liver cancer has not been observed in tree squirrels with evidence of hepadnaviral infection.

4. Other Relevant Data

4.1 Pathology

The pathology of infection by HBV involves an acute phase, which may be recognized clinically as acute viral hepatitis, and then a long chronic phase with development of chronic active hepatitis and often cirrhosis. HCC may evolve during some phase of chronic hepatitis, usually after cirrhosis has supervened but in some cases without cirrhosis. The majority of HCCs associated with HBV appear to arise in a clinically silent way, with few symptoms and no evidence of chronic hepatitis until the carcinoma is in a late stage.

4.1.1 *Acute hepatitis*

The histological features of acute viral hepatitis are highly variable, and hepatitis A, B, C, D and E viruses cannot be distinguished. They are also highly variable for the same agent in patients of different ages and immune status. In the neonate and other immunocompromised individuals, the histological changes are usually very mild with no significant hepatocellular cytopathic change and no significant hepatocellular hydropic swelling, acidophilic necrosis or cholestasis. The typical histological changes in an adult with symptomatic, icteric acute viral hepatitis include: (i) portal expansion with lymphoid hyperplasia (as occurs in many systemic viral infections); (ii) lobular inflammatory reaction with proliferation of sinusoidal lining cells; and (iii) marked hepatocellular changes, including hydropic change (especially in the perivenular areas), acidophilic necrosis and hepatocellular dropout. In the most severe cases of acute viral hepatitis, extensive hepatocellular necrosis occurs (called submassive or massive acute hepatic necrosis), and this is usually fatal. Such severe viral reactions are rare in the very young. In young people with severe viral hepatitis and necrosis, regeneration of hepatocytes is rapid, and recovery occurs with no chronic sequelae (Peters, 1975). There are few histopathological data on the transition of acute viral to chronic hepatitis in large series of patients.

4.1.2 *Chronic hepatitis*

Chronic hepatitis B is defined as HBV infection for more than six months; the corresponding histological features are extremely variable. Many different terms are applied to the histological patterns, which range from nearly normal to mild inflammatory changes to progressive fibrosis to severe necrotizing reactions. The terminology has been based not on large series of patients with carefully documented courses, virological studies and multiple biopsies but often on small numbers of patients with a few years of observation.

Two commonly used categories of chronic hepatitis are chronic persistent hepatitis and chronic active hepatitis. Chronic persistent hepatitis is used for portal lymphoid hyperplasia and HBV infection without lobular degenerative and inflammatory features (which are required for application of the term chronic active hepatitis). A third term for chronic hepatitis, chronic lobular hepatitis, was redefined by Scheuer and Thaler (1977) as predominantly intralobular inflammation and necrosis with no significant portal lymphoid hyperplasia or piecemeal necrosis (which is periportal hepatocellular necrosis and lymphocytic infiltration).

The separation of chronic hepatitis B into chronic persistent hepatitis and chronic active hepatitis may be misleading and has been challenged (Scheuer, 1991). As a single patient may demonstrate both patterns at the same time or over time, neither pattern is prognostically valid. Since cirrhosis is a common outcome of chronic active hepatitis and because it is irreversible and is not associated with evolution from chronic progressive hepatitis alone, chronic active hepatitis is valuable as a category. In 1989, an international group indicated that many factors, such as age, immunocompetence, infection status, drug use and cirrhosis, can be used to predict the severity of chronic hepatitis (Sherlock, 1989). Scheuer (1991) called for a reassessment of the classification and indicated that the terms chronic persistent hepatitis and chronic active hepatitis were introduced in the absence of adequate knowledge about the natural history of HBV. Cirrhosis is the major form of irreversible severe chronic liver disease that is related to chronic infection with HBV. The number of patients with acute viral hepatitis that progress to cirrhosis is not clear, and the rate of progression is highly variable. Many patients acquire HBV in childhood, and the initial episode (acute viral hepatitis) is clinically silent (Bortolotti *et al.*, 1990): of a series of 76 children with chronic hepatitis B, eight had a clinical event identified as acute viral hepatitis, but the rest were detected in screening programmes. Liver biopsy samples taken in this series showed a wide range of patterns, many having chronic persistent hepatitis and some having chronic active hepatitis. One of the patients had cirrhosis which subsequently became inactive (i.e. fewer inflammatory and hepatocellular degenerative changes). A few cases of cirrhosis resulting from HBV are detected in childhood, but the majority seem to be detected in late adult life. Clearly, many adults have chronic hepatitis B infection and do not progress to cirrhosis (Sugimura *et al.*, 1991).

4.1.3 *Cirrhosis*

Cirrhosis is defined as irreversible hepatic fibrosis with regenerative nodule formation (Popper, 1977). The etiology of cirrhosis may be very difficult to discern by histological means. The pattern of inflammatory activity may be a clue to the underlying agent, but

cirrhosis is often recognized at a late stage with little hepatocellular degeneration or inflammatory change. Hepatitis B viral cirrhosis is often identified on the basis of the presence of ground-glass cells, which are hepatocytes with a distinctive eosinophilic cytoplasmic change due to accumulated hepatitis B surface protein (Buendia, 1992), or by specific immunoperoxidase staining for HBV markers (Nayak & Sachdeva, 1975). The late stage of chronic hepatitis is associated with macronodular cirrhosis (formerly called postnecrotic cirrhosis). As cirrhosis progresses, the size of the nodules increases (Popper, 1977).

4.1.4 *Evolution of hepatocellular carcinoma from cirrhosis*

The strong correlation between HCC and cirrhosis is dependent on the etiology of the cirrhosis. Autopsy results suggest that HCC occurs with greatest frequency (38%) in association with cirrhosis due to chronic HBV infection, haemochromatosis and chronic HCV infection, with intermediate frequency (5–10%) in alcoholic cirrhosis and very low frequency in cirrhosis due to Wilson's disease, autoimmune chronic active hepatitis (< 5%) and primary biliary cirrhosis (Craig *et al.*, 1991).

The mechanism of progression of cirrhosis to HCC is much debated. Dysplastic hepatocellular changes have been described that may be associated with HCC in patients with HBV (Ho *et al.*, 1981). Liver-cell dysplasia is hepatocellular enlargement with nuclear pleomorphism and multinucleation; dysplastic cells occur in clumps or small nodules. Ho *et al.* (1981) reported that 60% of 558 cases of cirrhosis had liver-cell dysplasia and this change was strongly correlated with development of HCC; however, some nodules may be premalignant without liver-cell dysplasia. The transformation of regenerative nodules to atypical hyperplastic nodules and then to HCC is well described. The small regenerative nodule may have a 'nodule within the nodule' growth pattern, and some observers consider that such small nodules are precursors of carcinoma (Arakawa *et al.*, 1986).

Adenomatous hyperplasia (Takayama *et al.*, 1990), also called macroregenerative nodule, is a discrete nodule of hepatocytes that is apparent by gross examination because it is slightly larger and usually also has a different colour from the surrounding nodules of the cirrhotic liver. These nodules are usually 0.8–3.0 cm and not larger, as then the lesion is likely to be a small HCC. Light microscopy reveals that the nodule is composed of hepatocytes in regular trabeculae (not solid), lacks the overt features of carcinoma (such as acinar formation, mitosis and high nuclear:cytoplasmic ratios) and includes foci of small blood vessels and bile ducts. These 'trapped' bile ducts and vessels, usually at the periphery, distinguish adenomatous hyperplastic nodules from hepatocellular adenoma. Some enlarged nodules in cirrhotic livers are HCC, and other nodules demonstrate some features of both hepatocellular and adenomatous hyperplasia (and are thus called atypical adenomatous hyperplasia). These atypical nodules have small areas of acinar formation, an area of thicker cord development or focal hepatocytes with increased nuclear:cytoplasmic ratio. Additional levels of a tissue block and/or more sections of a single nodule reveal that some atypical nodules are small HCCs. The transition of benign hepatocytes to atypical hepatocytes to carcinoma has been observed in a few nodules of cirrhotic liver, whereas the usual histological examination of HCC reveals only masses of carcinoma with no transitional growth areas. In a series of 110 cirrhotic liver explants (of many etiologies, including HBV

cirrhosis but excluding known HCC) examined carefully for distinctive (grossly apparent) nodules, 40 nodules were identified in 19 livers (Ferrell *et al.*, 1992). Microscopic examination allowed classification of 12 as small HCCs and 28 as adenomatous hyperplasia (some with atypia) and liver-cell dysplasia.

Several examples of atypical adenomatous hyperplasia have been shown to have the same clonal integration pattern as the HCC within the same liver. This clonal growth provides a link between atypical adenomatous hyperplasia and the evolution of HCC (Tsuda *et al.*, 1988). Furthermore, the same HBV DNA integration pattern detected in multiple nodules of HCC in the same liver with chronic hepatitis B has been interpreted as proof of clonal growth of HCC and consequent metastasis within the liver. The detection of an additional, new integration pattern in the tumour cells during its course suggests additional HBV-related mutagenesis (Hsu, H.-C. *et al.*, 1991). In experimental models of HCC, e.g. woodchuck with chronic WHV infection (Ogston *et al.*, 1982) and rats with various chemicals, oval cells are identified adjacent to the tumour which are considered to be possible stem cells for HCC. Similar oval cells were detected in human HCC associated with HBV in one laboratory (Hsia *et al.*, 1992).

4.1.5 *Hepatocellular carcinoma*

The morphological pattern of human HCC is usually a homogeneous tumour composed of cells resembling hepatocytes with a trabecular or solid growth pattern. Several other growth patterns are recognized, including spindle-cell, clear-cell and fibrolamellar carcinoma, which are not associated with HBV (Colombo, 1992). A few reports have been made, however, of coexistence of fibrolamellar carcinoma with HBV. Because this variant almost always arises in noncirrhotic, normal livers, the few HBV-infected patients are probably carriers of HBV. Cholangiocarcinoma, the other common primary hepatic carcinoma, does not have detectable HBV markers in the tumour or in the surrounding non-tumorous tissue (Peters *et al.*, 1977).

4.2 Molecular biology

The molecular biology of HBV in relation to HCC has been reviewed (Rogler, 1991; Buendia, 1992; Feitelson, 1992; Slagle *et al.*, 1992). The virus was first associated with HCC in epidemiological studies. During the last 10 years, the role of HBV in liver-cell transformation has been investigated by different approaches, either directly in human HCC or in experimental models, including human HCC cells in culture, transgenic mice carrying part or all of the viral genome (see section 3.2) and naturally occurring animal models of hepadnavirus infection (see sections 3.3 and 3.4). The studies have focused on three main subjects:

(i) integration of HBV: the integrated state of HBV DNA in human HCCs, integration of other hepadnaviruses, structure of viral inserts, cellular target sites for viral integration and insertional mutagenesis;

(ii) expression of HBV genes in human HCCs and their role in the tumorigenic process (mainly surface proteins and X transactivator); and

(iii) genetic alterations in human HCCs, activation of cellular oncogenes and inactivation of tumour suppressor genes.

4.2.1 *Integration of HBV DNA*

Viral integration has been detected only in hepatocytes, despite the presence of viral DNA in extrahepatic tissues (Bréchot, 1987).

(*a*) *Integrated state of HBV DNA in chronic hepatitis and hepatocellular carcinomas from hepatitis B surface antigen-seropositive patients*

Initial studies using Southern blot analysis showed the presence of integrated HBV sequences in cellular DNA of established HCC cell lines and in human HCCs from HBsAg-seropositive patients (Bréchot *et al.*, 1980; Chakraborty *et al.*, 1980; Edman *et al.*, 1980).

It is at present uncertain whether or not integration occurs in the early stages of natural acute hepatitis. In contrast, multiple integrations have been observed in tissues from patients with chronic hepatitis (Bréchot *et al.*, 1981b; Shafritz *et al.*, 1981; Boender *et al.*, 1985; Tanaka, Y. *et al.*, 1988), indicating that viral integration takes place prior to tumour development. Analysis of 83 cirrhotic nodules from the livers of 11 HBV carriers with cirrhosis revealed discrete bands at a higher molecular weight region in 26 of 83 nodules (31.3%), indicating clonal outgrowth of altered hepatocytes with viral integration (Yasui *et al.*, 1992). Single-site HBV insertions are common in childhood HCC but are less common later in life (Chang *et al.*, 1991), suggesting that multiple-site integration occurring during the course of long-term HBV infections might accumulate within single cells, as indicated by sequence divergence among HBV inserts in the same tumour (Imai *et al.*, 1987).

Integrated HBV DNA has been identified in HCC specimens from chronic HBV carriers in numerous other studies (Bréchot *et al.*, 1981a; Shafritz *et al.*, 1981; Hino *et al.*, 1984, 1985; Horiike *et al.*, 1989; Sakamoto *et al.*, 1989), indicating that HBV DNA integration is present in more than 80% of HCCs developing in HBsAg-seropositive patients.

(*b*) *Presence of HBV DNA in hepatocellular carcinomas from hepatitis B surface antigen-seronegative patients*

The presence of serum anti-HBs and anti-HBc in HBsAg-seronegative HCC subjects has been reported (Kew *et al.*, 1986b; Bréchot, 1987; Blum *et al.*, 1990). In spite of the fact that these antibodies generally reflect past, resolved infection, HBV DNA sequences have nonetheless been detected in some tumours in this group (Bréchot *et al.*, 1982, 1985; Hino *et al.*, 1985; Bréchot, 1987; Tabor, 1989; Lai *et al.*, 1990). Improvements in the sensitivity of assays for HBsAg and HBV DNA have made it possible to identify cases of chronic HBV infection with low viral replication (Bréchot *et al.*, 1985; Liang *et al.*, 1989; Bréchot *et al.*, 1991; Kremsdorf *et al.*, 1991; Liang *et al.*, 1991b). The finding of HBV DNA in HCCs in HBsAg-seronegative patients (Pontisso *et al.*, 1987; Paterlini *et al.*, 1993) has been questioned because the estimated copy number of HBV DNA sequences per cell is only 0.001–0.1. The significance of these findings is unclear.

(*c*) *Integrated hepadnavirus DNA in animal models*

Integrated WHV sequences have been detected in chronically infected woodchuck liver and in a majority (> 90%) of woodchuck HCCs in chronic carriers (Ogston *et al.*, 1982; Rogler & Summers, 1984; Hsu *et al.*, 1990), although Korba *et al.* (1989) reported a lower frequency of integration. Viral integration appears to be less frequent in tumours associated

with GSHV and even less in those associated with DHBV (Yokosuka *et al.*, 1985; Marion *et al.*, 1986; Imazeki *et al.*, 1988; Transy *et al.*, 1992).

(d) Structure and expression of viral inserts

Studies of the organization of cloned HBV inserts in liver tissues and HCCs show that HBV sequences are fragmented and rearranged and that integration and recombination sites are dispersed over the viral genome. These data indicate that HBV integration does not occur through a unique mechanism, as is the case for other retroelements and retroviruses. Virtually all HBV inserts consist either of linear subgenomic fragments or of rearranged fragments in different orientations, in the absence of a complete HBV genome, showing that these integrated sequences cannot serve as a template for viral replication. Integrated HBV sequences may be rearranged both during the integration process and after formation of viral inserts (Mizusawa *et al.*, 1985; Nagaya *et al.*, 1987; Tokino *et al.*, 1987). Integrated forms, made up of a continuous genome or subgenomic fragment, which are frequent in tissues from children with HCC and chronic hepatitis (Yaginuma *et al.*, 1987), are believed to represent primary products of integration.

Highly preferred integration sites have been mapped in the HBV genome within the 'cohesive end' region that lies between two 11-base pair direct repeats (DR1 and DR2) which are highly conserved in hepadnaviruses (Koshy *et al.*, 1983; Dejean *et al.*, 1984; Nagaya *et al.*, 1987). A narrow region encompassing DR1 has been shown to be particularly prone to recombination (Yaginuma *et al.*, 1987; Hino *et al.*, 1989; Buendia, 1992; Quade *et al.*, 1992). This region coincides with a short terminal redundancy of the minus-strand DNA, which confers a triple-stranded structure to the circular viral genome (Shih *et al.*, 1987). Integration sites are tightly clustered at both the 5′ and 3′ ends of minus-strand DNA, suggesting that replication intermediates and specially relaxed circular DNA might be preferential preintegration substrates (Nagaya *et al.*, 1987; Shih *et al.*, 1987). Invasion of cellular DNA by single-stranded HBV DNA, using mainly free 3′ ends, might take place through a mechanism of illegitimate recombination, also suggested by frequent patch homology between HBV and cellular sequences at the recombination breakpoints (Matsubara & Tokino, 1990). Different minor changes in flanking cellular DNA have been associated with viral integration, including microdeletions and short duplications (Yaginuma *et al.*, 1985; Dejean *et al.*, 1986; Berger & Shaul, 1987; Nakamura *et al.*, 1988; Hino *et al.*, 1989). The hypothesis that topoisomerase I might promote illegitimate recombination of hepadnavirus DNA *in vivo* has been proposed (Wang & Rogler, 1991). The mechanisms underlying HBV DNA integration have still not been fully identified. Analysis of a limited number of WHV and DHBV insertions suggests that a similar mechanism of integration occurs in these hepadnaviruses (Ogston *et al.*, 1982; Rogler & Summers, 1984; Hsu *et al.*, 1988; Imazeki *et al.*, 1988; Fourel *et al.*, 1990). No similar information is available for GSHV.

As a consequence of the viral integration process, sequences of the S and X genes and of the enhancer I element are present almost systematically in HBV inserts, whereas those of the C gene are less frequently represented (Buendia, 1992). It has been shown that the pre-S2/S promoter is transcriptionally active in its integrated form in human HCC (Freytag von Loringhoven *et al.*, 1985; Caselmann *et al.*, 1990) and that HBsAg might be produced from viral inserts (Zhou *et al.*, 1987). Highly rearranged HBV inserts show virus–virus

junctions scattered throughout the viral genome (Nagaya *et al.*, 1987), and recombination breakpoints have been mapped in the S coding region of some of them (Buendia, 1992). Truncation of the S gene between residues 77 and 221 (Buendia, 1992) confers transcriptional activation activity on the mutated pre-S2/S products (Caselmann *et al.*, 1990; Kekulé *et al.*, 1990). Other studies have shown that a large percentage of virus–host junctions are located in the carboxy terminal of the viral X gene, predicting a fusion of the X open reading frame with flanking cellular sequences in a way that might preserve the functional capacity of the X transactivator. Transcripts have been demonstrated from integrated X sequences in tumours and chronically infected livers (Miyaki *et al.*, 1986; Wollersheim *et al.*, 1988; Takada & Koike, 1990; Hilger *et al.*, 1991).

(*e*) *Cellular targets for viral integration in human hepatocellular carcinomas*

Studies of different viral insertions in many human HCCs have revealed that integration can take place at multiple sites on various chromosomes; insertion sites have been mapped on many different human chromosomes, with higher than average rates on chromosomes 11 and 17 (Tokino & Matsubara, 1991; Slagle *et al.*, 1991; Quade *et al.*, 1992). These studies did not demonstrate the presence of known dominant oncogenes or tumour suppressor genes in the immediate vicinity of any integration site. *Alu*-type repeats and minisatellite-like, satellite III and variable-number terminal repeat sequences have frequently been identified near HBV insertion sites (Shaul *et al.*, 1986; Berger & Shaul, 1987; Nagaya *et al.*, 1987; Buendia, 1992). A small cellular DNA compartment (H3), characterized by a base composition similar to that of HBV DNA and a high concentration of *Alu* repeats, has been designated as a major target for stable HBV integration (Zerial *et al.*, 1986; Buendia, 1992).

In many tumours, large inverted duplications, deletions, amplifications and chromosomal translocations have been associated with HBV insertions, suggesting that HBV DNA integration may enhance chromosomal instability (Koch *et al.*, 1984; Mizusawa *et al.*, 1985; Rogler *et al.*, 1985; Yaginuma *et al.*, 1985; Hino *et al.*, 1986; Tokino *et al.*, 1987; Hatada *et al.*, 1988). It has also been shown that HBV DNA promotes homologous recombination at a distance from the insertion site (Hino *et al.*, 1991). Roles for most of these chromosomal abnormalities have not been assigned as yet, however, although amplification of *hst*-1 loci has been associated with HBV integration in the same chromosomal region (Hatada *et al.*, 1988).

(*f*) *Insertional mutagenesis by HBV DNA*

Evidence for a direct *cis*-acting promoter insertion mechanism in HCC has been provided. In two cases, viral integration disrupted the structure of the gene (Dejean *et al.*, 1986; de-Thé *et al.*, 1987; Dejean & de-Thé, 1990; Wang *et al.*, 1990) in early tumours that developed in non-cirrhotic livers from clonal proliferation of a cell containing a single, specific viral integration. In one case, the HBV insertion occurred in an exon of the retinoic acid receptor β gene (*RARβ*) and fused the amino terminal domain of the viral pre-S1 gene to the DNA and hormone binding domains of the gene (Dejean *et al.*, 1986; de-Thé *et al.*, 1987; Buendia, 1992). The predicted chimeric HBV/RARβ protein might have altered transcriptional capacity and thus participate in the tumorigenic process (Buendia, 1992). In the second case, HBV sequences were found to be integrated in an intron of the human cyclin A

gene, resulting in production of spliced HBV/cyclin A fusion mRNAs initiated at the pre-S2/S promoter (Wang *et al.*, 1990, 1992). In the deduced polypeptide, the amino terminal domain of cyclin A (a target for proteolytic degradation of cyclin A at the end of the M phase) was replaced by an amino acid sequence from the terminus of pre-S1. Cyclins are important in the control of cell division, and disruption of the cyclin A gene by viral insertion probably contributed to oncogenesis (Buendia, 1992).

In a third human HCC, integration of HBV DNA into a cellular gene related to the epidermal growth factor receptor (c-*erb*B) has been described (Zhang *et al.*, 1992).

(*g*) *Insertional activation of* myc *family genes in woodchuck hepatocellular carcinoma*

The search for transcriptional activation of already known proto-oncogenes and for viral insertion sites in woodchuck HCCs has revealed that WHV acts as an insertional mutagen which activates *myc* family genes (c-*myc* and N-*myc*) in more than half of the tumours examined (Möröy *et al.*, 1986; Hsu *et al.*, 1988; Fourel *et al.*, 1990). Analysis of the mutated c-*myc* alleles in two tumours showed integration of WHV sequences in the vicinity of the c-*myc* coding domain, either 5′ of the first exon or in the 3′ untranslated region (Hsu *et al.*, 1988). Deregulated expression of the oncogene driven by its normal promoters resulted from deletion or displacement of c-*myc* regulatory regions known to exert a negative effect on c-*myc* expression and their replacement by viral sequences encompassing the enhancer I element (Buendia, 1992).

Insertional activation of N-*myc* genes was observed more frequently. In particular, the woodchuck N-*myc*2 gene (a functional processed pseudogene or 'retroposon') represents by far the most frequent target for WHV DNA integration. In about 40% of tumours, viral insertions were detected either upstream of the gene or in a short sequence of the 3′ untranslated region (Fourel *et al.*, 1990; Wei *et al.*, 1992). The N-*myc*2 gene is also present in ground squirrels, although insertional mutagenesis has not been demonstrated in this animal model (Transy *et al.*, 1992). Furthermore, woodchucks infected with GSHV do not show insertional mutagenesis (Hansen *et al.*, 1993). Therefore, a direct role of WHV DNA integration in *myc* gene activation might account for the higher incidence and more rapid onset of HCC in the woodchuck model. Finally, there is no evidence that HBV integrates into *myc* family genes in human HCC (Buendia, 1992).

4.2.2 *Expression and potential oncogenic properties of HBV gene products*

The HBV genome encodes seven proteins from four open reading frames. Experimental evidence has been presented for an oncogenic role of two of the viral proteins, the large surface (HBs) protein and the transcriptional transactivator X.

(*a*) *Surface proteins*

In natural HBV infection, the production of infectious virions and HBsAg particles depends on tight regulation of the relative levels of the three envelope glycoproteins. Neither liver lesions nor HCCs have been observed in any of the published transgenic lineages that carry and replicate complete HBV genomes or produce the middle and major surface (HBs) proteins from HBV-derived regulatory sequences (Babinet *et al.*, 1985; Chisari *et al.*, 1985; Burk *et al.*, 1988; Farza *et al.*, 1988; Araki *et al.*, 1989). The appearance and rate of deve-

lopment of preneoplasic nodules and liver tumours following administration of carcinogens are, however, slightly increased in HBsAg-seropositive transgenic mice over that in sero-negative littermates, suggesting that HBsAg expression might enhance the effects of hepatocarcinogens (Dragani *et al.*, 1990).

In contrast, when the endogenous pre-S1 promoter is replaced by an exogenous promoter (the metallothionein or albumin promoter), the production of roughly equimolar ratios of large HBs protein with respect to middle and major HBs proteins leads to intracellular accumulation of nonsecretable filamentous envelope particles within the endoplasmic reticulum of transgenic mouse hepatocytes (Chisari *et al.*, 1986, 1987). This leads to histological and ultrastructural features similar to those of 'ground-glass' hepatocytes, which have been described in chronic hepatitis B in humans. Cell death follows, accompanied by mild persistent hepatitis, which is followed by the development of regenerative nodules and eventually HCC by 12 months of age (Chisari *et al.*, 1989). The preneoplastic nodules and tumours display a marked reduction in transgene expression, suggesting that hepatocytes that express low levels of the large HBs polypeptide would have a selective survival advantage. Chemical carcinogens are not required for tumour induction in this model, but exposure of adult transgenic mice to hepatocarcinogens produced more rapid and extensive development of preneoplastic lesions and HCCs under conditions that do not alter the liver morphology of nontransgenic controls (Sell *et al.*, 1991). These data show that inappropriate expression of the large HBs protein can be directly cytotoxic to hepatocytes and may initiate a cascade of events that ultimately progress to malignant transformation (Buendia, 1992).

Studies of integrated HBV sequences in human HCC suggest a possible role for abnormal expression of rearranged viral S genes in the development of HCC. Deletion of the carboxy terminal region of the S gene generates a novel transcriptional *trans*-activation activity (Caselmann *et al.*, 1990; Kekulé *et al.*, 1990; Lauer *et al.*, 1992). Both integrated HBV sequences from a human tumour and from a hepatoma-derived cell line and different constructs bearing similarly truncated pre-S2/S sequences can stimulate the SV40 promoter in transient transfection assays; transactivation occurs at the transcriptional level and is dependent on the SV40 enhancer. The c-*myc* P2 promoter is also activated *in trans*. These findings support the hypothesis that accidental 3′ truncation of integrated pre-S2/S genes could be a causative factor in HBV-associated oncogenesis (Buendia, 1992).

(*b*) *HBx: a transcriptional transactivator*

Evidence for expression of the HBV *X* gene was obtained by Moriarty *et al.* (1985) and by Kay *et al.* (1985), who reported that the sera of HBV-related HCC patients recognize synthetic peptides made on X sequences. Expression of the *X* reading frame in prokaryotic and eukaryotic cells, using various vectors, allowed identification of a 17-kDa polypeptide that reacted with serum samples from a number of HBV-infected individuals (Elfassi *et al.*, 1986; Meyers *et al.*, 1986; Schek *et al.*, 1991). Anti-HBx has been detected in a minor proportion of acutely infected patients about three to four weeks after the onset of clinical signs, and more frequently in chronic HBsAg carriers who have markers of active viral replication. Very few patients are seropositive for anti-HBx after seroconversion to anti-HBs or at the time an HCC is discovered (Levrero *et al.*, 1991). Conflicting results have been

obtained regarding the association between anti-HBx and other viral markers and with HCC. The problem may be related to the weak antigenicity of HBx protein or to its sequestration into cellular compartments that render it inaccessible to the host immune system. HBxAg has been detected in the livers of HBsAg carriers and has been correlated with current viral replication and chronic liver disease (Levrero *et al.*, 1990; Haruna *et al*, 1991; Wang *et al.*, 1991). The HBx protein is located mainly in the cytoplasm of cells infected *in vivo*, at or near the plasma membrane and at the nuclear periphery (Vitvitski *et al.*, 1988; Levrero *et al.*, 1990; Wang *et al.*, 1991). The HBx protein has been detected in the nuclear compartment only in transfected cell lines (Höhne *et al.*, 1990; Seifer *et al.*, 1990).

The recent finding that the X gene product can *trans*-activate transcription from a number of HBV and heterologous promoters is of considerable importance in defining its role in the pathogenesis of HCC (for review, see Rossner, 1992). More clues to the possible role of HBx protein in HBV-associated pathogenesis were provided by three lines of evidence: in studies *in vitro* and *in vivo* and by direct analysis of biopsy samples of human liver and of HCC (Buendia, 1992).

High levels of expression of the *X* gene may induce malignant transformation of certain cultured cells, such as the mouse fibroblast NIH3T3 cell line (Shirakata *et al.*, 1989) and immortalized hepatocytes that express the SV40 large tumour antigen (Höhne *et al.*, 1990; Seifer *et al.*, 1990). It has been shown that the c-*myc*, c-*jun* and c-*fos* proto-oncogene promoters can be *trans*-activated by the *X* gene product (Balsano *et al.*, 1991; Seifer *et al.*, 1991; Twu *et al.*, 1993). Activation of protein kinase C (Kekulé *et al.*, 1993) and formation of a p53–HBx protein complex have been reported (Feitelson *et al.*, 1993).

Studies of transgenic mice carrying the *X* reading frame controlled by its natural HBV enhancer and promoter sequences or by heterologous liver-specific promoters have given rise to conflicting results. In three lines of mice (C11, H9 and E1) derived from the outbred CD1 strain (see Table 7) carrying a 1.15-kilobase HBV fragment (spanning the enhancer, the complete *X* coding region and the polyadenylation signal), preneoplastic lesions were observed in the liver, which were followed by carcinomas at 8–10 months of age (Kim *et al*, 1991). In contrast, a transgene in which the *X* coding domain was placed under the control of the α-1-antitrypsin regulatory region failed to induce tumours in ICR × B6C3F1 transgenic mice, although *X* mRNAs were detected in liver tissues (Lee *et al.*, 1990).

Analysis of integrated viral sequences in tumour DNA has shed new light on one of the mechanisms leading to overexpression of the *X* gene in chronically infected livers and in HCCs. HBV sequences are frequently interrupted between or around the viral direct repeats DR1 and DR2 upon integration into host cell DNA (Buendia, 1992), and overproduction of hybrid viral and host transcripts may result from HBV DNA integration in a hepatoma cell line (Freytag von Loringhoven *et al.*, 1985; Ou & Rutter, 1985). The presence of viral and host transcripts containing a 3′ truncated version of the HBx coding region fused with flanking cellular sequences and retaining *trans*-activating capacity was first described in a human HCC (Wollersheim *et al.*, 1988). Moreover, enhanced *trans*-activating capacity of the integrated *X* gene product has been related to substitution of viral carboxy terminal residues by cellular amino acids (Koshy & Wells, 1991). *trans*-Activating ability of similarly truncated *X* gene products made from fusion of integrated HBV sequences with adjacent cell DNA has also been shown in many tissues from patients with chronic hepatitis (Takada & Koike, 1990).

This suggests that the integrated *X* gene might be essential for maintaining the tumour phenotype that develops at the early stages of carcinogenesis. Consistent with this model is the finding that viral and host junctions can be mapped in the carboxy terminal region of *X* in most human HCCs (Nagaya *et al.*, 1987; Buendia, 1992).

4.2.3 *Genetic alterations in hepatocellular carcinoma*

Genetic alterations that cannot be associated clearly with a direct effect of viral infection have been observed in human HCCs. Such somatic changes include allele losses on several chromosomal regions, mutation and activation of cellular genes that show oncogenic potential and deletion or mutation of tumour suppressor genes.

(*a*) *Chromosomal losses and tumour suppressor genes*

Several groups have reported loss of heterozygosity in a large number of HCCs (for a review, see Buendia, 1992). Loss of heterozygosity on the distal 1p region and on chromosomes 4q, 11p, 13q and 16q has frequently been detected in human HCCs by restriction fragment length polymorphism (Pasquinelli *et al.*, 1988; Wang & Rogler, 1988; Buetow *et al.*, 1989; Tsuda *et al.*, 1990; Simon *et al.*, 1991). These studies showed no relation to tumour histology or grade, presence of HBV, cirrhosis or ethnic origin of the patient. Thus, it has been suggested that these parts of the human genome might contain genes, the functional loss of which might be involved in hepatocellular carcinogenesis. Allele loss of the short arm of chromosome 17p, which includes the *p53* gene, has also been observed frequently in human HCC (Fujimori *et al.*, 1991; Slagle *et al.*, 1991). Viral insertions at chromosome 17p in some HCCs are not physically linked to the *p53* gene (Hino *et al.*, 1986; Tokino *et al.*, 1987; Zhou *et al.*, 1988). Accumulation of allelic loss on different chromosomes (e.g. 13q, 16q) has been associated with advanced stages of HCC (Nishida *et al.*, 1992). Whether HBV DNA integration, which has been shown to promote genetic instabilily, contributes to these events has not been elucidated.

Tumour suppressor genes are identified on the basis of their loss or inactivation in tumour cells, as the product of such genes are negative regulators of cell growth (Marshall, 1991). Mutational inactivation of *p53* is the commonest known genetic alteration in human cancer (Hollstein *et al.*, 1991). *p53* and retinoblastoma genes are the only genes known to be involved in HCC (Bressac *et al.*, 1990, 1991; Hsu, I.C. *et al.*, 1991; Murakami *et al.*, 1991). The frequency of *p53* mutations in HCC was reported to be high ($\geq 45\%$) in Qidong, China (Hsu, I.C. *et al.*, 1991; Scorsone *et al.*, 1992; Li *et al.*, 1993) and Mozambique (Bressac *et al.*, 1991; Ozturk *et al.*, 1991); intermediate (15–35%) in Japan (Murakami *et al.*, 1991; Oda *et al.*, 1992; Nishida *et al.*, 1993) and in Shanghai (Buetow *et al.*, 1992; Li *et al.*, 1993), Xian (Buetow *et al.*, 1992) and Taiwan, China (Hosono *et al.*, 1991; Sheu *et al.*, 1992); and low (0–15%) in Germany (Kress *et al.*, 1992), the United Kingdom (Challen *et al.*, 1992), Thailand (Hollstein *et al.*, 1993) and Alaska, USA (Buetow *et al.*, 1992). As shown in Table 12, the relationship between chronic infection with HBV (as determined by serum HBsAg) and the frequency of *p53* mutation in HCC was addressed directly in three studies, and *p53* mutations were shown to occur at similar frequencies in HbsAg-seropositive and HBsAg-seronegative patients.

Table 12. Frequency of *p53* mutations in patients seropositive and seronegative for hepatitis B surface antigen (HBsAg)

Location	All patients			HBsAg seropositive			HBsAg seronegative			Reference
	Total	With mutants		Total	With mutants		Total	With mutants		
		No.	%		No.	%		No.	%	
Japan[a]	140	46	33	30	10	33	98	32	33	Oda *et al.* (1992)
Taiwan, China	61	20	33	41	15	37	20	5	25	Sheu *et al.* (1992)
Thailand	15	2	13	7	2	29	6	0	0	Hollstein *et al.* (1993)
Total	216	68	31	78	27	35	124	37	30	

Numbers of seropositive and seronegative do not add up because the HBV status was not known for all patients.
[a]128 Japanese, six Korean, four Indonesian and two Taiwanese patients

A 'hot spot' mutation (Buendia, 1992) at the last guanine residue of codon 249 (AG*G* to AG*T*) was identified in more than 45% of HCCs from Mozambique and Qidong, China (Bressac *et al.*, 1991; Hsu, I.C. *et al.*, 1991). The codon 249 mutation (AGG→AGT) of *p53* is not a mutational hot spot in non-HCC tumours (Hollstein *et al.*, 1991). The presence of codon 249 mutations in HCC was reported in both HBsAg-seropositive and HBsAg-seronegative patients (Ozturk *et al.*, 1991; Buetow *et al.*, 1992; Oda *et al.*, 1992; Sheu *et al.*, 1992; Hollstein *et al.*, 1993; Yap *et al.*, 1993) as well as in the presence and absence of tumour HBV DNA (Scorsone *et al.*, 1992; Li *et al.*, 1993). An association between HBxAg and *p53* both *in vitro* and *in vivo* has been described (Feitelson *et al.*, 1993).

(*b*) *Activation of cellular oncogenes*

The search for activated oncogenes in DNA from HCCs using the NIH3T3 cell transformation assay has not been successful in most cases. A transforming DNA, *lca* (liver cancer), was characterized in a very small number of tumours (2/4) (Ochiya *et al.*, 1986). This new oncogene is expressed at a proliferative stage in fetal liver; its expression in liver cancer has not been associated with gross rearrangement of the gene (Shiozawa *et al.*, 1988). Another transforming gene, *hst*-1, was identified by this method in a tumour from an HBsAg-seronegative patient (Yuasa & Sudo, 1987); and co-amplification of integrated HBV sequences and *hst*-1 was reported in another case (Hatada *et al.*, 1988). Conflicting results have been obtained concerning the *ras* genes in the NIH3T3 cell transformation assay. Direct sequencing of the c-Ha-*ras*, c-Ki-*ras* and N-*ras* genes has shown a very low incidence of point mutations in liver tumours (Tsuda *et al.*, 1989; Tada *et al.*, 1990; Ogata *et al.*, 1991).

Increased expression of c-*myc* protooncogene has been described in a majority of human HCCs but also occurs in cirrhosis (Gu *et al.*, 1986; Himeno *et al.*, 1988). In rare cases, it was associated with genetic amplification of the c-*myc* locus (Trowbridge *et al.*, 1988).

4.3 Other observations relevant to possible mechanisms of action of HBV in carcinogenesis

4.3.1 *Cell division and tissue regeneration in response to HBV infection*

Increased incidences of HCC have been reported in association with liver diseases other than that caused by HBV infection, in which cirrhosis occurs with accompanying regenerative nodules (e.g. alcoholic cirrhosis, haemochromatosis, HCV-induced cirrhosis), suggesting that the active cell division associated with cirrhosis may contribute in an important way to the development of HCC (Colombo *et al.*, 1989; Simonetti *et al.*, 1992). Although cirrhosis is not essential to HCC (see above), the necrosis and inflammation associated with both chronic active hepatitis and cirrhosis may be important. This would explain the absence of tumours in chronically HBV-infected chimpanzees (see 3.1.1(*a*)) in which inflammatory changes are usually limited to the portal spaces and cell necrosis is normally absent or minimal (Shouval *et al.*, 1980). In support of this view is the high incidence of HCC observed in mutant LEC rats in which fulminant hepatitis occurs four months after birth, followed by chronic hepatitis and HCC in association with low levels of ceruloplasmin and heavy hepatic copper deposits (Ono *et al.*, 1991). The changes that occur in the lineages of transgenic mice in which HBsAg accumulates and induces cell necrosis and regeneration, described above, constitute yet another observation in support of this hypothesis (Chisari *et al.*, 1989).

4.3.2 *Immune response*

In patients with chronic HBV infection, it is now clear that immunological mechanisms are involved in the lysis of infected hepatocytes. At least in acute HBV infection, CD8 cytotoxic T lymphocytes that recognize hepatocytes which express HBV core peptides in association with major histocompatibility class I proteins have been demonstrated (Penna *et al.*, 1991). In chronic HBV infection, however, cytotoxic T cells are difficult to demonstrate. It is suggested that the secretion of processed nucleocapsid proteins, such as HBeAg, are involved in inducing tolerance of T cells that can react to HBe and HBc proteins (Thomas *et al.*, 1988). During the course of chronic infection, e antigen/antibody seroconversion may occur at a rate of approximately 5–10% of cases per year. This results in elimination of hepatocytes, indicating that HBV replication perhaps occurs by immune recognition of HBe by anti-HBe. In some patients, e-negative virus then emerges, and viraemia and inflammatory liver disease continue (Carman *et al.*, 1993). These patients progress to cirrhosis, and it has been argued that the associated cycles of liver-cell necrosis and regeneration regulated by growth factors contribute to the multi-step process that leads to the development of HCC in patients with chronic hepatitis with or without cirrhosis. The immunological selection pressure operating against the hepatocytes that support productive HBV infection (expressing nucleocapsid proteins) will favour selective regeneration of uninfected hepatocytes and of hepatocytes containing only integrated HBV sequences, not expressing c and e but perhaps expressing HBsAg (Fowler *et al.*, 1986). These cells are under a strong regenerative stimulus, and it is proposed that it is these cells that give rise to regenerative nodules and HCC (Shafritz *et al.*, 1981; Shafritz, 1982; Thomas *et al.*, 1982).

4.3.3 *Hepatocellular carcinoma-associated tumour markers*

The best-studied marker of HCC is α-fetoprotein, which is used to diagnose the tumour (Abelev, 1974). Whether there is a specific relationship between HBV and expression of this protein is uncertain (Chan *et al.*, 1980; Trichopoulos *et al.*, 1980b; Kew & Macerollo, 1988).

4.3.4 *Role of aflatoxins and possible modification of the effect of HBV*

Little information is available as to whether there is a biological interaction between HBV and aflatoxin, although it is established that aflatoxin is mutagenic, and both factors can induce liver injury resulting in increased cell proliferation. One hypothesis is that HBV infection and the associated hepatitis may alter carcinogen metabolism, and some evidence has been provided in humans and woodchucks to support this idea (De Flora *et al.*, 1985, 1989). Aflatoxin B_1 is immunosuppressive (Pier & McLoughlin, 1985; Richard, 1991), but its influence on the immune response to HBV infection has not been investigated.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Hepatitis B virus (HBV) is a small DNA virus made up of an outer envelope, bearing hepatitis B surface antigen (HBsAg), and an internal nucleocapsid. The nucleocapsid contains the hepatitis B core antigen (HBcAg), DNA polymerase/reverse transcriptase and the viral DNA genome. The viral genome is a circular, partially double-stranded DNA molecule about 3.2 kilobases long. It has four open reading frames, which encode for the different viral antigens, including hepatitis B e antigen (HBeAg) and hepatitis B x antigen (HBxAg), and replicates asymmetrically by reverse transcription of an RNA intermediate. Naturally occurring HBV mutants have been identified, but their pathobiological significance has not been defined. HBV belongs to a group of hepatotropic DNA viruses (hepadnaviruses) which include the hepatitis viruses of the woodchuck (*Marmota monax*), Beechey ground squirrel (*Spermophilus beecheyi*) and domestic duck (*Anas domesticus*). These viruses are highly species specific; they infect primarily hepatocytes.

Current serological methods of detection are highly sensitive and specific and are based on the detection of viral antigens, antibodies to viral antigens and viral DNA. The presence of HBsAg or HBV DNA indicates current HBV infection. The presence of HBeAg indicates a high level of viral replication. Seroconversion to anti-HBe is usually associated with reductions in replication and in disease activity. The presence of immunoglobulin M class anti-HBc indicates acute HBV infection; the immunoglobulin G class anti-HBc appears after acute HBV infection and persists during chronic HBV infection.

Transmission of infection in areas of high prevalence is predominantly between children; mother-to-child (perinatal) transmission plays a particularly important role in Asia. The modes of transmission in childhood are unclear. In areas of intermediate and low endemicity, the pattern of perinatal, childhood and adult infection is mixed. In adults, sexual transmission is a major mode of transmission, although intravenous use of drugs plays an important role in

some populations. In many cases in areas of low endemicity, the mode of transmission is unknown.

The course and clinical manifestations of HBV infection are highly variable and depend on age at infection, gender, the immune competence of the host and, possibly, viral factors. Infection perinatally and in early childhood is the major risk factor for chronicity, which frequently leads to progressive liver disease and cirrhosis.

The prevalence of chronic HBV infection varies markedly around the world. High rates of infection, defined as prevalences $\geq 8\%$, occur in China, Southeast Asia, the Pacific Basin, sub-Saharan Africa and the Amazon Basin. In western Europe, North America, Australia and New Zealand, the prevalences of chronic infection are low ($< 2\%$), and infection occurs predominantly in adults. Intermediate prevalences of infection, between 2 and 7%, occur elsewhere in the world.

The incidence of infection is reduced by vaccination with plasma-derived or recombinant vaccines, which are highly immunogenic and confer long-lasting protection against acute hepatitis and chronic infection. The efficacy of vaccines against chronic infection is in excess of 85% in regions where child and adult infection predominate and greater than 70% in regions where perinatal infection plays an important role. The efficacy of vaccination in preventing perinatal infection is improved by the addition of hepatitis B immunoglobulin administration soon after birth.

5.2 Human carcinogenicity data

In 15 cohort studies, carrier status for HBV was determined by the presence of HBsAg in serum. In all studies, the risk for hepatocellular carcinoma increased in association with HBsAg seropositivity, with estimates of relative risk ranging from 5.3 to 148.

Many case–control studies have been reported on the association between hepatocellular carcinoma and chronic infection with HBV, as determined by HBsAg seropositivity. Most of the studies were conducted in Asia and in Africa, but some have been reported from Europe and North America. The studies were of variable quality, but the majority showed a strong association, with relative risks between 5 and 30.

Potential confounding by aflatoxin, infection with hepatitis C virus, cigarette smoking and alcohol drinking appears to have been excluded in studies in which those factors were evaluated.

Serological patterns of HBV markers other than HBsAg, such as anti-HBc and anti-HBs, have been examined in many studies, but variability in methods of determination and reporting of results precluded evaluation of their association with hepatocellular carcinoma.

In general, cohort studies have not reported increased risks for cancers other than hepatocellular carcinoma. No consistent evidence of increased risk was found in case–control studies of other cancers (including cholangiocarcinoma of the liver).

5.3 Animal carcinogenicity data

Hepatitis B virus

Studies over the past two decades have shown that chimpanzees can be infected with HBV and can become carriers, exhibiting mild hepatitis. Progressive liver disease, including

hepatocellular carcinoma, is not known to develop in HBV-infected chimpanzees, although reporting of long-term studies of infected animals is sparse and inadequate. A single report suggested that Asian macaques are susceptible to HBV infection and to progressive liver lesions; a possible hepatocellular carcinoma developed in an HBV-infected macaque.

In three studies in transgenic mice on the expression of integrated HBV genes (pre-S, S and/or X genes) in hepatocytes, increased numbers of liver tumours were associated with a high level of expression of the large surface antigen and X proteins. The relevance of the finding that hepatocellular carcinomas are produced in these transgenic mice for evaluating the carcinogenicity of HBV is unclear.

Other hepadnaviruses

Woodchucks are susceptible to infection with the related hepadnaviruses, woodchuck and ground squirrel hepatitis viruses (WHV and GSHV), both of which lead to chronic hepatitis but not to cirrhosis. In one study of naturally infected, captive adults, one study of experimentally infected adults and newborns and one study of experimentally infected newborns, infection with WHV was associated with development of hepatocellular carcinoma in up to 85% of woodchucks with chronic infection. Uninfected animals did not develop hepatocellular carcinoma. Newborn woodchucks experimentally infected with GSHV also developed hepatocellular carcinoma. Beechey ground squirrels are susceptible to infection with GSHV, with the development of mild chronic hepatitis but not cirrhosis. In one study of Beechey ground squirrels captured in the wild, 11/24 (45%) animals naturally infected with GSHV developed hepatocellular carcinoma, while 2/26 (8%) uninfected animals developed the tumour. One study showed that captive Richardson ground squirrels may be infected with a similar but poorly characterized hepadnavirus, but the association of viral infection and hepatocellular carcinoma in this species has not been firmly demonstrated. Domestic ducks are susceptible to infection with a hepadnavirus, duck hepatitis B virus (DHBV). Hepatocellular carcinoma has been observed in free-ranging ducks infected with DHBV, but in three studies of experimentally infected animals and one study of congenitally infected ducks, no increase in the incidence of hepatocellular carcinoma was observed.

5.4 Other relevant data

The mechanisms whereby HBV may induce hepatocellular carcinoma are uncertain. HBV does not contain a known oncogene. HBV DNA is integrated into host DNA in the great majority of hepatocellular carcinomas in HBV carriers, and chromosomal translocations associated with integrated HBV sequences have been reported. In only three cases of hepatocellular carcinoma have HBV DNA sequences been shown to be integrated into any known host gene. This molecular event is, however, common in woodchucks: in about 50% of hepatocellular carcinomas arising in animals infected chronically with WHV, viral DNA sequences were integrated in or adjacent to c-*myc* or N-*myc* genes. In humans, sequences of the S and X genes of HBV are almost always present in integrated HBV DNA, and X gene protein has been shown to *trans*-activate both HBV and cellular genes. There is

no well documented evidence for overexpression of known oncogenes as a result of HBV DNA integration in human hepatocellular carcinoma. Deletions on multiple chromosomes and mutations of the *p53* tumour suppressor gene occur in hepatocellular carcinoma, but no pattern of these changes has been found to be specific to hepatocellular carcinomas arising in chronically HBV-infected humans.

The great majority of hepatocellular carcinomas that arise in association with chronic HBV infection occur in conjunction with cirrhosis or chronic hepatitis. Chronic HBV infection is generally established in early childhood, and several decades of chronic hepatitis usually precede development of the cancer. Studies of HBV integration have demonstrated that many regenerative nodules in cirrhotic liver have independent clonal origins; clonal regeneration reflects the extensive cell turnover that renders host DNA more susceptible to mutagenesis.

5.5 Evaluation[1]

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with hepatitis B virus.

There is *inadequate evidence* in experimental animals for the carcinogenicity of hepatitis B virus. Some hepadnaviruses closely related to hepatitis B virus produce hepatocellular carcinoma in susceptible species.

Overall evaluation

Chronic infection with hepatitis B virus *is carcinogenic to humans (Group 1).*

6. References

Abelev, G.I. (1974) Alpha-fetoprotein as a marker of embryo-specific differentiations in normal and tumor tissues. *Transplant. Rev.*, **20**, 3–37

Akahane, Y., Yamanaka, T., Suzuki, H., Sugai, Y., Tsuda, F., Yotsumoto, S., Omi, S., Okamoto, H., Miyakawa, Y. & Mayumi, M. (1990) Chronic active hepatitis with hepatitis B virus DNA and antibody against e antigen in the serum. Disturbed synthesis and secretion of e antigen from hepatocytes due to a point mutation in the precore region. *Gastroenterology*, **99**, 1113–1119

Alberts, S.R., Lanier, A.P., McMahon, B.J., Harpster, A., Bulkow, L.R., Heyward, W.L. & Murray, C. (1991) Clustering of hepatocellular carcinoma in Alaska native families. *Gen. Epidemiol.*, **8**, 127–139

Alexander, G.J.M., Brahm, J., Fagan, E.A., Smith, H.M., Daniels, H.M., Eddleston, A.L.W.F. & Williams, R. (1987) Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. *Lancet*, **ii**, 66–69

Al-Sarraf, M., Kithier, K., Sardesai, S., Vaitkevicius, V.K. & Poulik, M.D. (1973) The incidence and significance of Australia antigen in cancer patients. *Oncology*, **27**, 128–136

[1]For definition of the italicized terms, see Preamble, pp. 30–34.

Alter, M.J., Coleman, P.J., Alexander, W.J., Kramer, E., Miller, J.K., Mandel, E., Hadler, S.C. & Margolis, H.S. (1989) Importance of heterosexual activity in the transmission of hepatitis B and non-A, non-B hepatitis. *J. Am. med. Assoc.*, **262**, 1201–1205

Alter, M.J., Hadler, S.C., Margolis, H.S., Alexander, W.J., Hu, P.Y., Judson, F.N., Mares, A., Miller, J.K. & Moyer, L.A. (1990) The changing epidemiology of hepatitis B in the United States. Need for alternative vaccination strategies. *J. Am. med. Assoc.*, **263**, 1218–1222

Alward, W.L.M., McMahon, B.J., Hall, D.B., Heyward, W.L., Francis, D.P. & Bender, T.R. (1985) The long-term serological course of asymptomatic hepatitis B virus carriers and the development of primary hepatocellular carcinoma. *J. infect. Dis.*, **151**, 604–609

Anthony, P.P. (1984) Hepatocellular carcinoma: an overview. In: Williams, A.O., O'Conor, G.T., de-Thé, G.B. & Johnson, C.A., eds, *Virus-associated Cancers in Africa* (IARC Scientific Publications No. 63), Lyon, IARC, pp. 3–58

Arakawa, M., Kage, M., Sugihara, S., Nakashima, T., Suenaga, M. & Okuda, K. (1986) Emergence of malignant lesions within an adenomatous hyperplastic nodule in a cirrhotic liver. Observations in five cases. *Gastroenterology*, **91**, 198–208

Araki, K., Miyazaki, J.-I., Hino, O., Tomita, N., Chisaka, O., Matsubara, K. & Yamamura, K.-I. (1989) Expression and replication of hepatitis B virus genome in transgenic mice. *Proc. natl Acad. Sci. USA*, **86**, 207–211

Arya, S.C., Ashraf, S.J., Parande, C.M., Tobeiqi, M.S. & Ageel, A.R. (1988) Hepatitis B and delta markers in primary hepatocellular carcinoma patients in the Gizan area of Saudi Arabia. *Acta pathol. microbiol. immunol. Scand.*, **Suppl. 3**, 30–34

Austin, H., Delzell, E., Grufferman, S., Levine, R., Morrison, A.S., Stolley, P.D. & Cole, P. (1986) A case–control study of hepatocellular carcinoma and the hepatitis B virus, cigarette smoking, and alcohol consumption. *Cancer Res.*, **46**, 962–966

Babinet, C., Farza, H., Morello, D., Hadchouel, M. & Pourcel, C. (1985) Specific expression of hepatitis B surface antigen (HBsAg) in transgenic mice. *Science*, **230**, 1160–1163

Balsano, C., Avantaggiati, M.L., Natoli, G., De Marzio, E., Elfassi, E., Will, H. & Levrero, M. (1991) Transactivation of c-*fos* and c-*myc* protooncogenes by both full-length and truncated versions of the HBV-X protein. In: Hollinger, F.B., Lemon, S.M. & Margolis, H.S., eds, *Viral Hepatitis and Liver Disease*, Baltimore, Williams & Wilkins, pp. 572–576

Bancroft, W.H., Mundon, F.K. & Russell, P.K. (1972) Detection of additional antigenic determinants of hepatitis B antigen. *J. Immunol.*, **109**, 842–848

Barin, F., Perrin, J., Chotard, J., Denis, F., N'Doye, R., Mar, I.D., Chiron, J.-P., Coursaget, P., Goudeau, A. & Maupas, P. (1981) Cross-sectional and longitudinal epidemiology of hepatitis B in Senegal. *Prog. med. Virol.*, **27**, 148–162

Barker, L.F., Maynard, J.E., Purcell, R.H., Hoofnagle, J.H., Berquist, K.R., London, W.T., Gerety, R.J. & Krushak, D.H. (1975) Hepatitis B virus infection in chimpanzees: titration of subtypes. *J. infect. Dis.*, **132**, 451–458

Bartenschlager, R., Kuhn, C. & Schaller, H. (1992) Expression of the P-protein of the human hepatitis B virus in a vaccinia virus system and detection of the nucleocapsid-associated P-gene product by radiolabelling at newly introduced phosphorylation sites. *Nucleic Acids Res.*, **20**, 195–202

Bassendine, M.F., Della Seta, L., Salmeron, J., Thomas, H.C. & Sherlock, S. (1983) Incidence of hepatitis B virus infection in alcoholic liver disease, HBsAg negative chronic active liver disease and primary liver cell cancer in Britain. *Liver*, **3**, 65–70

Beasley, R.P. & Hwang, L.-Y. (1991) Overview on the epidemiology of hepatocellular carcinoma. In: Hollinger, F.B., Lemon, S.M. & Margolis, H.S., eds, *Viral Hepatitis and Liver Disease*, Baltimore, Williams & Wilkins, pp. 532–535

Beasley, R.P. & Lin, C.-C. (1978) Hepatoma risk among HBsAg carriers (Abstract). *Am. J. Epidemiol.*, **108**, 247

Beasley, R.P., Trepo, C., Stevens, C.E. & Szmuness, W. (1977) The e antigen and vertical transmission of hepatitis B surface antigen. *Am. J. Epidemiol.*, **105**, 94–98

Beasley, R.P., Hwang, L.-Y., Lin, C.-C. & Chien, C.-S. (1981) Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet*, **ii**, 1129-1133

Beasley, R.P., Lin, C.-C., Chien, C.-S., Chen, C.-J. & Hwang, L.-Y. (1982) Geographic distribution of HBsAg carriers in China. *Hepatology*, **2**, 553–556

Beasley, R.P., Hwang, L.-Y., Stevens, C.E., Lin, C.-C., Hsieh, F.-J., Wang, K.-Y., Sun, T.-S. & Szmuness, W. (1983) Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind, placebo-controlled trial. *Hepatology*, **3**, 135–141

Bensabath, G., Hadler, S.C., Pereira Soares, M.C., Fields, H. & Maynard, J.E. (1987) Epidemiologic and serologic studies of acute viral hepatitis in Brazil's Amazon basin. *Pan Am. Health Org. Bull.*, **21**, 16–27

Berger, I. & Shaul, Y. (1987) Integration of hepatitis B virus: analysis of unoccupied sites. *J. Virol.*, **61**, 1180–1186

Bernier, R.H., Sampliner, R., Gerety, R., Tabor, E., Hamilton, F. & Nathanson, N. (1982) Hepatitis B infection in households of chronic carriers of hepatitis B surface antigen. *Am. J. Epidemiol.*, **116**, 199–211

Bhat, R.A., Ulrich, P.P. & Vyas, G.N. (1990) Molecular characterization of a new variant of hepatitis B virus in a persistently infected homosexual man. *Hepatology*, **11**, 271–276

Blum, H.E., Stowring, L., Figus, A., Montgomery, C.K., Haase, A.T. & Vyas, G.N. (1983) Detection of hepatitis B virus DNA in hepatocytes, bile duct epithelium, and vascular elements by in situ hybridization. *Proc. natl Acad. Sci. USA*, **80**, 6685–6688

Blum, H.E., Gerok, W. & Vyas, G.N. (1989a) The molecular biology of hepatitis B virus. *Trends Genet.*, **5**, 154–158

Blum, H.E., Walter, E., Teubner, K., Offensperger, W.-B., Offensperger, S. & Gerok, W. (1989b) Hepatitis B virus in non-hepatocytes. In: Bannasch, P., Keppler, D. & Weber, G., eds, *Liver Cell Carcinoma*, Dordrecht, Kluwer Academic Publishers, pp. 169–183

Blum, H.E., Liang, T.J. & Wands, J.R. (1990) Hepatitis B virus carrier and hepatocellular carcinoma. In: Bianchi, L., Gerok, W., Maier, K.-P. & Deinhardt, F., eds, *Infectious Diseases of the Liver*, Dordrecht, Kluwer Academic Publishers, pp. 261–273

Blum, H.E., Galun, E., Liang, T.J., von Weizsäcker, F. & Wands, J.R. (1991) Naturally occurring missense mutation in the polymerase gene terminating hepatitis B virus replication. *J. Virol.*, **65**, 1836–1842

Blum, H.E., Zhang, Z.-S., Galun, E., von Weizsäcker, F., Garner, B., Liang, T.J. & Wands, J.R. (1992) Hepatitis B virus X protein is not central to the viral life cycle *in vitro*. *J. Virol.*, **66**, 1223–1227

Blumberg, B.S. & London, W.T. (1982) Hepatitis B virus: pathogenesis and prevention of primary cancer of the liver. *Cancer*, **50**, 2657–2665

Blumberg, B.S. & London, W.T. (1985) Hepatitis B virus and the prevention of primary cancer of the liver. *J. natl Cancer Inst.*, **74**, 267–273

Blumberg, B.S., Alter, H.J. & Visnich, S. (1965) A 'new' antigen in leukemia sera. *J. Am. med. Assoc.*, **191**, 101–106

Blumberg, B.S., Millman, I., Venkateswaran, P.S. & Thyagarajan, S.P. (1990) Hepatitis B virus and primary hepatocellular carcinoma: treatment of HBV carriers with *Phyllanthus amarus*. *Vaccine*, **8** (Suppl.), S86–S92

Boender, P.J., Schalm, S.W. & Heijtink, R.A. (1985) Detection of integration during active replication of hepatitis B virus in the liver. *J. med. Virol.*, **16**, 47–54

Bortolotti, F., Cadrobbi, P., Crivellaro, C., Guido, M., Rugge, M., Noventa, F., Calzia, R. & Realdi, G. (1990) Long-term outcome of chronic type B hepatitis in patients who acquire hepatitis B virus infection in childhood. *Gastroenterology*, **99**, 805–810

Bouffard, P., Lamelin, J.-P., Zoulim, F., Lepot, D. & Trepo, C. (1992) Phytohemagglutinin and concanavalin A activate hepatitis B virus in peripheral blood mononuclear cells of patients with chronic hepatitis B infection. *J. med. Virol.*, **37**, 255–262

Bowry, T.R. & Shah, M.V. (1980) A study of hepatitis Bs antigen titres and alpha-foetoprotein levels in primary hepatocellular carcinoma in Kenya. *East Afr. med. J.*, **57**, 382–389

Bowry, T.R., Shah, M.V. & Ahmad, Z. (1981) A controlled study of hepatitis core antibody (HBcAb) in primary hepatocellular carcinoma and liver cirrhosis in Kenya. *East Afr. med. J.*, **58**, 576–582

Bréchot, C. (1987) Hepatitis B virus (HBV) and hepatocellular carcinoma. HBV DNA status and its implications. *J. Hepatol.*, **4**, 269–279

Bréchot, C., Pourcel, C., Louise, A., Rain, B. & Tiollais, P. (1980) Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. *Nature*, **286**, 533–535

Bréchot, C., Hadchouel, M., Scotto, J., Fonck, M., Potet, F., Vyas, G.N. & Tiollais, P. (1981a) State of hepatitis B virus DNA in hepatocytes of patients with hepatitis B surface antigen-positive and -negative liver diseases. *Proc. natl Acad. Sci. USA*, **78**, 3906–3910

Bréchot, C., Hadchouel, M., Scotto, J., Degos, F., Charnay, P., Trépo, C. & Tiollais, P. (1981b) Detection of hepatitis B virus DNA in liver and serum: a direct appraisal of the chronic carrier state. *Lancet*, **ii**, 765–768

Bréchot, C., Pourcel, C., Hadchouel, M., Dejean, A., Louise, A., Scotto, J. & Tiollais, P. (1982) State of hepatitis B virus DNA in liver diseases. *Hepatology*, **2**, 27S–34S

Bréchot, C., Degos, F., Lugassy, C., Thiers, V., Zafrani, S., Franco, D., Bismuth, H., Trépo, C., Benhamou, J.-P., Wands, J., Isselbacher, K., Tiollais, P. & Berthelot, P. (1985) Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. *New Engl. J. Med.*, **312**, 270–276

Bréchot, C., Kremsdorf, D., Paterlini, P. & Thiers, V. (1991) Hepatitis B virus DNA in HBsAg-negative patients. Molecular characterization and clinical implications. *J. Hepatol.*, **13** (Suppl. 4), S49–S55

Bressac, B., Galvin, K.M., Liang, T.J., Isselbacher, K.J., Wands, J.R. & Ozturk, M. (1990) Abnormal structure and expression of p53 gene in human hepatocellular carcinoma. *Proc. natl Acad. Sci. USA*, **87**, 1973–1977

Bressac, B., Kew, M., Wands, J. & Ozturk, M. (1991) Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature*, **350**, 429–431

Brook, M.G., Karayiannis, P. & Thomas, H.C. (1989) Which patients with chronic hepatitis B virus infection will respond to alpha-interferon therapy? A statistical analysis of predictive factors. *Hepatology*, **10**, 761–763

Bruguera, M., Cremades, M., Salinas, R., Costa, J., Grau, M. & Sans, J. (1992) Impaired response to recombinant hepatitis B vaccine in HIV-infected persons. *J. clin. Gastroenterol.*, **14**, 27–30

Brunetto, M.R., Stemler, M., Bonino, F., Schodel, F., Oliveri, F., Rizzetto, M., Verme, G. & Will, H. (1990) A new hepatitis B virus strain in patients with severe anti-HBe positive chronic hepatitis B. *J. Hepatol.*, **10**, 258–261

Buendia, M.A. (1992) Hepatitis B viruses and hepatocellular carcinoma. *Adv. Cancer Res.*, **59**, 167–226

Buetow, K.H., Murray, J.C., Israel, J.L., London, W.T., Smith, M., Kew, M., Blanquet, V., Bréchot, C., Redeker, A. & Govindarajah, S. (1989) Loss of heterozygosity suggests tumor suppressor gene responsible for primary hepatocellular carcinoma. *Proc. natl Acad. Sci. USA*, **86**, 8852–8856

Buetow, K.H., Sheffield, V.C., Zhu, M., Zhou, T., Shen, F.-M., Hino, O., Smith, M., McMahon, B.J., Lanier, A.P., London, W.T., Redeker, A.G. & Govindarajan, S. (1992) Low frequency of p53 mutations observed in a diverse collection of primary hepatocellular carcinomas. *Proc. natl Acad. Sci. USA*, **89**, 9622–9626

Bunyaratvej, S., Rochanawutanon, M. & Chaimuangraj, S. (1979) Hepatitis B surface antigen in liver tissue and primary liver carcinomas in Thailand. *J. med. Assoc. Thailand*, **62**, 414–421

Burk, R.D., DeLoia, J.A., El Awady, M.K. & Gearhart, J.D. (1988) Tissue preferential expression of the hepatitis B virus (HBV) surface antigen gene in two lines of HBV transgenic mice. *J. Virol.*, **62**, 649–654

Carman, W.F., Jacyna, M.R., Hadziyannis, S., Karayiannis, P., McGarvey, M.J., Makris, A. & Thomas, H.C. (1989) Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet*, **ii**, 588–591

Carman, W.F., Zanetti, A.R., Karayiannis, P., Waters, J., Manzillo, G., Tanzi, E., Zuckerman, A.J. & Thomas, H.C. (1990) Vaccine-induced escape mutant of hepatitis B virus. *Lancet*, **336**, 325–329

Carman, W.F., Fagan, E.A., Hadziyannis, S., Karayiannis, P., Tassopoulos, N.C., Williams, R. & Thomas, H.C. (1991a) Association of a precore genomic variant of hepatitis B virus with fulminant hepatitis. *Hepatology*, **14**, 219–222

Carman, W.F., Hadziyannis, S., Karayiannis, P., Fagan, E.A., Tassopoulos, N.C., Williams, R. & Thomas, H.C. (1991b) Association of the precore variant of HBV with acute and fulminant hepatitis B infection. In: Hollinger, F.B., Lemon, S.M. & Margolis, H., eds, *Viral Hepatitis and Liver Disease*, Baltimore, Williams & Wilkins, pp. 216–219

Carman, W., Thomas, H. & Domingo, E. (1993) Viral genetic variation: hepatitis B virus as a clinical example. *Lancet*, **341**, 349–353

Carreño, V., Castillo, I., Molina, J., Porres, J.C. & Bartolomé, J. (1992) Long-term follow-up of hepatitis B chronic carriers who responded to interferon therapy. *J. Hepatol.*, **15**, 102–106

Caselmann, W.H., Eisenburg, J., Hofschneider, P.H. & Koshy, R. (1989) Beta- and gamma-interferon in chronic active hepatitis B. A pilot trial of short-term combination therapy. *Gastroenterology*, **96**, 449–455

Caselmann, W.H., Meyer, M., Kekulé, A.S., Lauer, U., Hofschneider, P.H. & Koshy, R. (1990) A trans-activator function is generated by integration of hepatitis B virus preS/S sequences in human hepatocellular carcinoma DNA. *Proc. natl Acad. Sci. USA*, **87**, 2970–2974

Catterall, A.P., Moyle, G.J., Hopes, E.A., Harrison, T.J., Gazzard, B.G. & Murray-Lyon, I.M. (1992) Dideoxyinosine for chronic hepatitis B infection. *J. med. Virol.*, **37**, 307–309

Chainuvati, T., Viranuvatti, V. & Pongpipat, D. (1975) Relationship of hepatitis B antigen in cirrhosis and hepatoma in Thailand. An etiological significance. *Gastroenterology*, **68**, 1261–1264

Chakraborty, P.R., Ruiz-Opazo, H., Shouval, D. & Shafritz, D.A. (1980) Identification of integrated hepatitis B virus DNA and expression of viral RNA in an HBsAg-producing human hepatocellular carcinoma cell line. *Nature*, **286**, 531–533

Challen, C., Lunec, J., Warren, W., Collier, J. & Bessendine, M.F. (1992) Analysis of the p53 tumor-suppressor gene in hepatocellular carcinomas from Britain. *Hepatology*, **16**, 1362–1366

Chan, S.H., Simons, M.J. & Oon, C.J. (1980) HLA antigen in Chinese patients with hepatocellular carcinomas. *J. natl Cancer Inst.*, **65**, 21–23

Chang, M.-H., Chen, P.-J., Chen, J.-Y., Lai, M.-Y., Hsu, H.-C., Lian, D.-C., Liu, Y.-G. & Chen, D.-S. (1991) Hepatitis B virus integration in hepatitis B virus-related hepatocellular carcinoma in childhood. *Hepatology*, **13**, 316–320

Chau, K.H., Hargie, M.P., Decker, R.H., Mushahwar, I.K. & Overby, L.R. (1983) Serodiagnosis of recent hepatitis B infection by IgM class anti-HBc. *Hepatology*, **3**, 142–149

Chen, D.-S. & Sung, J.L. (1978) Hepatitis B virus infection and chronic liver disease in Taiwan. *Acta hepato-gastroenterol.*, **25**, 423–430

Chen, D.-S., Hsu, N.H.-M., Sung, J.-L., Hsu, T.-C., Hsu, S.-T., Kuo, Y.-T., Lo, K.-J., Shih, Y.-T. & the Hepatitis Steering Committee and the Hepatitis Control Committee (1987) A mass vaccination program in Taiwan against hepatitis B virus infection in infants of hepatitis B surface antigen-carrier mothers. *J. Am. med. Assoc.*, **257**, 2597–2603

Chen, C.-J., Liang, K.-Y., Chang, A.-S., Chang, Y.-C., Lu, S.-N., Liaw, Y.-F., Chang, W.-Y., Sheen, M.-W. & Lin, T.-M. (1991) Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. *Hepatology*, **13**, 398–406

Chen, H.-S., Kew, M.C., Hornbuckle, W.E., Tennant, B.C., Cote, P.J., Gerin, J.L., Purcell, R.H. & Miller, R.H. (1992) The precore gene of the woodchuck hepatitis virus genome is not essential for viral replication in the natural host. *J. Virol.*, **66**, 5682–5684

Chen, Y., Robinson, W.S. & Marion, P.L. (1992) Naturally occurring point mutation in the C terminus of the polymerase gene prevents duck hepatitis B virus RNA packaging. *J. Virol.*, **66**, 1282–1287

Chen, H.-S., Kaneko, S., Girones, R., Anderson, R.W., Hornbuckle, W.E., Tennant, B.C., Cote, P.J., Gerin, J.L., Purcell, R.H. & Miller, R.H. (1993) The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. *J. Virol.*, **67**, 1218–1226

Chien, M.-C., Tong, M.J., Lo, K.-J., Lee, J.-K., Milich, D.R., Vyas, G.N. & Murphy, B.L. (1981) Hepatitis B viral markers in patients with primary hepatocellular carcinoma in Taiwan. *J. natl Cancer Inst.*, **66**, 475–479

Chisari, F.V. (1991) Analysis of hepadnavirus gene expression, biology and pathogenesis in the transgenic mouse. *Curr. Top. Microbiol. Immunol.*, **168**, 85–101

Chisari, F.V., Pinkert, C.A., Milich, D.R., Filippi, P., McLachlan, A., Palmiter, R.D. & Brinster, R.L. (1985) A transgenic mouse model of the chronic hepatitis B surface antigen carrier state. *Science*, **230**, 1157–1160

Chisari, F.V., Filippi, P., McLachlan, A., Milich, D.R., Riggs, M., Lee, S., Palmiter, R.D., Pinkert, C.A. & Brinster, R.L. (1986) Expression of hepatitis B virus large envelope polypeptide inhibits hepatitis B surface antigen secretion in transgenic mice. *J. Virol.*, **60**, 880–887

Chisari, F.V., Filippi, P., Buras, J., McLachlan, A., Popper, H., Pinkert, C.A., Palmiter, R.D. & Brinster, R.L. (1987) Structural and pathological effects of synthesis of hepatitis B virus large envelope polypeptide in transgenic mice. *Proc. natl Acad. Sci. USA*, **84**, 6909–6913

Chisari, F.V., Klopchin, K., Moriyama, T., Pasquinelli, C., Dunsford, H.A., Sell, S., Pinkert, C.A., Brinster, R.L. & Palmiter, R.D. (1989) Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. *Cell*, **59**, 1145–1156

Christenson, B. (1986) Epidemiological aspects of the transmission of hepatitis B by HBsAg positive adopted children. *Scand. J. infect. Dis.*, **18**, 105–109

Chuang, W.-L., Chang, W.-Y., Lu, S.-N., Su, W.-P., Lin, Z.-Y., Chen, S.-C., Hsieh, M.-Y., Wang, L.-Y., You, S.-L. & Chen, C.-J. (1992) The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area: a case-control study. *Cancer*, **69**, 2052–2054

Chung, W.K., Sun, H.S., Park, D.H., Minuk, G.Y. & Hoofnagle, J.H. (1983) Primary hepatocellular carcinoma and hepatitis B virus infection in Korea. *J. med. Virol.*, **11**, 99–104

Collier, A.C., Corey, L., Murphy, V.L. & Handsfield, H.H. (1988) Antibody to human immunodeficiency virus (HIV) and suboptimal response to hepatitis B vaccination. *Ann. intern. Med.*, **109**, 101–105

Colloredo Mels, G., Paris, B., Bertone, V. & Angeli, G. (1986) Primary hepatocellular carcinoma: epidemiological case–control study in the province of Bergamo. *Min. med.*, 77, 297–306 (in Italian)

Colombo, M. (1992) Hepatocellular carcinoma. *J. Hepatol.*, **15**, 225–236

Colombo, M., Kuo, G., Choo, Q.L., Donato, M.F., Del Ninno, E., Tommasini, M.A., Dioguardi, N. & Houghton, M. (1989) Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet*, **ii**, 1006–1008

Colombo, M., de Franchis, R., Del Ninno, E., Sangiovanni, A., De Fazio, C., Tommasini, M., Donato, M.F., Piva, A., Di Carlo, V. & Dioguardi, N. (1991) Hepatocellular carcinoma in Italian patients with cirrhosis. *New Engl. J. Med.*, **325**, 675–680

Cordier, S., Thuy, Le Thi Bich, Verger, P., Bard, D., Dai, Le Cao, Larouze, B., Dazza, M.C., Quinh, Hoang Trong & Abenhaim, L. (1993) Risk factors for hepatocellular carcinoma in Vietnam. *Int. J. Epidemiol.*, **55**, 196–201

Coursaget, P., Maupas, P., Goudeau, A., Chiron, J.-P., Drucker, J., Denis, F. & Diop-Mar, I. (1980) Primary hepatocellular carcinoma in intertropical Africa: relationship between age and hepatitis B virus etiology. *J. natl Cancer Inst.*, **65**, 687–690

Coursaget, P., Maupas, P., Goudeau, A., Chiron, J.-P., Raynaud, B., Drucker, J., Barin F., Denis, F., Diop-Mar, I. & Diop, B. (1981) A case/control study of hepatitis B virus serologic markers in Senegalese patients suffering from primary hepatocellular carcinoma. *Prog. med. Virol.*, **27**, 49–59

Coursaget, P., Chiron, J.P., Barres, J.L., Barin, F., Cottey, P., Tortey, E., Yvonnet, B., Diop, B., MBoup, S., Diop-Mar, I., Kocheleff, P., Perrin, J. & Duflo, B. (1984) Hepatitis B virus serological markers in Africans with liver cirrhosis and hepatocellular carcinoma. In: Williams, A.O., O'Conor, G.T., de-Thé, G.B. & Johnson, C.A., *Virus-associated Cancers in Africa* (IARC Scientific Publications No. 63), Lyon, IARC, pp. 181–198

Coursaget, P., Yvonnet, B., Barres, J.L., Perrin, J., Tortey, E., Diop, B., Kocheleff, P., Duflo, B., M'Boup, S., Diop-Mar, I., Bocande, J.E. & Chiron, J.-P. (1985) Hepatitis B virus and primary liver cancer in intertropical Africa. *Rev. Epidémiol. Santé publ.*, **33**, 267–275 (in French)

Coursaget, P., Yvonnet, B., Chotard, J., Sarr, M., Vincelot, P., N'Doye, R., Diop-Mar, I. & Chiron, J.-P. (1986) Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). *Lancet*, **ii**, 1143–1145

Coursaget, P., Yvonnet, B., Chotard, J., Vincelot, P., Sarr, M., Diouf, C., Chiron, J.-P. & Diop-Mar, I. (1987) Age- and sex-related study of hepatitis B virus chronic carrier state in infants from an endemic area (Senegal). *J. med. Virol.*, **22**, 1–5

Coursaget, P., Buisson, Y., Bourdil, C., Yvonnet, B., Molinié, C., Diop, M.T., Chiron, J.-P., Bao, O. & Diop-Mar, I. (1990) Antibody response to preS1 in hepatitis-B-virus-induced liver disease and after immunization. *Res. Virol.*, **141**, 563-570

Cova, L., Wild, C.P., Mehrotra, R., Turusov, V., Shirai, T., Lambert, V., Jacquet, C., Tomatis, L., Trépo, C. & Montesano, R. (1990) Contribution of aflatoxin B_1 and hepatitis B virus infection in the induction of liver tumors in ducks. *Cancer Res.*, **50**, 2156–2163

Craig, J.R., Klatt, E.C. & Yu, M. (1991) Role of cirrhosis and the development of HCC: evidence from histologic studies and large population studies. In: Tabor, E., Di Bisceglie, A.M. & Purcell, R.H., eds, *Etiology, Pathology, and Treatment of Hepatocellular Carcinoma in North America* (Advances in Applied Biotechnology Series Vol. 13), Houston, TX, Gulf Publishing, pp. 177–190

Craven, D.E., Awdeh, Z.L., Kunches, L.M., Yunis, E.J., Dienstag, J.L., Werner, B.G., Polk, B.F., Snydman, D.R., Platt, R., Crumpacker, C.S., Grady, G.F. & Alper, C.A. (1986) Nonresponsiveness to hepatitis B vaccine in health care workers. Results of revaccination and genetic typings. *Ann. intern. Med.*, **105**, 356–360

Cullen, J.M., Marion, P.L. & Newbold, J.E. (1989) A sequential histologic and immunohistochemical study of duck hepatitis B virus infection in Pekin ducks. *Vet. Pathol.*, **26**, 164–172

Cullen, J.M., Marion, P.L., Sherman, G.J., Hong, X. & Newbold, J.E. (1990) Hepatic neoplasms in aflatoxin B_1-treated, congenital duck hepatitis B virus-infected, and virus-free Pekin ducks. *Cancer Res.*, **50**, 4072–4080

Cullen, J.M., Newbold, J.E. & Sherman, G.J. (1991) Teratoma in a duck infected congenitally with duck hepatitis B virus. *Avian Dis.*, **35**, 61–64

D'Amelio, R., Matricardi, P.M., Biselli, R., Stroffolini, T., Mele, A., Spada, E., Chionne, P., Rapicetta, M., Ferrigno, L. & Pasquini, P. (1992) Changing epidemiology of hepatitis B in Italy: public health implications. *Am. J. Epidemiol.*, **135**, 1012–1018

Davis, G.L. (1989) Interferon treatment of viral hepatitis in immunocompromised patients. *Semin. Liver Dis.*, **9**, 267–272

De Flora, S., Romano, M., Basso, C., Serra, D., Astengo, M. & Picciotto, A. (1985) Metabolic activation of hepatocarcinogens in chronic hepatitis B. *Mutat. Res.*, **144**, 213–219

De Flora, S., Hietanen, E., Bartsch, H., Camoirano, A., Izzotti, A., Bagnasco, M. & Millman, I. (1989) Enhanced metabolic activation of chemical hepatocarcinogens in woodchucks infected with hepatitis B virus. *Carcinogenesis*, **10**, 1099–1106

De Franchis, R., Primignani, M., Vecchi, M., Zannier, P.M., Colombo, M., Colucci, G., Antoniozzi, F. & Tommasini, M. (1982) Serum HBV markers in hepatocellular carcinoma and chronic liver disease. *Ann. Sclavo*, **24**, 37–48 (in Italian)

De Franchis, R., Meucci, G., Vecchi, M., Tatarella, M., Colombo, M., Del Ninno, E., Rumi, M.G., Donato, M.F. & Ronchi, G. (1993) The natural history of asymptomatic hepatitis B surface antigen carriers. *Ann. intern. Med.*, **118**, 191–194

Degos, F. & Degott, C. (1989) Hepatitis in renal transplant recipients. *J. Hepatol.*, **9**, 114–123

Dejean, A. & de-Thé, H. (1990) Hepatitis B virus as an insertional mutagen in a human hepatocellular carcinoma. *Mol. biol. Med.*, **7**, 213–222

Dejean, A., Sonigo, P., Wain-Hobson, S. & Tiollais, P. (1984) Specific hepatitis B virus integration in hepatocellular carcinoma DNA through a viral 11-base-pair direct repeat. *Proc. natl Acad. Sci. USA*, **81**, 5350–5354

Dejean, A., Bougueleret, L., Grzeschik, K.-H. & Tiollais, P. (1986) Hepatitis B virus DNA integration in a sequence homologous to v-*erb*-A and steroid receptor genes in a hepatocellular carcinoma. *Nature*, **322**, 70–72

De Jongh, F.E., Janssen, H.L.A., De Man, R.A., Hop, W.C.J., Schalm, S.W. & Van Blankenstein, M. (1992) Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. *Gastroenterology*, **103**, 1630–1635

Diamantis, I.D., McGandy, C., Pult, I., Bühler, H., Schmid, M., Gudat, F. & Bianchi, L. (1992) Polymerase chain reaction detects hepatitis B virus DNA in paraffin-embedded liver tissue from patients sero- and histo-negative for active hepatitis B. *Virchows Arch. A. Pathol. Anat.*, **420**, 11–15

Di Bisceglie, A.M., Order, S.E., Klein, J.L., Waggoner, J.G., Sjogren, M.H., Kuo, G., Houghton, M., Choo, Q.-L. & Hoofnagle, J.H. (1991) The role of chronic viral hepatitis in hepatocellular carcinoma in the United States. *Am. J. Gastroenterol.*, **86**, 335–338

Dienstag, J.L., Stevens, C.E., Bhan, A.K. & Szmuness, W. (1982) Hepatitis B vaccine administered to chronic carriers of hepatitis B surface antigen. *Ann. intern. Med.*, **96**, 575–579

Ding, Z.G., Li, R.C., Gong, J., Feng, Y.Z., Cheng, K.L., Li, Y.P., Nong, Y.Z., Li, C.Z., Wei, L.D. & Li, Y.C. (1988) Epidemiological study on relationship between hepatitis B and liver cancer—a prospective study on development of liver cancer and distribution of HBsAg carriers and liver damage in persons in Guangxi. *Chin. J. Epidemiol.*, **9**, 220–223 (in Chinese)

Dodd, R.Y. & Nath, N. (1987) Increased risk for lethal forms of liver disease among HBsAg-positive blood donors in the United States. *J. virol. Methods*, **17**, 81–94

Dragani, T.A., Manenti, G., Farza, H., Della Porta, G., Tiollais, P. & Pourcel, C. (1990) Transgenic mice containing hepatitis B virus sequences are more susceptible to carcinogen-induced hepatocarcinogenesis. *Carcinogenesis*, **11**, 953–956

Dunsford, H.A., Sell, S. & Chisari, F.V. (1990) Hepatocarcinogenesis due to chronic liver cell injury in hepatitis B virus transgenic mice. *Cancer Res.*, **50**, 3400–3407

Dusheiko, G., Xu, J. & Zuckermann, A.J. (1992) Clinical diagnosis of hepatitis B infection: applications of the polymerase chain reaction. In: Becker, Y. & Darai, G., eds, *Frontiers of Virology*, Berlin, Springer Verlag, pp. 67–85

Edman, J.C., Gray, P., Valenzuela, P., Rall, L.B. & Rutter, W.J. (1980) Integration of hepatitis B virus sequences and their expression in a human hepatoma cell. *Nature*, **286**, 535–537

Edmunds, W.J., Medley, G.F., Nokes, D.J., Hall, A.J. & Whittle, H.C. (1993) The influence of age on the development of the hepatitis B carrier state. *Proc. R. Soc. Lond. B. Biol. Sci.*, **253**, 197–201

Ehata, T., Omata, M., Yokosuka, O., Hosoda, K. & Ohto, M. (1992) Variations in codons 84–101 in the core nucleotide sequence correlate with hepatocellular injury in chronic hepatitis B virus infection. *J. clin. Invest.*, **89**, 332–338

Elfassi, E., Haseltine, W.A. & Dienstag, J.L. (1986) Detection of hepatitis B virus X product using an open reading frame *Escherichia coli* expression vector. *Proc. natl Acad. Sci. USA*, **83**, 2219–2222

Fakunle, Y.M., Abdurrahman, M.B. & Whittle, H.C. (1980) Hepatitis-B virus infection in children and adults in northern Nigeria: a preliminary survey. *Trans. R. Soc. trop. Med. Hyg.*, **75**, 626–629

Farza, H., Hadchouel, M., Scotto, J., Tiollais, P., Babinet, C. & Pourcel, C. (1988) Replication and gene expression of hepatitis B virus in a transgenic mouse that contains the complete viral genome. *J. Virol.*, **62**, 4144–4152

Fattovich, G., Rugge, M., Brollo, L., Pontisso, P., Noventa, F., Guido, M., Alberti, A. & Realdi, G. (1986) Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. *Hepatology*, **6**, 167–172

Fattovich, G., Brollo, L., Alberti, A., Realdi, G., Pontisso, P., Giustina, G. & Ruol, A. (1990) Spontaneous reactivation of hepatitis B virus infection in patients with chronic type B hepatitis. *Liver*, **10**, 141–146

Fattovich, G., Brollo, L., Giustina, G., Noventa, F., Pontisso, P., Alberti, A., Realdi, G. & Ruol, A. (1991) Natural history and prognostic factors for chronic hepatitis type B. *Gut*, **32**, 294–298

Fattovich, G., Farci, P., Rugge, M., Brollo, L., Mandas, A., Pontisso, P., Giustina, G., Lai, M.E., Belussi, F., Busatto, G., Balestrieri, A., Ruol, A. & Alberti, A. (1992) A randomized controlled trial of lymphoblastoid interferon-alpha in patients with chronic hepatitis B lacking HBeAg. *Hepatology*, **15**, 584–589

Feitelson, M.A. (1992) Hepatitis B virus infection and primary hepatocellular carcinoma. *Clin. Microbiol. Rev.*, **5**, 275–301

Feitelson, M.A., Millman, I. & Blumberg, B.S. (1986a) Tree squirrel hepatitis B virus: antigenic and structural characterization. *Proc. natl Acad. Sci. USA*, **83**, 2994–2997

Feitelson, M.A., Millman, I., Halbherr, T., Simmons, H. & Blumberg, B.S. (1986b) A newly identified hepatitis B type virus in tree squirrels. *Proc. natl Acad. Sci. USA*, **83**, 2233–2237

Feitelson, M.A., Zhu, M., Duan, L.-X. & London, W.T. (1993) Hepatitis B x antigen and p53 are associated *in vitro* and in liver tissues from patients with primary hepatocellular carcinoma. *Oncogene*, **8**, 1109–1117

Féray, C., Zigneno, A.L., Samuel, D., Bismuth, A., Reynes, A., Tiollais, P., Bismuth, H. & Bréchot, C. (1990) Persistent hepatitis B virus infection of mononuclear blood cells without concomitant liver infection. *Transplantation*, **49**, 1155–1158

Ferrari, C., Penna, A., Giuberti, T., Tong, M.J., Ribera, E., Fiaccadori, F. & Chisari, F.V. (1987) Intrahepatic, nucleocapsid antigen-specific T cells in chronic active hepatitis B. *J. Immunol.*, **139**, 2050–2058

Ferrari, C., Penna, A., Bertoletti, A., Cavalli, A., Valli, A., Missale, G., Pilli, M., Marchelli, S., Giuberti, T. & Fiaccadori, F. (1992) Immune pathogenesis of hepatitis B. *Arch. Virol.*, **Suppl. 4**, 11–18

Ferrell, L., Wright, T., Lake, J., Roberts, J. & Ascher, N. (1992) Incidence and diagnostic features of macroregenerative nodules *vs.* small hepatocellular carcinoma in cirrhotic liver. *Hepatology*, **16**, 1372–1381

Filippazzo, M.G., Aragona, E., Cottone, M., Dardanoni, G., Lanzarone, F., Marenghini, A., Patti, C., Sciarrino, E., Simonetti, R., Tinè, F. & Pagliaro, L. (1985) Assessment of some risk factors for hepatocellular carcinoma: a case control study. *Stat. Med.*, **4**, 345–351

Fortuin, M., Chotard, J., Jack, A.D., Maine, N.P., Mendy, M., Hall, A.J., Inskip, H.M., George, M.O. & Whittle, H.C. (1993) Efficacy of hepatitis B vaccine in the Gambian expanded programme on immunization. *Lancet*, **341**, 1129–1131

Foster, O., Ajdukiewicz, A., Ryder, R., Whittle, H. & Zuckerman, A.J. (1984) Hepatitis B virus transmission in West Africa: a role for tropical ulcer? (Letter to the Editor). *Lancet*, **i**, 576-577

Fourel, G., Trépo, C., Bougueleret, L., Henglein, B., Ponzetto, A., Tiollais, P. & Buendia, M.-A. (1990) Frequent activation of N-*myc* genes by hepadnavirus insertion in woodchuck liver tumours. *Nature*, **347**, 294–298

Fowler, M.J.F., Thomas, H.C. & Monjardino, J. (1986) Cloning and analysis of integrated hepatitis B virus DNA of the *adr* subtype derived from a human primary liver cell carcinoma. *J. gen. Virol.*, **67**, 771-775

Fox, E., Abbatte, E.A., Said, S, Constantine, N.T., Wassef, H.H. & Woody, J.N. (1988) Viral hepatitis markers in Djibouti: an epidemiological survey. *Trans. R. Soc. trop. Med. Hyg.*, **82**, 750–752

Francis, D.P., Hadler, S.C., Thompson, S.E., Maynard, J.E., Ostrow, D.G., Altman, N., Braff, E.H., O'Malley, P., Hawkins, D., Judson, F.N., Penley, K., Nylund, T., Christie, G., Meyers, F., Moore, J.N., Jr, Gardner, A., Doto, I.L., Miller, J.H., Reynolds, G.H., Murphy, B.L., Schable, C.A., Clark, B.T., Curran, J.W. & Redeker, A.G. (1982) The prevention of hepatitis B with vaccine. Report of the Centers for Disease Control Multi-center Efficacy Trial among homosexual men. *Ann. intern. Med.*, **97**, 362–366

Freiman, J.S., Jilbert, A.R., Dixon, R.J., Holmes, M., Gowans, E.J., Burrell, C.J., Wills, E.J. & Cossart, Y.E. (1988) Experimental duck hepatitis B virus infection: pathology and evolution of hepatic and extrahepatic infection. *Hepatology*, **8**, 507–513

Freytag von Loringhoven, A., Koch, S., Hofschneider, P.H. & Koshy, R. (1985) Co-transcribed 3′ host sequences augment expression of integrated hepatitis B virus DNA. *EMBO J.*, **4**, 249–255

Fried, M.W., Korenman, J.C., Di Bisceglie, A.M., Park, Y., Waggoner, J.G., Mitsuya, H., Hartman, N.R., Yarchoan, R., Broder, S. & Hoofnagle, J.H. (1992) A pilot study of 2′,3′-dideoxyinosine for the treatment of chronic hepatitis B. *Hepatology*, **16**, 861–864

Friede, A., Harris, J.R., Kobayashi, J.M., Shaw, F.E., Jr, Shoemaker-Nawas, P.C. & Kane, M.A. (1988) Transmission of hepatitis B virus from adopted Asian children to their American families. *Am. J. public Health*, **78**, 26–29

Fujii, H., Moriyama, K., Sakamoto, N., Kondo, T., Yasuda, K., Hiraizumi, Y., Yamazaki, M., Sakaki, Y., Okochi, K. & Nakajima, E. (1992) Gly^{145} to Arg substitution in HBs antigen of immune escape mutant of hepatitis B virus. *Biochem. biophys. Res. Commun.*, **184**, 1152–1157

Fujimori, M., Tokino, T., Hino, O., Kitagawa, T., Imamura, T., Okamoto, E., Mitsunobu, M., Ishikawa, T., Nakagama, H., Harada, H., Yagura, M., Matsubara, K. & Nakamura, Y. (1991) Allelotype study of primary hepatocellular carcinoma. *Cancer Res.*, **51**, 89–93

Fukao, A. (1985) An epidemiological study on relationship between hepatitis B virus and hepatocellular carcinoma. *Jpn. J. Gastroenterol.*, **82**, 232–238 (in Japanese)

Ganem, D. & Varmus, H.E. (1987) The molecular biology of the hepatitis B virus. *Ann. Rev. Biochem.*, **56**, 651–693

Garcia, G., Smith, C.I., Weissberg, J.I., Eisenberg, M., Bissett, J., Nair, P.V., Mastre, B., Rosno, S., Roskamp, D., Waterman, K., Pollard, R.B., Tong, M.J., Brown, B.W., Jr, Robinson, W.S., Gregory, P.B. & Merigan, T.C. (1987) Adenine arabinoside monophosphate (vidarabine phosphate) in combination with human leukocyte interferon in the treatment of chronic hepatitis B. *Ann. intern. Med.*, **107**, 278–285

Gashau, W. & Mohammed, I. (1991) Hepatitis B viral markers in Nigerian patients with primary liver cancer. *Trop. geogr. Med.*, **43**, 64–67

Ge, X., Huang, G.-Y., Jie, C., Wang, S.-S., Huang, D.-C., Wei, L., Jiang, Y.-Z. & Li, R.-J. (1991) Studies on experimental infection of *Macaca assamensis* with human hepatitis B virus. In: Hollinger, F.B., Lemon, S.M. & Margolis, H.S., eds, *Viral Hepatitis and Liver Disease*, Baltimore, Williams & Wilkins, pp. 301–303

Gerin, J.L., Cote, P.J., Korba, B.E. & Tennant, B.C. (1989) Hepadnavirus-induced liver cancer in woodchucks. *Cancer Detect. Prev.*, **14**, 227–229

Girones, R. & Miller, R.H. (1989) Mutation rate of the hepadnavirus genome. *Virology*, **170**, 595–597

Gombe, C.M. (1984) HBsAg: marker of the relationship between hepatitis B virus and primary liver cancer: situation at Brazzaville in 1984. In: Williams, A.O., O'Conor, G.T., de-Thé, G.B. & Johnson, C.A., eds, *Virus-associated Cancers in Africa* (IARC Scientific Publications No. 63), Lyon, IARC, pp. 221–226 (in French)

Goudeau, A., Maupas, P., Dubois, F., Coursaget, P. & Bougnoux, P. (1981) Hepatitis B infection in alcoholic liver disease and primary hepatocellular carcinoma in France. *Prog. med. Virol.*, **27**, 26–34

Gu, J.-R., Hu, L.-F., Cheng, Y.-C. & Wan, D.-F. (1986) Oncogenes in human primary hepatic cancer. *J. cell. Physiol.*, **Suppl. 4**, 13–20

Guan, R., Yap, I., Wong, L., Tan, L.H., Oon, C.J. & Wee, A. (1989) Evidence of viral replication in HBsAg positive patients with hepatocellular carcinoma: measurement of serum hepatitis B virus deoxyribonucleic acid (HBV-DNA). *Ann. Acad. Med.*, **18**, 8–11

Günther, S., Meisel, H., Reip, A., Miska, S., Krüger, D.H. & Will, H. (1992) Frequent and rapid emergence of mutated pre-C sequences in HBV from e-antigen positive carriers who seroconvert to anti-HBe during interferon treatment. *Virology*, **187**, 271–279

Gyorkey, F., Melnick, J.L., Mirkovic, R., Cabral, G.A., Gyorkey, P. & Hollinger, F.B. (1977) Experimental carcinoma of liver in macaque monkeys exposed to diethylnitrosamine and hepatitis B virus. *J. natl Cancer Inst.*, **59**, 1451–1457

Hadler, S.C., Francis, D.P., Maynard, J.E., Thompson, S.E., Judson, F.N., Echenberg, D.F., Ostrow, D.G., O'Malley, P.M., Penley, K.A., Altman, N.L., Braff, E., Shipman, G.F., Coleman, P.J. & Mandel, E.J. (1986) Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. *New Engl. J. Med.*, **315**, 209–214

Hadziyannis, S.J., Merikas, G.E. & Afroudakis, A.P. (1970) Hepatitis-associated antigen in chronic liver disease (Letter to the Editor). *Lancet*, **i**, 100

Halevy, J., Achiron, A., Spiegel, D., Nili, M., Luboshits, J., Yerushalmi, Y. & Theodor, E. (1990) Recombinant alpha-interferon may be efficacious in acute hepatitis B. *Am. J. Gastroenterol.*, **85**, 210–212

Hall, A.J., Winter, P.D. & Wright, R. (1985) Mortality of hepatitis B positive blood donors in England and Wales. *Lancet*, **i**, 91–93

Halliday, M.L., Kang, L.-Y., Rankin, J.G., Coates, R.A., Corey, P.N.J., Hu, Z.-H., Zhou, T.-K., Yuan, G.-J. & Yao, F.-L. (1992) An efficacy trial of a mammalian cell-derived recombinant DNA hepatitis B vaccine in infants born to mothers positive for HBsAg, in Shanghai, China. *Int. J. Epidemiol.*, **21**, 564–573

Halpern, M.S., England, J.M., Deery, D.T., Petcu, D.J., Mason, W.S. & Molnar-Kimber, K.L. (1983) Viral nucleic acid synthesis and antigen accumulation in pancreas and kidney of Pekin ducks infected with duck hepatitis B virus. *Proc. natl Acad. Sci. USA*, **80**, 4865–4869

Halpern, M.S., Egan, J., Mason, W.S. & England, J.M. (1984) Viral antigen in endocrine cells of the pancreatic islets and adrenal cortex of Pekin ducks infected with duck hepatitis B virus. *Virus Res.*, **1**, 213–223

Han, K.-H., Hollinger, F.B., Noonan, C.A. & Yoffe, B. (1992) Simultaneous detection of HBV-specific antigens and DNA in paraffin-embedded liver tissue by immunohistochemistry and in situ hybridization using a digoxigenin-labeled probe. *J. virol. Methods*, **37**, 89–98

Hansen, L.J., Tennant, B.C., Seeger, C. & Ganem, D. (1993) Differential activation of *myc* gene family members in hepatic carcinogenesis by closely related hepatitis B viruses. *Mol. cell. Biol.*, **13**, 659–667

Harrison, T.J., Hopes, E.A., Oon, C.J., Zanetti, A.R. & Zuckerman, A.J. (1991) Independent emergence of a vaccine-induced escape mutant of hepatitis B virus. *J. Hepatol.*, **13** (Suppl. 4), S105–S107

Haruna, Y., Hayashi, N., Katamaya, K., Yuki, N., Kasahara, A., Sasaki, Y., Fusamoto, H. & Kamada, T. (1991) Expression of X protein and hepatitis B virus replication in chronic hepatitis. *Hepatology*, **13**, 417–421

Hasegawa, K., Huang, J., Wands, J.R., Obata, H. & Liang, T.J. (1991) Association of hepatitis B viral precore mutations with fulminant hepatitis B in Japan. *Virology*, **185**, 460–463

Hatada, I., Tokino, T., Ochiya, T. & Matsubara, K. (1988) Co-amplification of integrated hepatitis B virus DNA and transforming gene *hst-1* in a hepatocellular carcinoma. *Oncogene*, **3**, 537–540

Heyward, W.L., Lanier, A.P., McMahon, B.J., Fitzgerald, M.A., Kilkenny, S. & Paprocki, T.R. (1985) Early detection of primary hepatocellular carcinoma. Screening for primary hepatocellular carcinoma among persons infected with hepatitis B virus. *J. Am. med. Assoc.*, **254**, 3052–3054

Hilger, C., Velhagen, I., Zentgraf, H. & Schröder, C.H. (1991) Diversity of hepatitis B virus X gene-related transcripts in hepatocellular carcinoma: a novel polyadenylation site on viral DNA. *J. Virol.*, **65**, 4284–4291

Hilleman, M.R., Buynak, E.B., Roehm, R.R., Tytell, A.A., Bertland, A.U. & Lampson, G.P. (1975) Purified and inactivated human hepatitis B vaccine: progress report. *Am. J. med. Sci.*, **270**, 401–404

Himeno, Y., Fukuda, Y., Hatanaka, M. & Imura, H. (1988) Expression of oncogenes in human liver disease. *Liver*, **8**, 208–212

Hino, O., Kitagawa, T., Koike, K., Kobayashi, M., Hara, M., Mori, W., Nakashima, T., Hattori, N. & Sugano, H. (1984) Detection of hepatitis B virus DNA in hepatocellular carcinomas in Japan. *Hepatology*, **4**, 90–95

Hino, O., Kitagawa, T. & Sugano, H. (1985) Relationship between serum and histochemical markers for hepatitis B virus and rate of viral integration in hepatocellular carcinomas in Japan. *Int. J. Cancer*, **35**, 5–10

Hino, O., Shows, T.B. & Rogler, C.E. (1986) Hepatitis B virus integration site in hepatocellular carcinoma at chromosome 17;18 translocation. *Proc. natl Acad. Sci. USA*, **83**, 8338–8342

Hino, O., Ohtake, K. & Rogler, C.E. (1989) Features of two hepatitis B virus (HBV) DNA integrations suggest mechanisms of HBV integration. *J. Virol.*, **63**, 2638–2643

Hino, O., Tabata, S. & Hotta, Y. (1991) Evidence for increased in vitro recombination with insertion of human hepatitis B virus DNA. *Proc. natl Acad. Sci. USA*, **88**, 9248–9252

Ho, J.C.I., Wu, P.-C. & Mak, T.-K. (1981) Liver cell dysplasia in association with hepatocellular carcinoma, cirrhosis and hepatitis B surface antigen in Hong Kong. *Int. J. Cancer*, **28**, 571–574

Hohenberger, P. (1985) Pancreas as target organ of hepatitis virus—immunohistological demonstration of HBsAg in pancreatic carcinoma and in chronic pancreatitis. *Leber Magen Darm*, **15**, 58–63 (in German)

Höhne, M., Schaefer, S., Seifer, M., Feitelson, M.A., Paul, D. & Gerlich, W.H. (1990) Malignant transformation of immortalized transgenic hepatocytes after transfection with hepatitis B virus DNA. *EMBO J.*, **9**, 1137–1145

Hollstein, M.C., Sidransky, D., Vogelstein, B. & Harris, C.C. (1991) p53 Mutations in human cancers. *Science*, **253**, 49–53

Hollstein, M.C., Wild, C.P., Bleicher, F., Chutimataewin, S., Harris, C.C., Srivatanakul, P. & Montesano, R. (1993) p53 Mutations and aflatoxin B1 exposure in hepatocellular carcinoma patients from Thailand. *Int. J. Cancer*, **53**, 51–55

Holman, C.D.J., Quadros, C.F., Bucens, M.R. & Reid, P.M. (1987) Occurrence and distribution of hepatitis B infection in the aboriginal population of western Australia. *Aust. N.Z. J. Med.*, **17**, 518–525

Hoofnagle, J.H., Peters, M., Mullen, K.D., Jones, D.B., Rustgi, V., Di Bisceglie, A., Hallahan, C., Park, Y., Meschievitz, C. & Jones, E.A. (1988) Randomized, controlled trial of recombinant human alpha-interferon in patients with chronic hepatitis B. *Gastroenterology*, **95**, 1318–1325

Horiike, N., Michitaka, K., Onji, M., Murota, T. & Ohta, Y. (1989) HBV-DNA hybridization in hepatocellular carcinoma associated with alcohol in Japan. *J. med. Virol.*, **28**, 189–192

Horiike, N., Blumberg, B.S. & Feitelson, M.A. (1991) Characteristics of hepatitis B X antigen, antibodies to X antigen, and antibodies to the viral polymerase during hepatitis B virus infection. *J. infect. Dis.*, **164**, 1104–1112

Hosono, S., Lee, C.-S., Chou, M.-J., Yang, C.-S. & Shih, C. (1991) Molecular analysis of the p53 alleles in primary hepatocellular carcinomas and cell lines. *Oncogene*, **6**, 237–243

Howe, A.Y.M., Elliott, J.F. & Tyrrell, D.L.J. (1992) Duck hepatitis B virus polymerase produced by in vitro transcription and translation possesses DNA polymerase and reverse transcriptase activities. *Biochem. biophys. Res. Commun.*, **189**, 1170–1176

Hsia, C.C., Evarts, R.P., Nakatsukasa, H., Marsden, E.R. & Thorgeirsson, S.S. (1992) Occurrence of oval-type cells in hepatitis B virus-associated human hepatocarcinogenesis. *Hepatology*, **16**, 1327–1333

Hsieh, C.-C., Tzonou, A., Zavitsanos, X., Kaklamani, E., Lan, S.-J. & Trichopoulos, D. (1992) Age at first establishment of chronic hepatitis B virus infection and hepatocellular carcinoma risk: a birth order study. *Am. J. Epidemiol.*, **136**, 1115–1121

Hsing, A.W., Guo, W., Chen, J., Li, J.-Y., Stone, B.J., Blot, W.J. & Fraumeni, J.F., Jr (1991) Correlates of liver cancer mortality in China. *Int. J. Epidemiol.*, **20**, 54–59

Hsu, T.-Y., Möröy, T., Etiemble, J., Louise, A., Trépo, C., Tiollais, P. & Buendia, M.-A. (1988) Activation of c-*myc* by woodchuck hepatitis virus insertion in hepatocellular carcinoma. *Cell*, **55**, 627–635

Hsu, T.-Y., Fourel, G., Etiemble, J., Tiollais, P. & Buendia, M.-A. (1990) Integration of hepatitis virus DNA near c-*myc* in woodchuck hepatocellular carcinoma. *Gastroenterol. Jpn.*, **25**, 43–48

Hsu, H.-C., Chiou, T.-J., Chen, J.-Y., Lee, C.-S., Lee, P.-H. & Peng, S.-Y. (1991) Clonality and clonal evolution of hepatocellular carcinoma with multiple nodules. *Hepatology*, **13**, 923–928

Hsu, I.C., Metcalf, R.A., Sun, T., Welsh, J.A., Wang, N.J. & Harris, C.C. (1991) Mutational hotspot in the p53 gene in human hepatocellular carcinomas. *Nature*, **350**, 427–428

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 297–308

IARC (1988) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 44, *Alcohol Drinking*, Lyon

IARC (1993) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 56, *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*, Lyon, pp. 245–395

IARC (undated) *Hepatitis B and Liver Cancer. The Gambia Hepatitis Intervention Study*, Lyon

Iijima, T., Saitoh, N., Nobutomo, K., Nambu, M. & Sakuma, K. (1984) A prospective cohort study of hepatitis B surface antigen carriers in a working population. *Gann*, **75**, 571–573

Iizuka, H., Ohmura, K., Ishijima, A., Satoh, K., Tanaka, J., Tsuda, F., Okamoto, H., Miyakawa, Y. & Mayumi, M. (1992) Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. *Vox sang.*, **63**, 107–111

Imai, M., Hoshi, Y., Okamoto, H., Matsui, T., Tsurimoto, T., Matsubara, K., Miyakawa, Y. & Mayumi, M. (1987) Free and integrated forms of hepatitis B virus DNA in human hepatocellular carcinoma cells (PLC/342) propagated in nude mice. *J. Virol.*, **61**, 3555–3560

Imazeki, F., Yaginuma, K., Omata, M., Okuda, K., Kobayashi, M. & Koike, K. (1988) Integrated structures of duck hepatitis B virus DNA in hepatocellular carcinoma. *J. Virol.*, **62**, 861–865

Inaba, Y., Maruchi, N., Matsuda, M., Yoshihara, N. & Yamamoto, S.-I. (1984) A case–control study on liver cancer with special emphasis on the possible aetiological role of schistosomiasis. *Int. J. Epidemiol.*, **13**, 408–412

Inskip, H.M., Hall, A.J., Chotard, J., Loik, F. & Whittle, H. (1991) Hepatitis B vaccine in the Gambian Expanded Programme on Immunization: factors influencing antibody response. *Int. J. Epidemiol.*, **20**, 764–769

Itoh, Y., Takai, E., Ohnuma, H., Kitajima, K., Tsuda, F., Machida, A., Mishiro, S., Nakamura, T., Miyakawa, Y. & Mayumi, M. (1986) A synthetic peptide vaccine involving the product of the pre-S(2) region of hepatitis B virus DNA: protective efficacy in chimpanzees. *Proc. natl Acad. Sci. USA*, **83**, 9174–9178

Jackowska, T., Kalinowska, B., Rokicka-Milewska, R., Derulska, D., Sopyl/o, B. & Makowska, K. (1990) Hepatitis B virus infection in children with leukaemias and lymphomas. *Wiad. Lek.*, **43**, 645–651 (in Polish)

Janssen, H.L.A., Berk, L., Schalm, S.W., Heijtink, R.A., Hess, G., Rossol, S., Meyer zum Buschenfelde, K.-H., Chamuleau, R.A.F.M., Jansen, P.L.M., Reesink, H.W., Meyer, B., Beglinger, C., den Ouden-Muller, J.W., de Jong, M. & Mulder, C.J.J. (1992) Antiviral effect of prolonged intermittent lymphoblastoid alpha interferon treatment in chronic hepatitis B. *Gut*, **33**, 1094–1098

Jilg, W., Schmidt, M., Weinel, B., Küttler, T., Brass, H., Bommer, J., Müller, R., Schulte, B., Schwarzbeck, A. & Deinhardt, F. (1986) Immunogenicity of recombinant hepatitis B vaccine in dialysis patients. *J. Hepatol.*, **3**, 190–195

Johnson, P.J. (1991) Epidemiology of hepatocellular carcinoma in the West. *Pathol. Biol.*, **39**, 896–897

Joubert, J.J., Jupp, P.G., Prozesky, O.W. & Lourens, J.G.H. (1985) Infection of wild populations of the tampan, *Ornithodoros moubata* Murray, with hepatitis B virus in the Kavango, northern Namibia. *South Afr. J. Sci.*, **81**, 167–168

Junge, U. & Deinhardt, F. (1985) The acute manifestations of hepatitis B virus infection. In: Gerety, R.J., ed., *Hepatitis B*, New York, Academic Press, pp. 93–118

Kaklamani, E., Trichopoulos, D., Tzonou, A., Zavitsanos, X., Koumantaki, Y., Hatzakis, A., Hsieh, C.C. & Hatziyannis, S. (1991) Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. *J. Am. med. Assoc.*, **265**, 1974–1976

Kashala, L.O., Conne, B., Kapanci, Y., Frei, P.C., Lambert, P.H., Kalengayi, M.R., Essex, M. (1992) Hepatitis B virus, alpha-fetoprotein synthesis, and hepatocellular carcinoma in Zaire. *Liver*, **12**, 330–340

Kato, I., Tominaga, S. & Ikari, A. (1992) The risk and predictive factors for developing liver cancer among patients with decompensated liver cirrhosis. *Jpn. J. Clin. Oncol.*, **22**, 278–285

Kay, A., Mandart, E., Trépo, C. & Galibert, F. (1985) The HBV HBx gene expressed in *E. coli* is recognised by sera from hepatitis patients. *EMBO J.*, **4**, 1287–1292

Kekulé, A.S., Lauer, U., Meyer, M., Caselmann, W.H., Hofschneider, P.H. & Koshy, R. (1990) The preS2/S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. *Nature*, **343**, 457–461

Kekulé, A.S., Lauer, U., Weiss, L., Luber, B. & Hofschneider, P.H. (1993) Hepatitis B virus transactivator HBx uses a tumour promotor signalling pathway (Letter to the Editor). *Nature*, **361**, 742–745

Kent, G.P., Brondum, J., Keenlyside, R.A., LaFazia, L.M. & Denman Scott, H. (1988) A large outbreak of acupuncture-associated hepatitis B. *Am. J. Epidemiol.*, **127**, 591–598

Kew, M.C. & Macerollo, P. (1988) Effect of age on the etiologic role of the hepatitis B virus in hepatocellular carcinoma in blacks. *Gastroenterology*, **94**, 439–442

Kew, M.C., Geddes, E.W., Macnab, G.M. & Bersohn I. (1974) Hepatitis-B antigen and cirrhosis in Bantu patients with primary liver cancer. *Cancer*, **34**, 539–541

Kew, M.C., Desmyter, J., Bradburne, A.F. & Macnab, G.M. (1979) Hepatitis B virus infection in southern African blacks with hepatocellular cancer. *J. natl Cancer Inst.*, **62**, 517–520

Kew, M.C., Desmyter, J., De Groote, G., Frösner, G., Roggendorf, M. & Deinhardt, F. (1981) Hepatocellular cancer in southern African blacks: HBeAg, anti-HBe, IgM-anti-HBc and other markers of hepatitis B. *Prog. med. Virol.*, **27**, 41–48

Kew, M.C., Kassianides, C., Hodkinson, J., Coppin, A. & Paterson, A.C. (1986a) Hepatocellular carcinoma in urban born blacks: frequency and relation to hepatitis B virus infection. *Br. med. J.*, **293**, 1339–1341

Kew, M.C., Fujita, Y., Takahashi, H., Coppins, A. & Wands, J.R. (1986b) Comparison between polyclonal and first and second generation monoclonal radioimmunoassays in the detection of hepatitis B surface antigen in patients with hepatocellular carcinoma. *Hepatology*, **6**, 636–639

Kim, C.-M., Koike, K., Saito, I., Miyamura, T. & Jay, G. (1991) HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature*, **351**, 317–320

Kim, S.H., Hong, S.P., Kim, S.K., Lee, W.S. & Rho, H.M. (1992) Replication of a mutant hepatitis B virus with a fused X–C reading frame in hepatoma cells. *J. gen. Virol.*, **73**, 2421–2424

Kingsley, L.A., Rinaldo, C.R., Jr, Lyter, D.W., Valdiserri, R.O., Belle, S.H. & Ho, M. (1990) Sexual transmission efficiency of hepatitis B virus and human immunodeficiency virus among homosexual men. *J. Am. med. Assoc.*, **264**, 230–234

Koch, S., Freytag von Loringhoven, A., Hofschneider, P.H. & Koshy, R. (1984) Amplification and rearrangement in hepatoma cell DNA associated with integrated hepatitis B virus DNA. *EMBO J.*, **3**, 2185–2189

Kojima, M., Shimizu, M., Tsuchimochi, T., Koyasu, M., Tanaka, S., Iizuka, H., Tanaka, T., Okamoto, H., Tsuda, F., Miyakawa, Y. & Mayumi, M. (1991) Posttransfusion fulminant hepatitis B associated with precore-defective HBV mutants. *Vox sang.*, **60**, 34–39

Korba, B.E., Wells, F.V., Baldwin, B., Cote, P.J., Tennant, B.C., Popper, H. & Gerin, J.L. (1989) Hepatocellular carcinoma in woodchuck hepatitis virus-infected woodchucks: presence of viral DNA in tumor tissue from chronic carriers and animals serologically recovered from acute infections. *Hepatology*, **9**, 461–470

Kosaka, Y., Takase, K., Kojima, M., Shimizu, M., Inoue, K., Yoshiba, M., Tanaka, S., Akahane, Y., Okamoto, H., Tsuda, F., Miyakawa, Y. & Mayumi, M. (1991) Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. *Gastroenterology*, **100**, 1087–1094

Koshy, R. & Wells, J. (1991) Deregulation of cellular gene expression by HBV *trans*-activators in hepatocarcinogenesis. *Adv. appl. Biotechnol. Ser.*, **13**, 159–170

Koshy, R., Koch, S., Freytag von Loringhoven, A., Kahmann, R., Murray, K. & Hofschneider, P.H. (1983) Integration of hepatitis B virus DNA: evidence for integration in the single-stranded gap. *Cell*, **34**, 215–223

Kremsdorf, D., Thiers, V., Garreau, F., Nakajima, E., Chappey, C., Schellekens, H., Wands, J.R., Sninsky, J., Tiollais, P. & Bréchot, C. (1991) Nucleotide sequence analysis of hepatitis B virus genomes isolated from serologically negative patients. In: Hollinger, F.B., Lemon, S.M. & Margolis, H., eds, *Viral Hepatitis and Liver Disease*, Baltimore, Williams & Wilkins, pp. 222–226

Kress, S., Jahn, U.-R., Buchmann, A., Bannasch, P. & Schwarz, M. (1992) p53 Mutations in human hepatocellular carcinomas in Germany. *Cancer Res.*, **52**, 3220–3223

Krogsgaard, K., Kryger, P., Aldershvile, J., Andersson, P., Bréchot, C. & the Copenhagen Hepatitis Acuta Programme (1985) Hepatitis B virus DNA in serum from patients with acute hepatitis B. *Hepatology*, **5**, 10–13

Krugman, S., Giles, J.P. & Hammond, J. (1970) Hepatitis virus: effect of heat on the infectivity and antigenicity of the MS-1 and MS-2 strain. *J. infect. Dis.*, **122**, 432–436

Krugman, S., Overby, L.R., Mushahwar, I.K., Ling, C.-M., Frösner, G.G. & Deinhardt, F. (1979) Viral hepatitis, type B. Studies on natural history and prevention re-examined. *New Engl. J. Med.*, **300**, 101–106

Kruskall, M.S., Alper, C.A., Awdeh, Z., Yunis, E.J. & Marcus-Bagley, D. (1992) The immune response to hepatitis B vaccine in humans: inheritance patterns in families. *J. exp. Med.*, **175**, 495–502

Kubo, Y., Okuda, K., Hashimoto, M., Nagasaki, Y., Ebata, H., Nakajima, Y., Musha, H., Sakuma, K. & Ohtake, H. (1977) Antibody to hepatitis B core antigen in patients with hepatocellular carcinoma. *Gastroenterology*, **72**, 1217–1220

Lai, M.-Y., Chen, P.-J., Yang, P.-M., Sheu, J.-C., Sung, J.-L. & Chen, D.-S. (1990) Identification and characterization of intrahepatic hepatitis B virus DNA in HBsAg-seronegative patients with chronic liver disease and hepatocellular carcinoma in Taiwan. *Hepatology*, **12**, 575–581

Lai, M.E., Melis, A., Mazzoleni, A.P., Farci, P. & Balestrieri, A. (1991) Sequence analysis of hepatitis B virus genome of a new mutant of ayw subtype isolated in Sardinia. *Nucleic Acids Res.*, **19**, 5078

Lam, K.C., Yu, M.C., Leung, J.W.C. & Henderson, B.E. (1982) Hepatitis B virus and cigarette smoking: risk factors for hepatocellular carcinoma in Hong Kong. *Cancer Res.*, **42**, 5246–5248

Lampertico, P., Malter, J.S., Colombo, M. & Gerber, M.A. (1990) Detection of hepatitis B virus DNA in formalin-fixed, paraffin-embedded liver tissue by the polymerase chain reaction. *Am. J. Pathol.*, **137**, 253–258

Larouzé, B., London, W.T., Saimot, G., Werner, B.G., Lustbader, E.D., Payet, M. & Blumberg, B.S. (1976) Host responses to hepatitis-B infection in patients with primary hepatic carcinoma and their families: a case/control study in Senegal, West Africa. *Lancet*, **ii**, 534–538

Lauer, U., Weiss, L., Hofschneider, P.H. & Kekulé, A.S. (1992) The hepatitis B virus *pre*-S/S^t transactivator is generated by 3′ truncations within a defined region of the *S* gene. *J. Virol.*, **66**, 5284–5289

Laukamm-Josten, U., von Laer, G., Feldmeier, H., Bienzle, U., Uy, A., Thomssen, R. & Guggenmoos-Holzmann, I. (1987) Active immunization against hepatitis B: immunogenicity of a recombinant DNA vaccine in females, heterosexual and homosexual males. *Postgrad. med. J.*, **63** (Suppl. 2), 143–146

Leandro, G. & Duca, P. (1993) The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area: a case–control study (Letter to the Editor). *Cancer*, **71**, 510–511

Leandro, G., Colloredo, G., Cozzolongo, R. & Elba, S. (1990) Hepatocellular carcinoma in cirrhotic patients: role of hepatitis B virus. *Ital. J. Gastroenterol.*, **22**, 66–69

Le Bouvier, G.L. (1971) The heterogeneity of Australia antigen. *J. infect. Dis.*, **123**, 671–675

Lee, A.K.Y. (1975) Hepatitis B antigen and auto-antibodies in chronic liver diseases in Hong Kong. *Aust. N.Z. J. Med.*, **5**, 235–239

Lee, T.-H., Finegold, M.J., Shen, R.-F., DeMayo, J.L., Woo, S.L.C. & Butel, J.S. (1990) Hepatitis B virus transactivator X protein is not tumorigenic in transgenic mice. *J. Virol.*, **64**, 5939–5947

Levrero, M., Jean-Jean, O., Balsano, C., Will, H. & Perricaudet, M. (1990) Hepatitis B virus (HBV) X gene expression in human cells and anti-HBx antibodies detection in chronic HBV infection. *Virology*, **174**, 299–304

Levrero, M., Stemler, M., Pasquinelli, C., Alberti, A., Jean-Jean, O., Franco, A., Balsano, C., Diop, D., Bréchot, C., Melegari, M., Villa, E., Barnaba, V., Perricaudet, M. & Will, H. (1991) Significance of anti-HBx antibodies in hepatitis B virus infection. *Hepatology*, **13**, 143–149

Li, D., Cao, Y., He, L., Wang, N.J. & Gu, J.-R. (1993) Aberrations of p53 gene in human hepatocellular carcinoma from China (Accelerated paper). *Carcinogenesis*, **14**, 169–173

Liang, T.J., Isselbacher, K.J. & Wands, J.R. (1989) Rapid identification of low level hepatitis B-related viral genome in serum (Rapid publication). *J. clin. Invest.*, **84**, 1367–1371

Liang, T.J., Hasegawa, K., Rimon, N., Wands, J.R. & Ben-Porath, E. (1991a) A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *New Engl. J. Med.*, **324**, 1705–1709

Liang, T.J., Baruch, Y., Ben-Porath, E., Enat, R., Bassan, L., Brown, N.V., Rimon, N., Blum, H.E. & Wands, J.R. (1991b) Hepatitis B virus infection in patients with idiopathic liver disease. *Hepatology*, **6**, 1044–1051

Liaw, Y.-F., Tai, D.-I., Chu, C.-M., Lin, D.-Y., Sheen, I.-Y., Chen, T.-J. & Pao, C.C. (1986) Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. *Gastroenterology*, **90**, 263–267

Lin, T.-M., Tsu, W.T. & Chen, C.J. (1986) Mortality of hepatoma and cirrhosis of liver in Taiwan. *Br. J. Cancer*, **54**, 969–976

Lin, T.-M., Chen, C.-J., Lu, S.-N., Chang, A.-S., Chang, Y.-C., Hsu, S.-T., Liu, J.-Y., Liaw, Y.-F. & Chang, W.-Y. (1991) Hepatitis B virus e antigen and primary hepatocellular carcinoma. *Anticancer Res.*, **11**, 2063–2066

Lingao, A.L. (1989) The relationship of hepatocellular carcinoma and liver cirrhosis to hepatitis B virus infection in the Philippines. *Gastroenterol. Jpn.*, **24**, 425–433

Lingao, A.L., Domingo, E.O. & Nishioka, K. (1981) Hepatitis B virus profile of hepatocellular carcinoma in the Philippines. *Cancer*, **48**, 1590–1595

Lok, A.S.F., Lai, C.-L. & Wu, P.-C. (1986) Interferon therapy of chronic hepatitis B virus infection in Chinese. *J. Hepatol.*, **3** (Suppl. 2), S209–S215

Lok, A.S.F., Lai, C.-L., Wu, P.-C., Leung, E.K.Y. & Lam, T.-S. (1987) Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. *Gastroenterology*, **92**, 1839–1843

Lok, A.S.F., Liang, R.H.S., Chiu, E.K.W., Wong, K.-L., Chan, T.-K. & Todd, D. (1991) Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. *Gastroenterology*, **100**, 182–188

Lok, A.S.F., Wu, P.-C., Lai, C.-L., Lau, J.Y.N., Leung, E.K.Y., Wong, L.S.K., Ma, O.C.K., Lauder, I.J., Ng, C.P.L. & Chung, H.-T. (1992) A controlled trial of interferon with or without prednisone priming for chronic hepatitis B. *Gastroenterology*, **102**, 2091–2097

Loncarevic, I.F., Zentgraf, H. & Schröder, C.H. (1990) Sequence of a replication competent hepatitis B virus genome with a pre-X open reading frame. *Nucleic Acids Res.*, **18**, 4940

London, W.T. (1979) Sex differences in response to hepatitis B virus. Introduction. *Arth. Rheum.*, **22**, 1258–1260

London, W.T. (1981) Primary hepatocellular carcinoma—etiology, pathogenesis, and prevention. *Hum. Pathol.*, **12**, 1085–1097

Lu, C.Q., Chung, H., Liu, H., Ping, H. & Tsa, C. (1988) A study of correlation between primary hepatocellular carcinoma and hepatitis B. *Chin. J. Pathol.*, **9**, 5–7 (in Chinese)

Lu, S.N., Lin, T.M., Chen, C.J., Chen, J.S., Liaw, Y.F., Chang, W.Y. & Hsu, S.T. (1988) A case–control study of primary hepatocellular carcinoma in Taiwan. *Cancer*, **62**, 2051–2055

Luo, S., Ye, F.-S., Mo, Z.-C., Su, Y.-X., He, J.-S., Zhou, C.-Q., Tong, M.J., Henderson, B.E. & Yu, M.C. (1988) Prevalence of hepatitis B viral markers in hepatitis B endemic areas of China. *Chin. med. J.*, **101**, 654–658

Maddrey, W.C. & Van Thiel, D.H. (1988) Liver transplantation: an overview. *Hepatology*, **8**, 948–959

Marion, P.L. (1991) Ground squirrel hepatitis virus. In: McLachlan, A., ed., *Molecular Biology of the Hepatitis B Virus*, Boca Raton, FL, CRC Press, pp. 39–51

Marion, P.L. & Cullen, J.M. (1992) Animal models of human disease: hepatitis B virus infection. *Comp. Pathol. Bull.*, **24**, 3–4

Marion, P.L., Oshiro, L.S., Regnery, D.C., Scullard, G.H. & Robinson, W.S. (1980) A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. *Proc. natl Acad. Sci. USA*, **77**, 2941–2945

Marion, P.L., Knight, S.S., Salazar, F.H., Popper, H. & Robinson, W.S. (1983) Ground squirrel hepatitis virus infection. *Hepatology*, **3**, 519–527

Marion, P.L., Knight, S.S., Ho, B.-K., Guo, Y.-Y., Robinson, W.S. & Popper, H. (1984) Liver disease associated with duck hepatitis B virus infection of domestic ducks. *Proc. natl Acad. Sci. USA*, **81**, 898–902

Marion, P.L., Van Davelaar, M.J., Knight, S.S., Salazar, F.H., Garcia, G., Popper, H. & Robinson, W.S. (1986) Hepatocellular carcinoma in ground squirrels persistently infected with ground squirrel hepatitis virus. *Proc. natl Acad. Sci. USA*, **83**, 4543–4546

Marion, P.L., Popper, H., Azcarraga, R.R., Steevens, C., Van Davelaar, M.J., Garcia, G. & Robinson, W.S. (1987) Ground squirrel hepatitis virus and hepatocellular carcinoma. In: Koike, K., Robinson, W. & Will, H., eds, *Hepadna Viruses* (UCLA Symposia on Molecular and Cellular Biology Vol. 70), New York, Alan R. Liss, pp. 337–348

Marshall, C.J. (1991) Tumor suppressor genes. *Cell*, **64**, 313–326

Martin, P., Munoz, S.J. & Friedman L.S. (1992) Liver transplantation for viral hepatitis: current status. *Am. J. Gastroenterol.*, **87**, 409–418

Mason, W.S. & Taylor, J.M. (1989) Experimental systems for the study of hepadnavirus and hepatitis delta virus infections. *Hepatology*, **9**, 635–645

Mason, W.S., Seal, G. & Summers, J. (1980) Virus of Pekin ducks with structural and biological relatedness to human hepatitis B virus. *J. Virol.*, **36**, 829–836

Mason, A., Yoffe, B., Noonan, C., Mearns, M., Campbell, C., Kelley, A. & Perrillo, R.P. (1992) Hepatitis B virus DNA in peripheral-blood mononuclear cells in chronic hepatitis B after HBsAg clearance. *Hepatology*, **16**, 36–41

Matsubara, K. & Tokino, T. (1990) Integration of hepatitis B virus DNA and its implications for hepatocarcinogenesis. *Mol. biol. Med.*, **7**, 243–260

Maupas, P. & Melnick, J.L. (1981) Hepatitis B infection and primary liver cancer. *Prog. med. Virol.*, **27**, 1–5

Maupas, P., Coursaget, P., Goudeau, A., Drucker, J., Sankale, M., Linhard, J. & Diebolt, G. (1977) Hepatitis B virus and primary liver carcinoma: evidences for a filiation hepatitis B, cirrhosis and primary liver cancer. *Ann. Microbiol. (Inst. Pasteur)*, **128A**, 245–253

Maupas, P., Chiron, J.-P., Barin, F., Coursaget, P., Goudeau, A., Perrin, J., Denis, F. & Diop-Mar, I. (1981) Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children. Controlled trial in an endemic area (Senegal). *Lancet*, **i**, 289–292

Maynard, J.E., Kane, M.A. & Hadler, S.C. (1989) Global control of hepatitis B through vaccination: role of hepatitis B vaccine in the Expanded Programme on Immunization. *Rev. infect. Dis.*, **11** (Suppl. 3), S574–S578

McLean, A.A., Guess, H.A. & Scolnick, E.M. (1985) Suboptimal response to hepatitis B vaccine given by injection into the buttock. *Morb. Mortal. Wkly Rep.*, **34**, 105–108, 113

McMahon, B.J., Alward, W.L.M., Hall, D.B., Heyward, W.L., Bender, T.R., Francis, D.P. & Maynard, J.E. (1985) Acute hepatitis B virus infection: relation of age to the clinical expression of the disease and subsequent development of the carrier state. *J. infect. Dis.*, **151**, 599–603

McMahon, B.J., Rhoades, E.R., Heyward, W.L., Tower, E., Ritter, D., Lanier, A.P., Wainwright, R.B. & Helminiak, C. (1987) A comprehensive programme to reduce the incidence of hepatitis B virus infection and its sequelae in Alaskan natives. *Lancet*, **ii**, 1134–1136

McMahon, B.J., Alberts, S.R., Wainwright, R.B., Bulkow, L. & Lanier, A.P. (1990a) Hepatitis B-related sequelae. Prospective study in 1400 hepatitis B surface antigen-positive Alaska native carriers. *Arch. intern. Med.*, **150**, 1051–1054

McMahon, B.J., Lanier, A.P., Wainwright, R.B. & Kilkenny S.J. (1990b) Hepatocellular carcinoma in Alaska Eskimos: epidemiology, clinical features, and early detection. *Prog. Liver Dis.*, **9**, 643–655

McMahon, B.J., Parkinson, A.J., Helminiak, C., Wainwright, R.B., Bulkow, L., Kellerman-Douglas, A., Schoenberg, S. & Ritter, D. (1992) Response to hepatitis B vaccine of persons positive for antibody to hepatitis B core antigen. *Gastroenterology*, **103**, 590–594

McMahon, G., Ehrlich, P.H., Moustafa, Z.A., McCarthy, L.A., Dottavio, D., Tolpin, M.D., Nadler, P.I. & Östberg, L. (1992) Genetic alterations of the gene encoding the major HBsAg: DNA and immunological analysis of recurrent HBsAg derived from monoclonal antibody-treated liver transplant patients. *Hepatology*, **15**, 757–766

McNair, A.N.B., Main, J. & Thomas, H.C. (1992) Interactions of the human immunodeficiency virus and the hepatotropic viruses. *Semin. Liver Dis.*, **12**, 188–196

McQuillan, G.M., Townsend, T.R., Fields, H.A., Carroll, M., Leahy, M. & Polk, B.F. (1989) Seroepidemiology of hepatitis B virus infection in the United States. *Am. J. Med.*, **87** (Suppl. 3A), 5S–10S

McSweeney, P.A., Carter, J.M., Green, G.J. & Romeril, K.R. (1988) Fatal aplastic anemia associated with hepatitis B viral infection. *Am. J. Med.*, **85**, 255–256

Melegari, M., Jung, M.-C., Schneider, R., Santantonio, T., Bagnulo, S., Luchena, N., Pastore, G., Pape, G., Scaglioni, P.P., Villa, E. & Will, H. (1991) Conserved core protein sequences in hepatitis B virus infected patients without anti-HBc. *J. Hepatol.*, **13**, 187–191

Meyers, M.L., Vitvitski Trépo, L., Nath, N. & Sninsky, J.J. (1986) Hepatitis B virus polypeptide X: expression in *Escherichia coli* and identification of specific antibodies in sera from hepatitis B virus-infected humans. *J. Virol.*, **57**, 101–109

Michon, J., Prince, A.M., Szmuness, W., Demaille, J., Diebolt, G., Linhard, J., Quenum, C. & Sankale, M. (1975) Primary liver cancer and hepatitis B infection in Senegal. Comparison between cancer patients and two controls. *Biomedicine*, **23**, 263–266 (in French)

Mikhailov, M.I., Papernova, N.Y., Zinovyeva, L.I., Durnov, L.A. & Makhonova, L.A. (1986) Hepatitis B markers in infant cancer patients. *Vopr. Onkol.*, **32**, 70–74 (in Russian)

Milich, D.R., Hughes, J.L., Houghten, R., McLachlan, A. & Jones, J.E. (1989) Functional identification of agretopic and epitopic residues within an HBcAg T cell determinant. *J. Immunol.*, **143**, 3141–3147

Miller, R.H. & Robinson, W.S. (1986) Common evolutionary origin of hepatitis B virus and retroviruses. *Proc. natl Acad. Sci. USA*, **83**, 2531–2535

Millman, I., Southam, L., Halbherr, T., Simmons, H. & Kang, C.M. (1984) Woodchuck hepatitis virus: experimental infection and natural occurrence. *Hepatology*, **4**, 817–823

Milne, A., Allwood, G.K., Moyes, C.D., Pearce, N.E. & Lucas, C.R. (1985) Prevalence of hepatitis B infections in a multiracial New Zealand community. *N.Z. med. J.*, **98**, 529–532

Minuk, G.Y., Shaffer, E.A., Hoar, D.I. & Kelly, J. (1986) Ground squirrel hepatitis virus (GSHV) infection and hepatocellular carcinoma in the Canadian Richardson ground squirrel (*Spermophilus richardsonii*). *Liver*, **6**, 350–356

Mitamura, K., Hoyer, B.H., Ponzetto, A., Nelson, J., Purcell, R.H. & Gerin, J.L. (1982) Woodchuck hepatitis virus DNA in woodchuck liver tissues. *Hepatology*, **2**, 47S–50S

Mitsuda, T., Mori, T., Ookawa, N., Aihara, Y., Kosuge, K., Yokota, S., Ibe, M., Shimizu, H., Yoshida, N. & Matsuyama, S. (1989) Demonstration of mother-to-infant transmission of hepatitis B virus by means of polymerase chain reaction. *Lancet*, **ii**, 886–888

Mitsui, T., Iwano, K., Suzuki, S., Yamazaki, C., Masuko, K., Tsuda, F., Aihara, S., Akahane, Y., Miyakawa, Y. & Mayumi, M. (1989) Combined hepatitis B immune globulin and vaccine for postexposure prophylaxis of accidental hepatitis B virus infection in hemodialysis staff members: comparison with immune globulin without vaccine in historical controls. *Hepatology*, **10**, 324–327

Miyaki, M., Sato, C., Gotanda, T., Matsui, T., Mishiro, S., Imai, M. & Mayumi, M. (1986) Integration of region X of hepatitis B virus genome in human primary hepatocellular carcinomas propagated in nude mice. *J. gen. Virol.*, **67**, 1449–1454

Mizusawa, H., Taira, M., Yaginuma, K., Kobayashi, M., Yoshida, E. & Koike, K. (1985) Inversely repeating integrated hepatitis B virus DNA and cellular flanking sequences in the human hepatoma-derived cell line huSP. *Proc. natl Acad. Sci. USA*, **82**, 208–212

Mohamed, A.E., Kew, M.C & Groeneveld, H.T. (1992) Alcohol consumption as a risk factor for hepatocellular carcinoma in urban southern African blacks. *Int. J. Cancer*, **51**, 537–541

Mondelli, M., Vergani, G.M., Alberti, A., Vergani, D., Portmann, B., Eddleston, A.L.W.F. & Williams, R. (1982) Specificity of T lymphocytes cytotoxicity to autologous hepatocytes in chronic hepatitis B virus infection: evidence that T cells are directed against HBV core antigen expressed on hepatocytes. *J. Immunol.*, **129**, 2773–2778

Moriarty, A.M., Alexander, H., Lerner, R.A. & Thornton, G.B. (1985) Antibodies to peptides detect new hepatitis B antigen: serological correlation with hepatocellular carcinoma. *Science*, **227**, 429–433

Möröy, T., Marchio, A., Etiemble, J., Trépo, C., Tiollais, P. & Buendia, M.-A. (1986) Rearrangement and enhanced expression of c-*myc* in hepatocellular carcinoma of hepatitis virus infected woodchucks. *Nature*, **324**, 276–279

Muchmore, E., Socha, W.W. & Krawczynski, C. (1990) HCC in chimpanzees. In: Sung, J.L., ed., *Viral Hepatitis and Hepatocellular Carcinoma*, Hong Kong, Excerpta Medica, pp. 698–702

Murakami, Y., Hayashi, K., Hirohashi, S. & Sekiya, T. (1991) Aberrations of the tumor suppressor p53 and retinoblastoma genes in human hepatocellular carcinomas. *Cancer Res.*, **51**, 5520–5525

Mutchnick, M.G., Appelman, H.D., Chung, H.T., Aragona, E., Gupta, T.P., Cummings, G.D., Waggoner, J.G., Hoofnagle, J.H. & Shafritz, D.A. (1991) Thymosin treatment of chronic hepatitis B: a placebo-controlled pilot trial. *Hepatology*, **14**, 409–415

Nagaya, T., Nakamura, T., Tokino, T., Tsurimoto, T., Imai, M., Mayumi, T., Kamino, K., Yamamura, K. & Matsubara, K. (1987) The mode of hepatitis B virus DNA integration in chromosomes of human hepatocellular carcinoma. *Genes Dev.*, **1**, 773–782

Nakamura, T., Tokino, T., Nagaya, T. & Matsubara, K. (1988) Microdeletion associated with the integration process of hepatitis B virus DNA. *Nucleic Acids Res.*, **16**, 4865–4873

Naoumov, N.V., Schneider, R., Grötzinger, T., Jung, M.C., Miska, S., Pape, G.R. & Will, H. (1992) Precore mutant hepatitis B virus infection and liver disease. *Gastroenterology*, **102**, 538–543

Nasidi, A., Harry, T.O., Vyazov, S.O., Manube, G.M.R., Azzan, B.B. & Ananiev, V.A. (1986) Prevalence of hepatitis B infection markers in representative areas of Nigeria. *Int. J. Epidemiol.*, **15**, 274–276

Nayak, N.C. & Sachdeva, R. (1975) Localization of hepatitis B surface antigen in conventional paraffin sections of the liver. *Am. J. Pathol.*, **81**, 479–492

Nayak, N.C., Dhar, A., Sachdeva, R., Mittal, A., Seth, H.N., Sudarsanam, D., Reddy, B., Wagholikar, U.L. & Reddy, C.R.R.M. (1977) Association of human hepatocellular carcinoma and cirrhosis with hepatitis B virus surface and core antigens in the liver. *Int. J. Cancer*, **20**, 643–654

Neurath, A.R., Kent, S.B.H., Strick, N. & Parker, K. (1986) Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. *Cell*, **46**, 429–436

Neurath, A.R., Strick, N., Sproul, P., Ralph, H.E. & Valinsky, J. (1990) Detection of receptors for hepatitis B virus on cells of extrahepatic origin. *Virology*, **176**, 448–457

Nielsen, J.O., Dietrichson, O., Elling, P. & Christoffersen, P. (1971) Incidence and meaning of persistence of Australia antigen in patients with acute viral hepatitis: development of chronic hepatitis. *New Engl. J. Med.*, **285**, 1157–1160

Nishida, N., Fukuda, Y., Kokuryu, H., Sadamoto, T., Isowa, G., Honda, K., Yamaoka, Y., Ikenaga, M., Imura, H. & Ishizaki, K. (1992) Accumulation of allelic loss on arms of chromosomes 13q, 16q and 17p in the advanced stages of human hepatocellular carcinoma. *Int. J. Cancer*, **51**, 862–868

Nishida, N., Fukuda, Y., Kokuryu, H., Toguchida, J., Yandell, D.W., Ikenega, M., Imura, H. & Ishizaki, K. (1993) Role and mutational heterogeneity of the p53 gene in hepatocellular carcinoma. *Cancer Res.*, **53**, 368–372

Nishioka, K., Levin, A.G. & Simons, M.J. (1975) Hepatitis B antigen, antigen subtypes and hepatitis B antibody in normal subjects and patients with liver disease. Results of a collaborative study. *Bull. World Health Organ.*, **52**, 293–300

Nomura, A., Stemmermann, G.N. & Wasnich, R.D. (1982) Presence of hepatitis B surface antigen before primary hepatocellular carcinoma. *J. Am. med. Assoc.*, **247**, 2247–2249

Ochiya, T., Fujiyama, A., Fukushige, S., Hatada, I. & Matsubara, K. (1986) Molecular cloning of an oncogene from a human hepatocellular carcinoma. *Proc. natl Acad. Sci. USA*, **83**, 4993–4997

Oda, T., Tsuda, H., Scarpa, A., Sakamoto, M. & Hirohashi, S. (1992) *p53* Gene mutation spectrum in hepatocellular carcinoma. *Cancer Res.*, **52**, 6358–6364

Ogata, N., Kamimura, T. & Asakura, H. (1991) Point-mutation, allelic loss and increased methylation of c-Ha-*ras* gene in human hepatocellular carcinoma. *Hepatology*, **13**, 31–37

Ogston, C.W., Jonak, G.J., Rogler, C.E., Astrin, S.M. & Summers, J. (1982) Cloning and structural analysis of integrated woodchuck hepatitis virus sequences from hepatocellular carcinomas of woodchucks. *Cell*, **29**, 385–394

Ohaki, Y., Misugi, K., Sasaki, Y. & Tsunoda, A. (1983) Hepatitis B surface antigen positive hepatocellular carcinoma in children. Report of a case and review of the literature. *Cancer*, **51**, 822–828

Okamoto, H., Imai, M., Tsuda, F., Tanaka, T., Miyakawa, Y. & Mayumi, M. (1987) Point mutation in the *S* gene of hepatitis B virus for a d/y or w/r subtypic change in two blood donors carrying a surface antigen of compound subtype adyr or adwr. *J. Virol.*, **61**, 3030–3034

Okamoto, H., Yano, K., Nozaki, Y., Matsui, A., Miyazaki, H., Yamamoto, K., Tsuda, F., Machida, A. & Mishiro, S. (1992) Mutations within the *S* gene of hepatitis B virus transmitted from mothers to babies immunized with hepatitis B immune globulin and vaccine. *Pediatr. Res.*, **32**, 264–268

Okochi, K. & Murakami, S. (1968) Observations on Australia antigen in Japanese. *Vox sang.*, **15**, 374–385

Okuda, K., Kasai, Y., Sugawara, K., Mizumoto, R., Tsuzuki, T., Ichida, F., Sasaki, H., Endo, Y., Hattori, N., Nakashima, T., Mori, W., Tobe, T., Maetani, S., Matsumoto, Y., Suzuki, N. & Hiarkawa, A. (1980) Primary liver cancers in Japan. *Cancer*, **45**, 2663–2669

Omata, M. (1990) Significance of extrahepatic replication of hepatitis B virus. *Hepatology*, **12**, 364–366

Omata, M., Ashcavai, M., Liew, C.-T. & Peters, R.L. (1979) Hepatocellular carcinoma in the USA, etiologic considerations. Localization of hepatitis B antigens. *Gastroenterology*, **76**, 279–287

Omata, M., Uchiumi, K., Ito, Y., Yokosuka, O., Mori, J., Terao, K., Ye, W.-F., O'Connell, A., London, W.T. & Okuda, K. (1983) Duck hepatitis B virus and liver diseases. *Gastroenterology*, **85**, 260–267

Omata, M., Yokosuka, O., Imazeki, F., Matsuyama, Y., Uchiumi, K., Ito, Y., Mori, J. & Okuda, K. (1984) Transmission of duck hepatitis B virus from Chinese carrier ducks to Japanese ducklings: a study of viral DNA in serum and tissue. *Hepatology*, **4**, 603–607

Omata, M., Zhou, Y.-Z., Uchiumi, K., Hirota, K., Ito, Y., Yokosuka, O. & Okuda, K. (1987) Hepatitis B virus DNA, antigen, and liver pathology in ducks: an animal model of human liver disease. In: Koike, K., Robinson, W. & Will, H., eds, *Hepadna Viruses* (UCLA Symposia on Molecular and Cellular Biology Vol. 70), New York, Alan R. Liss, pp. 349–356

Ono, T., Abe, S. & Yoshida, M.C. (1991) Hereditary low level of plasma ceruloplasmin in LEC rats associated with spontaneous development of hepatitis and liver cancer. *Jpn. J. Cancer Res.*, **82**, 486–489

Oshima, A., Tsukuma, H., Hiyama, T., Fujimoto, I., Yamano, H. & Tanaka, M. (1984) Follow-up study of HBsAg-positive blood donors with special reference to effect of drinking and smoking on development of liver cancer. *Int. J. Cancer*, **34**, 775–779

Osmond, D.H., Charlebois, E., Sheppard, H.W., Page, K., Winkelstein, W., Moss, A.R. & Reingold, A. (1993) Comparison of risk factors for hepatitis C and hepatitis B virus infection in homosexual men. *J. infect. Dis.*, **167**, 66–71

Otu, A.A. (1987) Hepatocellular carcinoma, hepatic cirrhosis, and hepatitis B virus infection in Nigeria. *Cancer*, **60**, 2581–2585

Ou, J.-H. & Rutter, W.J. (1985) Hybrid hepatitis B virus–host transcripts in a human hepatoma cell. *Proc. natl Acad. Sci. USA*, **82**, 83–87

Ozturk, M., Bressac, B., Puisieux, A., Kew, M., Volkmann, M., Bozcall, S., Mura, J.M., de la Monte, S., Carlson, R., Blum, H., Wands, J., Takahashi, H., von Weizsäcker, F., Galun, E., Siddhartha, K., Carr, B.L., Schröder, C.H., Erken, E., Varinii, S., Rustgi, V.K., Prat, J., Toda, G., Koch, H.K., Liang, X.H., Tang, Z.-Y., Shouval, D., Lee, H.-S., Vyas, G.N. & Sarosi, I. (1991) p53 Mutation in hepatocellular carcinoma after aflatoxin exposure. *Lancet*, **338**, 1356–1359

Pagliaro, L., Saracci, R., Bardelli, D., Filippazzo, G. & a group of the Italian Association for the Study of the Liver (AISF) (1982) Chronic liver disease, alcohol consumption and HBsAg antigen in Italy: a multiregional case–control study. *Ital. J. Gastroenterol.*, **14**, 90–95

Pagliaro, L., Simonetti, R.G., Craxì, A., Spanò, C., Filippazzo, M.G., Palazzo, U., Patti, S., Giannuoli, G., Marraffa, A., Colombo, M., Tommasini, M., Bellentani, S., Villa, E., Manenti, F., Caporaso, N., Coltorti, M., Del Vecchio-Blanco, C., Farsi, P., Smedile, A. & Verme, G. (1983) Alcohol and HBV infection as risk factors for hepatocellular carcinoma in Italy: a multicentric, controlled study. *Hepato-gastroenterology*, **30**, 48–50

Parkin, D.M., Srivatanakul, P., Khlat, M., Chenvidhya, D., Chotiwan, P., Insiripong, S., L'Abbé, K.A. & Wild, C.P. (1991) Liver cancer in Thailand. I. A case–control study of cholangiocarcinoma. *Int. J. Cancer*, **48**, 323–328

Paronetto, F. & Tennant, B.C. (1990) Woodchuck hepatitis virus infection: a model of human hepatic diseases and hepatocellular carcinoma. *Prog. Liver Dis.*, **9**, 463–483

Pasquinelli, C., Garreau, F., Bougueleret, L., Cariani, E., Grzeschik, K.H., Thiers, V., Croissant, O., Hadchouel, M., Tiollais, P. & Bréchot, C. (1988) Rearrangement of a common cellular DNA domain on chromosome 4 in human primary liver tumors. *J. Virol.*, **62**, 629–632

Pastore, G., Santantonio, T., Milella, M., Monno, L., Mariano, N., Moschetta, R. & Pollice, L. (1992) Anti-HBe-positive chronic hepatitis B with HBV-DNA in the serum response to a 6-month course of lymphoblastoid interferon. *J. Hepatol.*, **14**, 221–225

Paterlini, P., Driss, F., Nalpas, B., Pisi, E., Franco, D., Berthelot, P. & Bréchot, C. (1993) Persistence of hepatitis B and hepatitis C viral genomes in primary liver cancers from HBsAg-negative patients: a study of a low-endemic area. *Hepatology*, **17**, 20–29

Payet, M., Camain, R. & Pene, P. (1956) Primary liver cancer. Critical study of 240 cases. *Rev. int. Hepatol.*, **6**, 1–20 (in French)

Pedreira, J.D., Vargas, V., Vilaseca, J., Esteban, R., Hernández-Sánchez, J.M., Guardia, J. & Bacardí, R. (1980) Primary liver carcinoma and markers of virus of type B hepatitis. *Med. clín. (Barcelona)*, **75**, 58–60 (in Spanish)

Penna, A., Chisari, F.V., Bertoletti, A., Missale, G., Fowler, P., Giuberti, T., Fiaccadori, F. & Ferrari, C. (1991) Cytotoxic T lymphocytes recognize an HLA-A2-restricted epitope within the hepatitis B virus nucleocapsid antigen. *J. exp. Med.*, **174**, 1565–1570

Penner, E., Maida, E., Mamoli, B. & Gangl, A. (1982) Serum and cerebrospinal fluid immune complexes containing hepatitis B surface antigen in Guillain–Barré syndrome. *Gastroenterology*, **82**, 576–580

Perrillo, R.P., Chau, K.H., Overby, L.R. & Decker, R.H. (1983) Anti-hepatitis B core immunoglobulin M in the serologic evaluation of hepatitis B virus infection and simultaneous infection with type B, delta agent and non-A, non-B viruses. *Gastroenterology*, **85**, 163–167

Perrillo, R.P., Schiff, E.R., Davis, G.L., Bodenheimer, H.C., Jr, Lindsay, K., Payne, J., Dienstag, J.L., O'Brien, C., Tamburro, C., Jacobson, I.M., Sampliner, R., Feit, D., Lefkowitch, J., Kuhns, M., Meschievitz, C., Sanghvi, B., Albrecht, J., Gibas, A. and the Hepatitis Interventional Therapy Group (1990) A randomized, controlled trial of interferon alfa-2a alone and after prednisone withdrawal for the treatment of chronic active hepatitis B. *New Engl. J. Med.*, **323**, 295–301

Peters, R.L. (1975) Viral hepatitis: a pathologic spectrum. *Am. J. med. Sci.*, **270**, 17–31

Peters, R.L., Afroudakis, A.P. & Tatter, D. (1977) The changing incidence of association of hepatitis B with hepatocellular carcinoma in California. *Am. J. clin. Pathol.*, **68**, 1–7

Pier, A.C. & McLoughlin, M.E. (1985) Mycotoxic suppression of immunity. In: Lacey, J., ed., *Trichothecenes and Other Mycotoxins*, New York, John Wiley & Sons, pp. 507–519

Pirovino, M., Heer, M., Joller-Jemelka, H.P., Altorfer, J., Akovbiantz, A. & Schmid, M. (1983) Hepatocellular carcinoma and hepatitis B virus infection. Analysis of 75 cases from Switzerland. *Liver*, **3**, 398–402

Planes, N., De Somoza, N. & Morgenfeld, M.C. (1976) Surface antigen of hepatitis B in malignant lymphoma. *Sangre*, **21**, 492–498 (in Spanish)

Polakoff, S. (1990) Acute viral hepatitis B, reported to the public health laboratory service. *J. Infect.*, **20**, 163–168

Pontisso, P., Locasciulli, A., Schiavon, E., Cattoretti, G., Schirò, R., Stenico, D. & Alberti, A. (1987) Detection of hepatitis B virus DNA sequences in bone marrow of children with leukemia. *Cancer*, **59**, 292–296

Popper, H. (1977) Pathologic aspects of cirrhosis. A review. *Am. J. Pathol.*, **87**, 228–264

Popper, H., Shih, J.W.-K., Gerin, J.L., Wong, D.C., Hoyer, B.H., London, W.T., Sly, D.L. & Purcell, R.H. (1981) Woodchuck hepatitis and hepatocellular carcinoma: correlation of histologic with virologic observations. *Hepatology*, **1**, 91–98

Popper, H., Roth, L., Purcell, R.H., Tennant, B.C. & Gerin, J.L. (1987) Hepatocarcinogenicity of the woodchuck hepatitis virus. *Proc. natl Acad. Sci. USA*, **84**, 866–870

Prince, A.M. (1970) Prevalence of serum-hepatitis-related-antigen (SH) in different geographic regions. *Am. J. trop. Med. Hyg.*, **19**, 872–879

Prince, A.M. & Alcabes, P. (1982) The risk of development of hepatocellular carcinoma in hepatitis B virus carriers in New York. A preliminary estimate using death-records matching. *Hepatology*, **2**, 15S–20S

Prince, A.M., Leblanc, L., Krohn, K., Masseyeff, R. & Alpert, M.E. (1970) SH antigen and chronic liver disease (Letter to the Editor). *Lancet*, **ii**, 717–718

Prince, A.M., Metselaar, D., Kafuko, G.W., Mukwaya, L.G., Ling, C.M. & Overby, L.R. (1972) Hepatitis B antigen in wild-caught mosquitoes in Africa. *Lancet*, **ii**, 247–250

Prince, A.M., Szmuness, W., Michon, J., Demaille, J., Diebolt, G., Linhard, J., Quenum, C. & Sankale, M. (1975) A case/control study of the association between primary liver cancer and hepatitis B infection in Senegal. *Int. J. Cancer*, **16**, 376–383

Quade, K., Saldanha, J., Thomas, H. & Monjardino, J. (1992) Integration of hepatitis B virus DNA through a mutational hot spot within the cohesive region in a case of hepatocellular carcinoma. *J. gen. Virol.*, **73**, 179–182

Redeker, A.G. (1975) Viral hepatitis: clinical aspects. *Am. J. med. Sci.*, **270**, 9–16

Repp, R., Keller, C., Borkhardt, A., Csecke, A., Schaefer, S., Gerlich, W.H. & Lampert, F. (1992) Detection of a hepatitis B virus variant with a truncated X gene and enhancer II. *Arch. Virol.*, **125**, 299–304

Reys, L.L., Purcell, R.H., Holland, P.V. & Alter, H.J. (1977) The relationship between hepatitis B virus infection and hepatic cell carcinoma in Mozambique. *Trop. geogr. Med.*, **29**, 251–256

Richard, J.L. (1991) Mycotoxins as immunomodulators in animal systems. In: Bray, G.A. & Ryan, D., eds, *Mycotoxins, Cancer and Health*, Baton Rouge, LA, Louisiana State University Press, pp. 197–220

Röckelein, G. & Hecken-Emmel, M. (1988) Risk factors of hepatocellular carcinoma in Germany: hepatitis B or liver cirrhosis? *Hepato-gastroenterology*, **35**, 151–157

Rogler, C.E. (1991) Cellular and molecular mechanisms of hepatocarcinogenesis associated with hepadnavirus infection. *Curr. Top. Microbiol. Immunol.*, **168**, 103–140

Rogler, C.E. & Summers, J. (1984) Cloning and structural analysis of integrated woodchuck hepatitis virus sequences from a chronically infected liver. *J. Virol.*, **50**, 832–837

Rogler, C.E., Sherman, M., Su, C.Y., Shafritz, D.A., Summers, J., Shows, T.B., Henderson, A. & Kew, M. (1985) Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. *Science*, **230**, 319–322

Rosenblum, L., Darrow, W., Witte, J., Cohen, J., French, J., Gil, P.S., Potterat, J., Sikes, K., Reich, R. & Hadler, S. (1992) Sexual practices in the transmission of hepatitis B virus and prevalence of delta virus infection in female prostitutes in the United States. *J. Am. med. Assoc.*, **267**, 2477–2481

Ross, R.K., Yuan, J.-M., Yu, M.C., Wogan, G.N., Qian, G.-S., Tu, J.-T., Groopman, J.D., Gao, Y.-T. & Henderson, B.E. (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*, **339**, 943–946

Rossner, M.T. (1992) Review: hepatitis B virus X-gene product: a promiscuous transcriptional activator. *J. med. Virol.*, **36**, 101–117

Roth, L., King, J.M., Hornbuckle, W.E., Harvey, H.J. & Tennant, B.C. (1985) Chronic hepatitis and hepatocellular carcinoma associated with persistent woodchuck hepatitis virus infection. *Vet. Pathol.*, **22**, 338–343

Ruiz-Moreno, M., Rúa, M.J., Moraleda, G., Guardia, L., Moreno, A. & Carreño, V. (1992) Treatment with interferon gamma versus interferons alfa and gamma in children with chronic hepatitis B. *Pediatrics*, **90**, 254–258

Ryder, R.W., Whittle, H.C., Sanneh, A.B.K., Ajdukiewicz, A.B., Tulloch, S. & Yvonnet, B. (1992) Persistent hepatitis B virus infection and hepatoma in the Gambia, West Africa. A case–control study of 140 adults and their 603 family contacts. *Am. J. Epidemiol.*, **136**, 1122–1131

Sakamoto, M., Hirohashi, S., Tsuda, H., Shimosato, Y., Makuuchi, M. & Hosoda, Y. (1989) Multicentric independent development of hepatocellular carcinoma revealed by analysis of hepatitis B virus integration pattern. *Am. J. surg. Pathol.*, **13**, 1064–1067

Sakuma, K., Saitoh, N., Kasai, M., Jitsukawa, H., Yoshino, I., Yamaguchi, M., Nobutomo, K., Yamumi, M., Tsuda, F., Komazawa, T., Nakamura, T., Yoshida, Y. & Okuda, K. (1988) Relative risks of death due to liver disease among Japanese male adults having various statuses for hepatitis B s and e antigen/antibody in serum: a prospective study. *Hepatology*, **8**, 1642–1646

Santantonio, T., Jung, M.-C., Miska, S., Pastore, G., Pape, G.R. & Will, H. (1991a) High prevalence and heterogeneity of HBV preC mutants in anti-HBe-positive carriers with chronic liver disease in southern Italy. *J. Hepatol.*, **13** (Suppl. 4), S78–S81

Santantonio, T., Jung, M.-C., Schneider, R., Pastore, G., Pape, G.R. & Will, H. (1991b) Selection for a pre-C stop codon mutation during interferon treatment. *J. Hepatol.*, **13**, 368–371

Schek, N., Fischer, M. & Schaller, H. (1991) The hepadnaviral X protein. In: McLachlan, A., ed., *The Hepadnaviral X Protein*, Boca Raton, FL, CRC Press, pp. 181–192

Scheuer, P.J. (1991) Classification of chronic viral hepatitis: a need for reassessment. *J. Hepatol.*, **13**, 372–374

Scheuer, P.J. & Thaler, H. (1977) Acute and chronic hepatitis revisited. Review by an international group. *Lancet*, **ii**, 914–919

Schödel, F., Sprengel, R., Weimer, T., Fernholz, D., Schneider, R. & Will, H. (1989) Animal hepatitis B viruses. In: Klein, G., ed., *Advances in Viral Oncology*, Vol. 8, New York, Raven Press, pp. 73–102

Schödel, F., Weimer, T., Fernholz, D., Schneider, R., Sprengel, R., Wildner, G. & Will, H. (1991) The biology of avian hepatitis B viruses. In: McLachlan, A., ed., *Molecular Biology of the Hepatitis B Virus*, Boca Raton, FL, CRC Press, pp. 53–80

Scorsone, K.A., Zhou, Y.-Z., Butel, J.S. & Slagle, B.L. (1992) p53 Mutations cluster at codon 249 in hepatitis B virus-positive hepatocellular carcinomas from China. *Cancer Res.*, **52**, 1635–1638

Sebti, M.F. (1984) Acute viral hepatitis and markers of HB virus in chronic liver diseases and in primary liver cancers. In: Williams, A.O., O'Conor, G.T., de-Thé, G.B. & Johnson, C.A., eds, *Virus-associated Cancers in Africa* (IARC Scientific Publications No. 63), Lyon, IARC, pp. 227–236 (in French)

Seeger, C., Ganem, D. & Varmus H.E. (1986) Biochemical and genetic evidence for the hepatitis B virus replication strategy. *Science*, **232**, 477–484

Seeger, C., Baldwin, B., Hornbuckle, W.E., Yeager, A.E., Tennant, B.C., Cote, P., Ferrell, L., Ganem, D. & Varmus, H.E. (1991) Woodchuck hepatitis virus is a more efficient oncogenic agent than ground squirrel hepatitis virus in a common host. *J. Virol.*, **65**, 1673–1679

Seelig, R., Renz, M. & Seelig, H.P. (1992) PCR (polymerase chain reaction) in the diagnosis of viral hepatitis. *Ann. Med.*, **24**, 225–230

Seifer, M., Heermann, K.H. & Gerlich, W.H. (1990) Expression pattern of the hepatitis B virus genome in transfected mouse fibroblasts. *Virology*, **179**, 287–299

Seifer, M., Höhne, M., Schaefer, S. & Gerlich, W.H. (1991) In vitro tumorigenicity of hepatitis B virus DNA and HBx protein. *J. Hepatol.*, **13** (Suppl. 4), S61–S65

Sell, S., Hunt, J.M., Dunsford, H.A. & Chisari, F.V. (1991) Synergy between hepatitis B virus expression and chemical hepatocarcinogens in transgenic mice. *Cancer Res.*, **51**, 1278–1285

Shafritz, D.A. (1982) Hepatitis B virus DNA molecules in the liver of HBsAg carriers: mechanistic considerations in the pathogenesis of hepatocellular carcinoma. *Hepatology*, **2**, 35S–41S

Shafritz, D.A., Shouval, D., Sherman, H.I., Hadziyannis, S.J. & Kew, M.C. (1981) Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. *New Engl. J. Med.*, **305**, 1067–1073

Shaul, Y., Garcia, P.D., Schonberg, S. & Rutter, W.J. (1986) Integration of hepatitis B virus DNA in chromosome-specific satellite sequences. *J. Virol.*, **59**, 731–734

Sherker, A.H. & Marion, P.L. (1991) Hepadnaviruses and hepatocellular carcinoma. *Ann. Rev. Microbiol.*, **45**, 475–508

Sherlock, S. (1989) Classifying chronic hepatitis. *Lancet*, **ii**, 1168–1170

Sherlock, S., Fox, R.A., Niazi, S.P. & Scheurer, P.J. (1970) Chronic liver disease and primary liver-cell cancer with hepatitis-associated (Australia) antigen in serum. *Lancet*, **i**, 1243–1247

Sheu, J.-C., Huang, G.-T., Lee, P.-H., Chung, J.-C, Chou, H.-C., Lai, M.-Y., Wang, J.-T., Lee, H.-S., Shih, L.-N., Yang, P.-M., Wang, T.-H. & Chen, D.-S. (1992) Mutation of p53 gene in hepatocellular carcinoma in Taiwan. *Cancer Res.*, **52**, 6098–6100

Shih, C., Burke, K., Chou, M.-J., Zeldis, J.B., Yang, C.-S., Lee, C.-S., Isselbacher, K.J., Wands, J.R. & Goodman, H.M. (1987) Tight clustering of human hepatitis B virus integration sites in hepatomas near a triple-stranded region. *J. Virol.*, **61**, 3491–3498

Shikata, T., Uzawa, T., Yoshiwara, N., Akatsaka, T. & Yamasaki, S. (1974) Staining method of Australia antigen in paraffin section. Detection of cytoplasmic inclusion bodies. *Jpn. J. exp. Med.*, **44**, 25–36

Shimoda, T., Uchida, T., Miyata, H., Abe, K., Ariga, H., Shikata, T. & Fujii, Y. (1980) A 6-year-old boy having hepatocelllar carcinoma associated with hepatitis B surface antigenemia. *Am. J. clin. Pathol.*, **74**, 827–831

Shiozawa, M., Ochiya, T., Hatada, I., Imamura, T., Okudaira, Y., Hiraoka, H. & Matsubara, K. (1988) The *lca* as an onco-fetal gene: its expression in human fetal liver. *Oncogene*, **2**, 523–526

Shirakata, Y., Kawada, M., Fujiki, Y., Sano, H., Oda, M., Yaginuma, K., Kobayashi, M. & Koike, K. (1989) The X gene of hepatitis B virus induced growth stimulation and tumorigenic transformation of mouse NIH3T3 cells. *Jpn. J. Cancer Res.*, **80**, 617–621

Shouval, D., Chakraborty, P.R., Ruiz-Opazo, N., Baum, S., Spigland, I., Muchmore, E., Gerber, M.A., Thung, S.N., Popper, H. & Shafritz, D.A. (1980) Chronic hepatitis in champanzee carriers of hepatitis B virus: morphologic, immunologic, and viral DNA studies. *Proc. natl Acad. Sci. USA*, **77**, 6147–6151

Shusterman, N. & London, W.T. (1984) Hepatitis B and immune-complex disease. *New Engl. J. Med.*, **310**, 43–46

Simon, D., Knowles, B.B. & Weith, A. (1991) Abnormalities of chromosome 1 and loss of heterozygosity on 1p in primary hepatomas. *Oncogene*, **6**, 765–770

Simonetti, R.G., Cammà, C., Fiorello, F., Cottone, M., Rapicetta, M., Marino, L., Fiorentino, G., Craxì, A., Ciccaglione, A., Giuseppetti, R., Stroffolini, T. & Pagliaro, L. (1992) Hepatitis C virus infection as a risk factor for hepatocellular carcinoma in patients with cirrhosis. A case–control study. *Ann. intern. Med.*, **116**, 97–102

Simons, M.J., Yap, E.H., Yu, M., Seah, C.S., Chew, B.K., Fung, W.P., Tan, A.Y.O. & Shanmugaratnam, K. (1971) Australia antigen in Singapore Chinese patients with hepatocellular carcinoma. *Lancet*, **i**, 1149–1151

Simons, M.J., Yap, E.H., Yu, M. & Shanmugaratnam, K. (1972) Australia antigen in Singapore Chinese patients with hepatocellular carcinoma and comparison groups: influence of technique sensitivity on differential frequencies. *Int. J. Cancer*, **10**, 320–325

Sjøgren, M.H., Lemon, S.M., Chung, W.K., Sun, H.S. & Hoofnagle, J.H. (1984) IgM antibody to hepatitis B core antigen in Korean patients with hepatocellular carcinoma. *Hepatology*, **4**, 615–618

Sjøgren, M.H., Dusheiko, G.M., Kew, M.C. & Song, E. (1988) Hepatitis B virus infection and hepatocellular carcinoma: correlation between IgM antibody to hepatitis B core antigen, hepatitis B e antigen, and hepatitis B DNA. *Am. J. trop. Med. Hyg.*, **39**, 582–585

Slagle, B.L., Zhou, Y.-Z. & Butel, J.S. (1991) Hepatitis B virus integration event in human chromosome 17p near the p53 gene identifies the region of the chromosome commonly deleted in virus-positive hepatocellular carcinomas. *Cancer Res.*, **51**, 49–54

Slagle, B.L., Lee, T.-H. & Butel, J.S. (1992) Hepatitis B virus and hepatocellular carcinoma. *Prog. med. Virol.*, **39**, 167–203

Smith, J.B. & Blumberg, B.S. (1969) Viral hepatitis, postnecrotic cirrhosis, and hepatocellular carcinoma (Letter to the Editor). *Lancet*, **ii**, 953

Snyder, R.L. (1968) Hepatomas of captive woodchucks (Abstract No. 68). *Am. J. Pathol.*, **52**, 32a–33a

Soběslavský, O. (1980) Prevalence of markers of hepatitis B virus infection in various countries: a WHO collaborative study. *Bull. World Health Organ.*, **58**, 621–628

Sokal, E.M., Ulla, L. & Otte, J.B. (1992) Hepatitis B vaccine response before and after transplantation in 55 extrahepatic biliary atresia children. *Dig. Dis. Sci.*, **37**, 1250–1252

Sprengel, R., Kaleta, E.F. & Will, H. (1988) Isolation and characterization of a hepatitis B virus endemic in herons. *J. Virol.*, **62**, 3832-3839

Srivatanakul, P., Parkin, D.M., Khlat, M., Chenvidhya, D., Chotiwan, P., Insiripong, S., L'Abbé, K.A. & Wild, C.P. (1991) Liver cancer in Thailand. II. A case–control study of hepatocellular carcinoma. *Int. J. Cancer*, **48**, 329–332

Stevens, C.E., Toy, P.T., Tong, M.J., Taylor, P.E., Vyas, G.N., Nair, P.V., Gudavalli, M. & Krugman, S. (1985) Perinatal hepatitis B virus transmission in the United States. Prevention by passive–active immunization. *J. Am. med. Assoc.*, **253**, 1740–1745

Stevens, C.E., Taylor, P.E., Tong, M.J., Toy, P.T., Vyas, G.N., Nair, P.V., Weissman, J.Y. & Krugman, S. (1987) Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. *J. Am. med. Assoc.*, **257**, 2612–2616

Stevens, C.E., Toy, P.T., Taylor, P.E., Lee, T. & Yip, H.-Y. (1992) Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long-term protection. *Pediatrics*, **90**, 170–173

Stroffolini, T., Chiaramonte, M., Tiribelli, C., Villa, E., Simonetti, R.G., Rapicetta, M., Stazi, M.A., Bertin, T., Crocè, S.L., Trande, P., Magliocco, A. & Chionne, P. (1992) Hepatitis C virus infection, HBsAg carrier state and hepatocellular carcinoma: relative risk and population attributable risk from a case–control study in Italy. *J. Hepatol.*, **16**, 360–363

Struve, J. (1992) Hepatitis B virus infection among Swedish adults: aspects on seroepidemiology, transmission and vaccine response. *Scand. J. infect. Dis.*, **Suppl. 82**, 1–57

Sugimura, H., Fukazawa, T., Saitoh, M., Nakamura, K.-I., Shimada, H. & Ohtsubo, K. (1991) The histopathology of the liver in older patients with hepatitis B surface antigenaemia. *Virchow's Arch. A Pathol. Anat.*, **419**, 409–415

Summers, J. (1981) Three recently described animal virus models for human hepatitis B virus. *Hepatology*, **1**, 179–183

Summers, J. & Mason, W.S. (1982) Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. *Cell*, **29**, 403–415

Summers, J., Smolec, J.M. & Snyder, R. (1978) A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. *Proc. natl Acad. Sci. USA*, **75**, 4533–4537

Sun, T.-T., Chu, Y.-R., Ni, Z.-Q., Lu, J.-H., Huang, F., Ni, Z.-P., Pei, X.-F., Yu, Z.-I. & Liu, G.-T. (1986) A pilot study on universal immunization of newborn infants in an area of hepatitis B virus and primary hepatocellular carcinoma prevalence with a low dose of hepatitis B vaccine. *J. Cell. Physiol.*, **Suppl. 4**, 83–90

Sun, Z.(T), Zhu, Y., Stjernsward, J., Hilleman, M., Collins, R., Zhen, Y., Hsia, C.C., Lu, J., Huang, F., Ni, Z., Ni, T., Liu, G.T., Yu, Z., Liu, Y., Chen, J.M. & Peto, R. (1991) Design and compliance of HBV vaccination trial on newborns to prevent hepatocellular carcinoma and 5-year results of its pilot study. *Cancer Detect. Prev.*, **15**, 313–318

Sureau, C., Eichberg, J.W., Hubbard, G.B., Romet-Lemonne, J.L. & Essex, M. (1988) A molecular cloned hepatitis B virus produced *in vitro* is infectious in a chimpanzee. *J. Virol.*, **62**, 3064-3067

Suwangool, P. (1979) Orcein staining for detection of hepatitis B surface antigen in cirrhosis and hepatic carcinoma in Thailand. *J. med. Assoc. Thai*, **62**, 422–428

Szmuness, W. (1975) Recent advances in the study of the epidemiology of hepatitis B. *Am. J. Pathol.*, **81**, 629–649

Szmuness, W. (1978) Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. *Prog. med. Virol.*, **24**, 40–69

Szmuness, W., Much, M.I., Prince, A.M., Hoofnagle, J.H., Cherubin, C.E., Harley, E.J. & Block, G.H. (1975a) On the role of sexual behavior in the spread of hepatitis B infection. *Ann. intern. Med.*, **83**, 489–495

Szmuness, W., Harley, E.J. & Prince, A.M. (1975b) Intrafamilial spread of asymptomatic hepatitis B. *Am. J. med. Sci.*, **270**, 293–304

Szmuness, W., Harley, E.J., Ikram, H. & Stevens, C.E. (1978a) Sociodemographic aspects of the epidemiology of hepatitis B. In: Vyas, G.N., Cohen, S.N. & Schmidt, R., eds, *Viral Hepatitis*, Philadelphia, Franklin Institute Press, pp. 297–320

Szmuness, W., Stevens, C.E., Ikram, H., Much, M.I., Harley, E.J. & Hollinger, B. (1978b) Prevalence of hepatitis B virus infection and hepatocellular carcinoma in Chinese-Americans. *J. infect. Dis.*, **137**, 822–829

Szmuness, W., Stevens, C.E., Harley, E.J., Zang, E.A., Oleszko, W.R., William, D.C., Sadovsky, R., Morrison, J.M. & Kellner, A. (1980) Hepatitis B vaccine. Demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *New Engl. J. Med.*, **303**, 833–841

Tabor, E. (1987) Guillain-Barré syndrome and other neurologic syndromes in hepatitis A, B and non-A, non-B. *J. med. Virol.*, **21**, 207–216

Tabor, E. (1989) Hepatocellular carcinoma: possible etiologies in patients without serologic evidence of hepatitis B virus infection. *J. med. Virol.*, **27**, 1–6

Tabor, E., Gerety, R.J., Vogel, C.L., Bayley, A.C., Anthony, P.P., Chan, C.H. & Barker, L.F. (1977) Hepatitis B virus infection and primary hepatocellular carcinoma. *J. natl Cancer Inst.*, **58**, 1197–1200

Tabor, E., Bayley, A.C., Cairns, J., Pelleu, L. & Gerety, R.J. (1985) Horizontal transmission of hepatitis B virus among children and adults in five rural villages in Zambia. *J. med. Virol.*, **15**, 113–120

Tada, M., Omata, M. & Ohto, M. (1990) Analysis of *ras* gene mutations in human hepatic malignant tumors by polymerase chain reaction and direct sequencing. *Cancer Res.*, **50**, 1121–1124

Tagawa, M., Omata, M., Yokosuka, D., Uchiumi, K., Imazeki, F. & Okuda, K. (1985) Early events in duck hepatitis B virus infection. Sequential appearance of viral deoxyribonucleic acid in liver, pancreas, kidney and spleen. *Gastroenterology*, **89**, 1224–1229

Takada, S. & Koike, K. (1990) Trans-activation function of a 3′ truncated X gene–cell fusion product from integrated hepatitis B virus DNA in chronic hepatitis tissues. *Proc. natl Acad. Sci. USA*, **87**, 5628–5632

Takayama, T., Makuuchi, M., Hirohashi, S., Sakamoto, M., Okazaki, N., Takayasu, K., Kosuge, T., Motoo, Y., Yamazaki, S. & Hasegawa, H. (1990) Malignant transformation of adenomatous hyperplasia to hepatocellular carcinoma. *Lancet*, **336**, 1150–1153

Takeda, K., Akahane, Y., Suzuki, H., Okamoto, H., Tsuda, F., Miyakawa, Y. & Mayumi, M. (1990) Defects in the precore region of the HBV genome in patients with chronic hepatitis B after sustained seroconversion from HBeAg to anti-HBe induced spontaneously or with interferon therapy. *Hepatology*, **12**, 1284–1289

Tan, A.Y.O., Law, C.H. & Lee, Y.S. (1977) Hepatitis B antigen in the liver cells in cirrhosis and hepatocellular carcinoma. *Pathology*, **9**, 57–64

Tanaka, K. & Mori, W. (1985) Hepatitis B surface antigen in hepatocellular carcinoma. *Jpn. J. Cancer Res. (Gann)*, **76**, 603–607

Tanaka, T., Miyamoto, H., Hino, O., Kitagawa, T., Koike, M., Iizuka, T., Sakamoto, H. & Ooshima, A. (1986) Primary hepatocellular carcinoma with hepatitis B virus–DNA integration in a 4-year-old boy. *Hum. Pathol.*, **17**, 202–204

Tanaka, K., Hirohata, T. & Takeshita, S. (1988) Blood transfusion, alcohol consumption, cigarette smoking in causation of hepatocellular carcinoma: a case–control study in Fukuoka, Japan. *Jpn. J. Cancer Res. (Gann)*, **79**, 1075–1082

Tanaka, Y., Esumi, M. & Shikata, T. (1988) Frequent integration of hepatitis B virus DNA in non-cancerous liver tissue from hepatocellular carcinoma patients. *J. med. Virol.*, **26**, 7–14

Tanaka, K., Hirohata, T., Takeshita, S., Hirohata, I., Koga, S., Sugimachi, K., Kanematsu, T., Ohryohji, F. & Ishibashi, H. (1992) Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma: a case–control study in Fukuoka, Japan. *Int. J. Cancer*, **51**, 509–514

Taylor, P.E. & Stevens, C.E. (1988) Persistence of antibody to hepatitis B surface antigen after vaccination with hepatitis B vaccine. In: Zuckerman, A.J., ed., *Viral Hepatitis and Liver Disease*, New York, Alan R. Liss, pp. 995-997

Taylor, P.E., Stevens, C.E., de Cordoba, S.R. & Rubinstein, P. (1988) Hepatitis B virus and human immunodeficiency virus: possible interactions. In: Zuckerman, A.J., ed., *Viral Hepatitis and Liver Disease*, New York, Alan R. Liss, pp. 198–200

Tennant, B.C., Hornbuckle, W.E., Baldwin, B.H., King, J.M., Cote, P., Popper, H., Purcell, R.H. & Gerin, J.L. (1988) Influence of age on the response to experimental woodchuck hepatitis virus infection. In: Zuckerman, A.J., ed., *Viral Hepatitis and Liver Disease*, New York, Alan R. Liss, pp. 462–464

Tennant, B.C., Hornbuckle, W.E., Yeager, A.E., Baldwin, B.H., Sherman, W.K., Anderson, W.I., Steinberg, H., Cote, P.J., Korba, B.E. & Gerin, J.L. (1990) Effects of aflatoxin B_1 on experimental woodchuck hepatitis virus infection and hepatocellular carcinoma. In: Hollinger, F.B., Lemon, S.M. & Margolis, H., ed., *Viral Hepatitis and Liver Disease*, Baltimore, Williams & Wilkins, pp. 599–600

Tennant, B.C., Mrosovsky, N., McLean, K., Cote, P.J., Korba, B.E., Engle, R.E., Gerin, J.L., Wright, J., Michener, G.R., Uhl, E. & King, J.M. (1991) Hepatocellular carcinoma in Richardson's ground squirrels (*Spermophilus richardsonii*): evidence for association with hepatitis B-like virus infection. *Hepatology*, **13**, 1215–1221

Terazawa, S., Kojima, M., Yamanaka, T., Yotsumoto, S., Okamoto, H., Tsuda, F., Miyakawa, Y. & Mayumi, M. (1990) Hepatitis B virus mutants with precore-region defects in two babies with fulminant hepatitis and their mothers positive for antibody to hepatitis B e antigen. *Pediatr. Res.*, **29**, 5–9

de-Thé, H., Marchio, A., Tiollais, P. & Dejean, A. (1987) A novel steroid thyroid hormone receptor-related gene inappropriately expressed in human hepatocellular carcinoma. *Nature*, **330**, 667–670

The Gambia Hepatitis Study Group (1987) The Gambia Hepatitis Intervention Study. *Cancer Res.*, **47**, 5782–5787

Thomas, H.C., Montano, L., Goodall, A., de Koning, R., Oladapo, J. & Wiedman, K.H. (1982) Immunological mechanisms in chronic hepatitis B virus infection. *Hepatology*, **2**, 116S–121S

Thomas, H.C., Jacyna, M., Waters, J. & Main, J. (1988) Virus–host interaction in chronic hepatitis B virus infection. *Semin. Liver Dis.*, **8**, 342–349

Thyagarajan, S.P., Subramanian, S., Thirunalasundari, T., Venkateswaran, P.S. & Blumberg, B.S. (1988) Effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. *Lancet*, **ii**, 764–766

Tiollais, P. & Buendia, M.-A. (1991) Hepatitis B virus. *Sci. Am.*, **April**, 48–54

Tiollais, P., Pourcel, C. & Dejean, A. (1985) The hepatitis B virus. *Nature*, **317**, 489–495

Todo, S., Demetris, A.J., Van Thiel, D., Teperman, L., Fung, J.J. & Starzl, T.E. (1991) Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. *Hepatology*, **13**, 619–626

Toh, H., Hayashida, H. & Miyata, T. (1983) Sequence homology between retroviral reverse transcriptase and putative polymerases of hepatitis B virus and cauliflower mosaic virus. *Nature*, **305**, 827–829

Tokino, T. & Matsubara, K. (1991) Chromosomal sites for hepatitis B virus integration in human hepatocellular carcinoma. *J. Virol.*, **65**, 6761–6764

Tokino, T., Fukushige, S., Nakamura, T., Nagaya, T., Murotsu, T., Shiga, K., Aoki, N. & Matsubara, K. (1987) Chromosomal translocation and inverted duplication associated with integrated hepatitis B virus in hepatocellular carcinomas. *J. Virol.*, **61**, 3848–3854

Tokudome, S., Ikeda, M., Matsushita, K., Maeda, Y. & Yoshinari, M. (1987) Hepatocellular carcinoma among female Japanese hepatitis B virus carriers. *Hepato-gastroenterology*, **34**, 246–248

Tokudome, S., Ikeda, M., Matsushita, K., Maeda, Y. & Yoshinari, M. (1988) Hepatocellular carcinoma among HBsAg positive blood donors in Fukuoka, Japan. *Eur. J. Cancer clin. Oncol.*, **24**, 235–239

Tong, M.J. & Govindarajan, S. (1988) Primary hepatocellular carcinoma following perinatal transmission of hepatitis B. *West. J. Med.*, **148**, 205–208

Tong, M.J., Sun, S.-C., Schaeffer, B.T., Chang, N.-K., Lo, K.-J. & Peters, R.L. (1971) Hepatitis-associated antigen and hepatocellular carcinoma in Taiwan. *Ann. intern. Med.*, **75**, 687–691

Tong, M.J., Weiner, J.M., Ashcavel, M.W. & Vyas, G.N. (1979) Evidence for clustering of hepatitis B virus infection in families of patients with primary hepatocellular carcinoma. *Cancer*, **44**, 2338–2342

Tong, S., Li, J., Vitvitski, L. & Trépo, C. (1990) Active hepatitis B virus replication in the presence of anti-HBe is associated with viral variants containing an inactive pre-C region. *Virology*, **176**, 596–603

Toukan, A.U. (1987) Hepatitis B virus infection in urban residents of Jordan with particular reference to socioeconomic factors. *Trop. Gastroenterol.*, **8**, 161–166

Toukan, A.U., Sharaiha, Z.K., Abu-El-Rub, O.A., Hmoud, M.K., Dahbour, S.S., Abu-Hassan, H., Yacoub, S.M., Hadler, S.C., Margolis, H.S., Coleman, P.J. & Maynard, J.E. (1990) The epidemiology of hepatitis B virus among family members in the Middle East. *Am. J. Epidemiol.*, **132**, 220–232

Transy, C., Fourel, G., Robinson, W.S., Tiollais, P., Marion, P.L. & Buendia, M.-A. (1992) Frequent amplification of c-*myc* in ground squirrel liver tumors associated with past or ongoing infection with a hepadnavirus. *Proc. natl Acad. Sci. USA*, **89**, 3874–3878

Trichopoulos, D., Tabor, E., Gerety, R.J., Xirouchaki, E., Sparros, L., Muñoz, N. & Linsell, C.A. (1978) Hepatitis B and primary hepatocellular carcinoma in a European population. *Lancet*, **ii**, 1217–1219

Trichopoulos, D., MacMahon, B., Sparros, L. & Merikas, G. (1980a) Smoking and hepatitis B-negative primary hepatocellular carcinoma. *J. natl Cancer Inst.*, **65**, 111–114

Trichopoulos, D., Sizaret, P., Tabor, E., Gerety, R.J., Martel, N., Muñoz, N. & Theodoropoulos, G. (1980b) Alphafetoprotein levels of liver cancer patients and controls in a European population. *Cancer*, **46**, 736–740

Trichopoulos, D., Day, N.E., Kaklamani, E., Tzonou, A., Muñoz, N., Zavitsanos, X., Koumantaki, Y. & Trichopoulou, A. (1987) Hepatitis B virus, tobacco smoking and ethanol consumption in the etiology of hepatocellular carcinoma. *Int. J. Cancer*, **39**, 45–49

Trowbridge, R., Fagan, E.A., Davison, F., Eddleston, A.L.W.F., Williams, R., Linskens, M.H.K. & Farzaneh, F. (1988) Amplification of the c-*myc* gene locus in a human hepatic tumour containing integrated hepatitis B virus DNA. In: Zuckerman, A.J., ed., *Viral Hepatitis and Liver Disease*, New York, Alan R. Liss, pp. 764–768

Tsai, S.L., Chen, P.J., Lai, M.Y., Yang, P.M., Sung, J.L., Huang, J.H., Hwang, L.H., Chang, T.H. & Chen, D.S. (1992) Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. *J. clin. Invest.*, **89**, 87–96

Tsega, E. (1977) Hepatocellular carcinoma in Ethiopia. A prospective clinical study of 100 patients. *East Afr. med. J.*, **54**, 281–292

Tsega, E., Gold, P., Shuster, J., Whittemore, B. & Lester, F.T. (1976) Hepatitis B antigen, alpha-fetoglobulins and primary hepatocellular carcinoma in Ethiopia. *J. trop. Med. Hyg.*, **79**, 230–234

Tsuda, H., Hirohashi, S., Shimosato, Y., Terada, M. & Hasegawa, H. (1988) Clonal origin of atypical adenomatous hyperplasia of the liver and clonal identity with hepatocellular carcinoma. *Gastroenterology*, **95**, 1664–1666

Tsuda, H., Hirohashi, S., Shimosato, Y., Ino, Y., Yoshida, T. & Terada, M. (1989) Low incidence of point mutation of c-Ki-*ras* and N-*ras* oncogenes in human hepatocellular carcinoma. *Jpn. J. Cancer Res.*, **80**, 196–199

Tsuda, H., Zhang, W., Shimosato, Y., Yokota, J., Terada, M., Sugimura, T., Miyamura, T. & Hirohashi, S. (1990) Allele loss on chromosome 16 associated with progression of human hepatocellular carcinoma. *Proc. natl Acad. Sci. USA*, **87**, 6791–6794

Tsukuma, H., Hiyama, T., Oshima, A., Sobue, T., Fujimoto, I., Kasugai, H., Kojima, J., Sasaki, Y., Imaoka, S., Horiuchi, N. & Okuda, S. (1990) A case–control study of hepatocellular carcinoma in Osaka, Japan. *Int. J. Cancer*, **45**, 231–236

Tswana, S.A. & Moyo, S.R. (1992) The interrelationship between HBV-markers and HIV antibodies in patients with hepatocellular carcinoma. *J. med. Virol.*, **37**, 161–164

Tu, J.-T., Gao, R.-N., Zhang, D.-H. & Gu, B.-C. (1985) Hepatitis B virus and primary liver cancer on Chongming Island, People's Republic of China. *Natl Cancer Inst. Monogr.*, **69**, 213–215

Turbitt, M.L., Patrick, R.S., Goudie, R.B. & Buchanan, W.M. (1977) Incidence in south-west Scotland of hepatitis B surface antigen in the liver of patients with hepatocellular carcinoma. *J. clin. Pathol.*, **30**, 1124–1128

Twu, J.-S., Lai, M.-Y., Chen, D.-S. & Robinson, W.S. (1993) Activation of proto-oncogene c-*jun* by the X protein of hepatitis B virus. *Virology*, **192**, 346–350

Tygstrup, N., Andersen P.K., Juhl, E. & a Trial Group of the European Association for the Study of the Liver (1986) Steroids in chronic B-hepatitis. A randomized, double-blind, multinational trial on the effect of low-dose, long-term treatment on survival. *Liver*, **6**, 227–232

Tzonou, A., Trichopoulos, D., Kaklamani, E., Zavitsanos, X., Koumantaki, Y. & Hsieh, C.-C. (1991) Epidemiologic assessment of interactions of hepatitis-C virus with seromarkers of hepatitis-B and -D viruses, cirrhosis and tobacco smoking in hepatocellular carcinoma. *Int. J. Cancer*, **49**, 377–380

Uchida, T., Suzuki, K., Esumi, M., Arii, M. & Shikata, T. (1988) Influence of aflatoxin B_1 intoxication on duck livers with duck hepatitis B virus infection. *Cancer Res.*, **48**, 1559–1565

Ulrich, P.P., Bhat, R.A., Seto, B., Mack, D., Sninsky, J. & Vyas, G.N. (1989) Enzymatic amplification of hepatitis B virus DNA in serum compared with infectivity testing in chimpanzees. *J. infect. Dis.*, **160**, 37–43

Vall Mayans, M., Calvet, X., Bruix, J., Bruguera, M., Costa, J., Estève, J., Bosch, F.X., Bru, C. & Rodés, J. (1990) Risk factors for hepatocellular carcinoma in Catalonia, Spain. *Int. J. Cancer*, **46**, 378–381

Van Den Heever, A., Pretorius, F.J., Falkson, G. & Simson, I.W. (1978) Hepatitis B surface antigen and primary liver cancer. *South Afr. med. J.*, **24**, 359–361

Vento, S. & Eddleston, A.L.W.F. (1987) Immunological aspects of chronic active hepatitis. *Clin. exp. Immunol.*, **68**, 225–232

Vento, S., Di Perri, G., Luzzati, R., Cruciani, M., Garofano, T., Mengoli, C., Concia, E. & Bassetti, D. (1989) Clinical reactivation of hepatitis B in anti-HBs-positive patients with AIDS (Letter to the Editor). *Lancet*, **i**, 332–333

Vijayakumar, T., Sasidharan, V.K., Ankathil, R., Remani, P., Kumari, T.V. & Vasudevan, D.M. (1984) Incidence of hepatitis B surface antigen (HBsAg) in oral cancer and carcinoma of uterine cervix. *Indian J. Cancer*, **21**, 7–10

Villarrubia, V.G., Valladolid, J.M., Elorza, F.L., Sada, G., Vilchez, J.G., Jimenez, M. & Herrerias, J.M. (1992) Therapeutic response of chronic active hepatitis B (CAHB) to a new immunomodulator: AM3. Immunohematological effects. *Immunopharmacol. Immunotoxicol.*, **14**, 141–164

Viola, M.V., Prince, A.M. & Alpert, E. (1972) Hepatitis-associated antigen in patients with cancer. *Yale J. Biol. Med.*, **45**, 64–69

Vitvitski, L., Meyers, M.L., Sninsky, J.J., Berthillon, P., Chevalier, P., Sells, M.A., Acs, G. & Trépo, C. (1988) Expression of the X gene product of hepatitis B virus and WHV in infected livers and transfected 3T3 cells: evidence for cross-reactivity and correlation with core/e gene expression. In: Zuckerman, A.J., ed., *Viral Hepatitis and Liver Disease*, New York, Alan R. Liss, pp. 341–344

Vogel, C.L., Anthony, P.P., Mody, N. & Barker, L.F. (1970) Hepatitis-associated antigen in Ugandan patients with hepatocellular carcinoma. *Lancet*, **ii**, 621–624

Vogel, C.L., Anthony, P.P., Sadikali, F., Barker, L.F. & Peterson, M.R. (1972) Hepatitis-associated antigen and antibody in hepatocellular carcinoma: results of a continuing study. *J. natl Cancer Inst.*, **48**, 1583–1588

Wain-Hobson, S. (1984) Molecular biology of the hepadnaviruses. In: Chisari, F.V., ed., *Advances in Hepatitis Research*, New York, Masson, pp. 49–53

Wainwright, R.B., McMahon, B.J., Bulkow, L.R., Hall, D.B., Fitzgerald, M.A., Harpster, A.P., Hadler, S.C., Lanier, A.P. & Heyward, W.L. (1989) Duration of immunogenicity and efficacy of hepatitis B vaccine in a Yupik Eskimo population. *J. Am. med. Assoc.*, **261**, 2362–2366

Wang, H.-P. & Rogler, C.E. (1988) Deletions in human chromosome arms 11p and 13q in primary hepatocellular carcinomas. *Cytogenet. Cell Genet.*, **48**, 72–78

Wang, H.-P. & Rogler, C.E. (1991) Topoisomerase I-mediated integration of hepadnavirus DNA *in vitro*. *J. Virol.*, **65**, 2381–2392

Wang, G.-H. & Seeger, C. (1992) The reverse transcriptase of hepatitis B virus acts as a protein primer for viral DNA synthesis. *Cell*, **71**, 663–670

Wang, N., Sun, Z., Pang, Q., Zhu, Y. & Xia, Q. (1980) Liver cancer, liver disease background and virus-like particles in serum among ducks from high incidence area of human hepatocellular carcinoma. *Chin. J. Oncol.*, **2**, 176–178 (in Chinese)

Wang, J., Chenivesse, X., Henglein, B. & Bréchot, C. (1990) Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. *Nature*, **343**, 555–557

Wang, W., London, W.T., Lega, L. & Feitelson, M.A. (1991) HBxAg in the liver from carrier patients with chronic hepatitis and cirrhosis. *Hepatology*, **14**, 29–37

Wang, J., Zindy, F., Chenivesse, X., Lamas, E., Henglein, B. & Bréchot, C. (1992) Modification of cyclin A expression by hepatitis B virus DNA integration in a hepatocellular carcinoma. *Oncogene*, **7**, 1653–1656

Waters, J.A., Kennedy, M., Voet, P., Hauser, P., Petre, J., Carman, W. & Thomas, H.C. (1992) Loss of the common 'a' determinant of hepatitis B surface antigen by a vaccine-induced escape mutant. *J. clin. Invest.*, **90**, 2543–2547

Wei, Y., Fourel, G., Ponzetto, A., Silvestro, M., Tiollais, P. & Buendia, M.-A. (1992) Hepadnavirus integration: mechanisms of activation of the N-*myc*2 retrotransposon in woodchuck liver tumors. *J. Virol.*, **66**, 5265–5276

Whittle, H.C., Bradley, A.K., McLauchlan, K., Ajdukiewicz, A.B., Howard, C.R., Zuckerman, A.J. & McGregor, I.A. (1983) Hepatitis B virus infection in two Gambian villages. *Lancet*, **i**, 1203–1206

Whittle, H.C., Lamb, W.H. & Ryder, R.W. (1987) Trials of intradermal hepatitis B vaccines in Gambian children. *Ann. trop. Paediatr.*, **7**, 6–9

Whittle, H.C., Inskip, H., Bradley, A.K., McLaughlan, K., Shenton, F., Lamb, W., Eccles, J., Baker, B.A. & Hall, A.J. (1990) The pattern of childhood hepatitis B infection in two Gambian villages. *J. infect. Dis.*, **161**, 1112–1115

Whittle, H.C., Inskip, H., Hall, A.J., Mendy, M., Downes, R. & Hoare, S. (1991) Vaccination against hepatitis B and protection against chronic viral carriage in the Gambia. *Lancet*, **337**, 747–750

Will, H., Reiser, W., Weimer, T., Pfaff, E., Büscher, M., Sprengel, R., Cattaneo, R. & Schaller, H. (1987) Replication strategy of human hepatitis B virus. *J. Virol.*, **61**, 904–911

Wills, W., Larouzé, B., London, W.T., Millman, I., Werner, B.G., Ogston, W., Pourtaghva, M., Diallo, S. & Blumberg, B.S. (1977) Hepatitis B virus in bedbugs (*Cimex hemipterus*) from Senegal. *Lancet*, **ii**, 217–220

Wollersheim, M., Debelka, U. & Hofschneider, P.H. (1988) A transactivating function encoded in the hepatitis B virus X gene is conserved in the integrated state. *Oncogene*, **3**, 545–552

Wong, V.C.W., Ip, H.M.H., Reesink, H.W., Nco Lelie, P., Reerink-Brongers, E.E., Yeung, C.Y. & Ma, H.K. (1984) Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HbeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. *Lancet*, **i**, 921–926

Wright, R., McCollum, R.W. & Klatskin, G. (1969) Australia antigen in acute and chronic liver disease. *Lancet*, **ii**, 117–121

Wright, H.I., Gavaler, J.S. & Van Thiel, D.H. (1992) Preliminary experience with alpha-2b-interferon therapy of viral hepatitis in liver allograft recipients. *Transplantation*, **53**, 121–124

Xu, J., Brown, D., Harrison, T., Lin, Y. & Dusheiko, G. (1992) Absence of hepatitis B virus precore mutants in patients with chronic hepatitis B responding to interferon-alpha. *Hepatology*, **15**, 1002–1006

Yaginuma, K., Kobayashi, M., Yoshida, E. & Koike, K. (1985) Hepatitis B virus integration in hepatocellular carcinoma DNA: duplication of cellular flanking sequences at the integration site. *Proc. natl Acad. Sci. USA*, **82**, 4458–4462

Yaginuma, K., Kobayashi, H., Kobayashi, M., Morishima, T., Matsuyama, K. & Koike, K. (1987) Multiple integration site of hepatitis B virus DNA in hepatocellular carcinoma and chronic active hepatitis tissues from children. *J. Virol.*, **61**, 1808–1813

Yap, E.P.H., Cooper, K., Maharaj, B. & McGee, J.O'D. (1993) p53 Codon 249 scr hot-spot mutation in HBV-negative hepatocellular carcinoma (Letter to the Editor). *Lancet*, **341**, 251

Yarrish, R.L., Werner, B.G. & Blumberg, B.S. (1980) Association of hepatitis B virus infection with hepatocellular carcinoma in American patients. *Int. J. Cancer*, **26**, 711–715

Yasui, H., Hino, O., Ohtake, K., Machinami, R. & Kitagawa, T. (1992) Clonal growth of hepatitis B virus-integrated hepatocytes in cirrhotic liver nodules. *Cancer Res.*, **52**, 6810–6814

Yeh, F.-S., Mo, C.-C., Luo, S., Henderson, B.E., Tong, M.J. & Yu, M.C. (1985a) A serological case-control study of primary hepatocellular carcinoma in Guangxi, China. *Cancer Res.*, **45**, 872–873

Yeh, F.-S., Mo, C.-C. & Yen, R.-C. (1985b) Risk factors for hepatocellular carcinoma in Guangxi, People's Republic of China. *Natl Cancer Inst. Monogr.*, **69**, 47–48

Yeh, F.-S., Yu, M.C., Mo, C.-C., Luo, S., Tong, M.J. & Henderson, B.E. (1989) Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Res.*, **49**, 2506–2509

Yoffe, B., Burns, D.K., Bhatt, H.S. & Combes, B. (1990) Extrahepatic hepatitis B virus DNA sequences in patients with acute hepatitis B infection. *Hepatology*, **12**, 187–192

Yokosuka, O., Omata, M., Zhou, Y.-Z., Imazeki, F. & Okuda, K. (1985) Duck hepatitis B virus DNA in liver and serum of Chinese ducks: integration of viral DNA in a hepatocellular carcinoma. *Proc. natl Acad. Sci. USA*, **82**, 5180–5184

Yoshizawa, K., Itoh, Y., Akahane, Y. & Tsuda, F. (1977) A direct immunofluorescence method for the detection of hepatitis B core antigen in formalin-fixed and gelatin-embedded liver specimens. *J. clin. Pathol.*, **30**, 776–778

Yu, M.C., Tong, M.J., Coursaget, P., Ross, R.K., Govindarajan, S. & Henderson, B.E. (1990) Prevalence of hepatitis B and C viral markers in black and white patients with hepatocellular carcinoma in the United States. *J. natl Cancer Inst.*, **82**, 1038–1041

Yuasa, Y. & Sudo, K. (1987) Transforming genes in human hepatomas detected by a tumorigenicity assay. *Jpn. J. Cancer Res. (Gann)*, **78**, 1036–1040

Zerial, M., Salinas, J., Filipski, J. & Bernardi, G. (1986) Genomic localization of hepatitis B virus in a human hepatoma cell line. *Nucleic Acids Res.*, **14**, 8373–8386

Zhang, X.-K., Egan, J.O., Huang, D.-P., Sun, Z.-L., Chien, V.K.Y. & Chiu, J.-F. (1992) Hepatitis B virus DNA integration and expression of an erb B-like gene in human hepatocellular carcinoma. *Biochem. biophys. Res. Commun.*, **188**, 344–351

Zhou, Y.-Z., Butel, J.S., Li, P.-J., Finegold, M.J. & Melnick, J.L. (1987) Integrated state of subgenomic fragments of hepatitis B virus DNA in hepatocellular carcinoma from mainland China. *J. natl Cancer Inst.*, **79**, 223–231

Zhou, Y.-Z., Slagle, B.L., Donehower, L.A., vanTuinen, P., Ledbetter, D.H. & Butel, J.S. (1988) Structural analysis of a hepatitis B virus genome integrated into chromosome 17p of a human hepatocellular carcinoma. *J. Virol.*, **62**, 4224–4231