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Advances in viral hepatitis

Report of the WHO Expert Committee on Viral Hepatitis

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CORRIGENDA

Page 24, lines 2–4

Delete : Three glycopeptides of molecular weights 19 000, 24 000, and 27 000 and 2 larger non-glycosylated polypeptides failed to elicit an antibody response in guineapigs.

Insert : Antibody and cell-mediated immune responses to 3 glycopeptides of molecular weights 19 000, 24 000, and 27 000 and several larger non-glycosylated polypeptides have been studied in immunized guineapigs. The 19 000 molecular weight polypeptide derived from *ayw* particles and the 27 000 molecular weight polypeptide from both subtypes *adw* and *ayw* failed to elicit an antibody response.

Page 48, line 4

Delete : — segregation of HBsAg-negative patients from susceptible patients

Insert : — segregation of HBsAg-positive patients from susceptible patients

Page 55, last sentence

Delete : Eventually an appropriate immunogenicity extinction test in nonhuman primates may be substituted for this criterion if . . .

Insert : Eventually an appropriate immunogenicity extinction test in other laboratory animals may be substituted for this criterion if . . .

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Geneva, 11–16 October 1976

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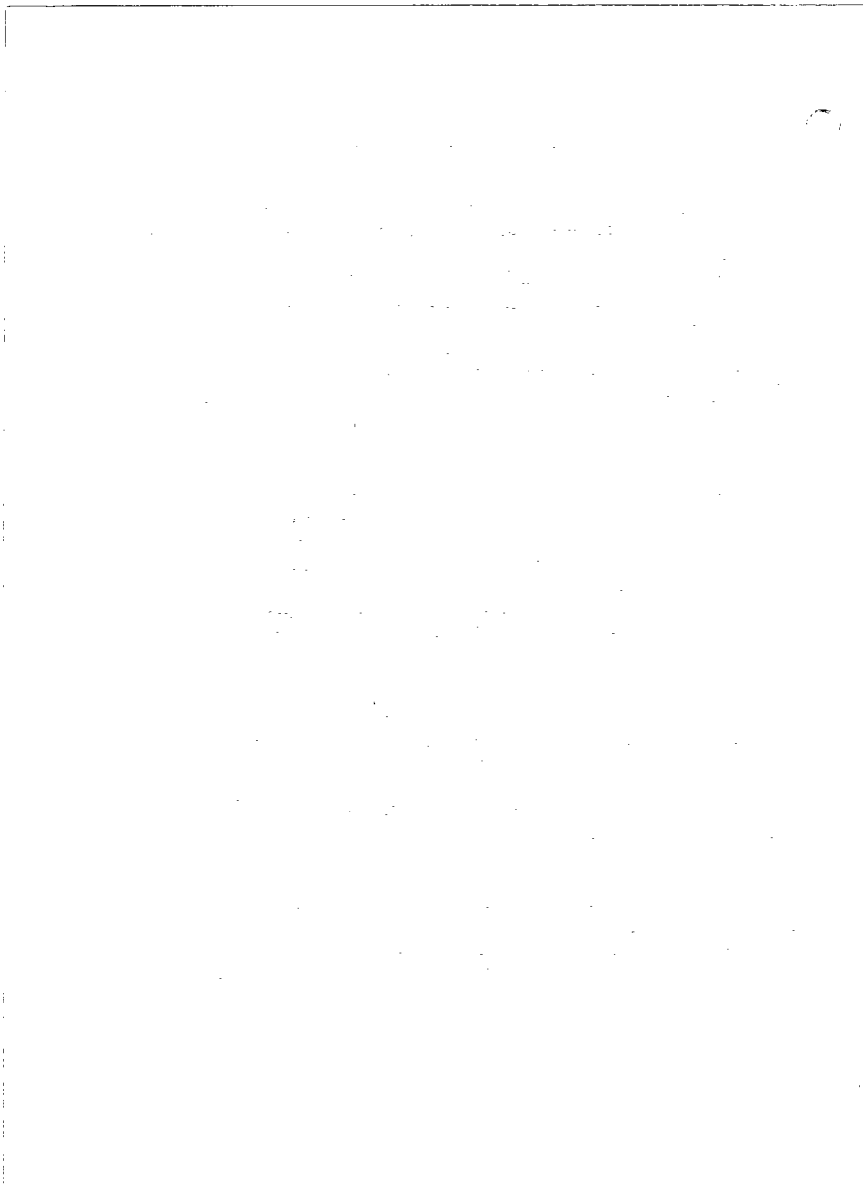

ADVANCES IN VIRAL HEPATITIS

Report of the WHO Expert Committee on Viral Hepatitis

A WHO Expert Committee on Viral Hepatitis met in Geneva from 11 to 16 October 1976. Dr I. D. Ladnyi, Assistant Director-General, opened the meeting on behalf of the Director-General.

1. INTRODUCTION

Twenty-five years have passed since the Third World Health Assembly originally requested that a WHO Expert Committee be convened on hepatitis. The first report of the Committee in 1953 called attention to the public health importance of the disease and to the limited knowledge of its etiology and epidemiology.[a] The next decade saw progress in the control of hepatitis A by passive immunization but few other advances, as the second report of the Committee in 1964 made clear.[b] Laboratories throughout the world were able to develop methods for studying the etiology, epidemiology, and immunology of hepatitis B only when it was realized in the late 1960s that the Australia antigen was an indicator of infection by hepatitis B virus. Three groups of experts subsequently convened by the Organization discussed this important finding and made recommendations for its application to control of the disease.[c,d,e]

Through WHO, international collaboration and the sharing of new information before its publication in scientific journals have become regular features of the global research efforts towards controlling hepatitis. The present report attempts to bring readers up to date on the rapid advances being made in this field, particularly those that have taken place since the last WHO meeting.[e] One advance is a simplified nomenclature based on the direct visualization of both hepatitis A and hepatitis B viruses and on their biochemical and biophysical properties. The report also reviews the significant progress that has been made in

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[a] WHO Technical Report Series, No. 62, 1953.

[b] WHO Technical Report Series, No. 285, 1964.

[c] *Bulletin of the World Health Organization*, **42** : 957 (1970).

[d] WHO Technical Report Series, No. 512, 1973.

[e] WHO Technical Report Series, No. 570, 1975.

the specific diagnosis of viral hepatitis, thanks to which a new type of hepatitis unrelated to hepatitis A or B virus has been recognized. This new type is now the most common form of post-transfusion hepatitis in some areas. In another development, experimental animal models of infections with hepatitis A and B viruses are making it possible to study the infectivity of both agents in the laboratory and to evaluate the safety and effectiveness of experimental hepatitis B vaccines before testing in man. New studies on passive immunization against hepatitis B and the first promising attempts at therapy for this disease are also covered in the present report. The suggested role of hepatitis B virus in primary liver carcinoma offers hope that through the control of this viral infection the incidence not only of hepatitis B but also of this form of cancer may be reduced.

2. TERMINOLOGY OF HEPATITIS VIRUSES AND ANTIGENS

Intensive research efforts all over the world are leading to a better understanding of the viruses of hepatitis and the antigens associated with them. In this report, the Expert Committee suggests modifications in nomenclature that take into account recent findings from many laboratories. In addition, to make the abbreviations for hepatitis antigens and antibodies easier to read, the Committee proposes to eliminate the use of subscript letters. Hepatitis B surface antigen, for example, thus would be abbreviated HBsAg rather than HB_sAg.

2.1 Hepatitis A virus

HAV	Hepatitis A virus. A small virus in the range of 25–28 nm possessing cubic symmetry. As with other viruses of this size, empty as well as full particles exist. Both full and empty particles are identified by immune electron microscopy. Other serological tests for hepatitis A virus include complement fixation, immune adherence haemagglutination, and radioimmunoassay.
anti-HAV	Antibody to hepatitis A virus.

2.2 Hepatitis B virus

HBV	Hepatitis B virus. A 42-nm double-shelled virus, originally known as the Dane particle.
HBsAg	Hepatitis B surface antigen. The hepatitis B antigen found on the surface of the virus and on the accompanying unattached spherical (22-nm) and tubular particles.

HBcAg	Hepatitis B core antigen. The hepatitis B antigen found within the core of the virus.
HBeAg	The *e* antigen that is closely associated with hepatitis B infection.
anti-HBs	Antibody to hepatitis B surface antigen.
anti-HBc	Antibody to hepatitis B core antigen.
anti-HBe	Antibody to the *e* antigen.

Subdeterminants of hepatitis B surface antigen

HBsAg carries a common determinant *a* and a number of major subdeterminants, which are coded by the virus genome and not by the host. The subdeterminants can be demonstrated by the development of " spurs " in immunodiffusion tests with appropriate reagents. Eight distinct categories and 2 categories of mixed subtypes have been recognized.[a] These consist of various combinations of the subdeterminants *d*, *y*, *w*, and *r*, and, in addition, other variants originally described as being related to the common determinant *a* but better designated as variants of the specificity *w* since they always behave as alleles of *r*. The 10 categories are as follows :

ayw1 (a_1yw)	*adw2* (a_2^1dw)
ayw2 (a_2^1yw)	*adw4* (a_3dw)
ayw3 (a_2^3yw)	*adr*
ayw4 (a_3yw)	*adyw*
ayr	*adyr*

The major subdeterminants behave as though they comprise 2 allelic groups : *d* and *y*, on the one hand, and *w1*, *w2*, *w3*, *w4*, and *r* on the other. However, these systems are probably not completely independent since only 2 of the 4 variants of *w* (found with *y*) have been demonstrated with *d*. The 2 mixed subtype categories are rare and may possibly result from phenotypic or genotypic mixing of determinants during simultaneous infection with viruses associated with more than one subtype of HBsAg.

Other surface antigenic reactivities, such as *q*, *x*, *f*, *t*, *j*, *n*, and *g*, have also been described. The necessary serological comparisons between these reactivities have not yet been made.

Subdeterminants of hepatitis B e *antigen*

Two antigens have been identified. These are designated HBeAg/1 and HBeAg/2.

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[a] Le Bouvier, G. L. & Williams, A. *American journal of the medical sciences*, **270** : 165 (1975).

2.3 Other hepatitis viruses

A new form of hepatitis has been recognized that is clinically indistinguishable from hepatitis type A or type B infection but antigenically unrelated to either type. In some areas, it is now the most common form of hepatitis occurring after blood transfusion. Secondary cases appear to be uncommon. Since no laboratory tests are yet available for identifying the agents or antigens associated with this new form of hepatitis, it would be premature to propose any special terms for it.

3. CLINICAL AND LABORATORY FINDINGS

While differences between the clinical syndromes of type A and type B viral hepatitis become apparent on analysis of large numbers of cases, these differences are not reliable for the diagnosis of individual patients with icteric disease.

Hepatitis A is frequently heralded by nonspecific symptoms such as fever, chills, headache, fatigue, generalized weakness, and aches and pains. A few days later, anorexia, nausea, vomiting, and right upper quadrant pain appear, followed closely by the passage of dark urine and light stools and jaundice of the sclera and skin.

The prodrome of hepatitis B is often prolonged and more insidious. Low-grade fever, arthralgias, and skin rashes, usually urticarial, are common prodromal features.

The well known clinical features of the established disease are similar for type A and type B viral hepatitis. Liver function tests also show a similar picture in both types, although the serum enzyme and bilirubin elevations tend to be more prolonged in hepatitis B. The mortality is probably similar in the two diseases, of the order of 1 : 500–1000, with the important exceptions of hepatitis B following blood transfusion and hepatitis A during pregnancy, for which much higher mortality rates have been reported.

In view of the difficulty of differentiating between these diseases on clinical and biochemical grounds, the advent of specific serological diagnostic tests for infection with both hepatitis A and hepatitis B viruses is of great importance.

As mentioned above, a new form of post-transfusion viral hepatitis antigenically unrelated to infection with hepatitis A or B virus has become apparent. This syndrome is not readily distinguishable from hepatitis B on clinical grounds, including incubation period and the duration and degree of bilirubin and transaminase elevation. However,

there are indications that severe acute disease and fulminant fatal disease are less likely to occur in patients with this syndrome than in those with hepatitis B.

4. PROPERTIES OF HEPATITIS A VIRUS

Hepatitis A virus (HAV) has been identified as a small (25–28 nm) virus possessing cubic symmetry.[a] In appearance, HAV resembles the RNA-containing picornaviruses and the DNA-containing parvoviruses. HAV has been detected in the faeces of man, chimpanzees, and marmosets, in the bile of chimpanzees, and in serum from man, chimpanzees, and marmosets with acute hepatitis A infection. Although virus-like particles of other sizes and morphological characteristics have been found in various faecal specimens from infected individuals, only the approximately 27-nm particles appear to be serologically and temporally related to HAV infection. Both " full " particles that are unpenetrated by stain and " empty " particles that are penetrated by stain have been detected by electron microscopy. However, the two variants are antigenically indistinguishable and aggregate when mixed with serum containing antibody to the virus. A viral envelope has not been found and subunit components have not been identified.

In limited studies, HAV was shown to be relatively resistant to inactivation by ether, heating at 60°C for 1 h, and acid at pH 3, but was inactivated by formaldehyde solution (0.25 ml/l) at 37°C for 72 h and by chlorine (1 mg/l) for 30 min. Nonionic detergents did not alter the morphology or destroy the infectivity of the virus, nor did storage at 4°C, −20°C, or −70°C.

HAV separates into 3 distinct populations of particles when banded in cesium chloride. The mean buoyant densities of these populations vary from 1.4 g/ml to 1.27 g/ml, with major peaks at densities of approximately 1.38–1.41 g/ml, 1.31–1.34 g/ml, and 1.24–1.29 g/ml. The lightest of the 3 major populations appears to consist of " empty " particles that are probably deficient in or devoid of nucleic acid. The relative sizes of the different populations vary from sample to sample and experiment to experiment, but heavy and intermediate-density particles predominate in faeces whereas the particles found in liver, serum, and bile are mainly of intermediate density. Low-density particles have also been detected in liver and bile. All 3 density populations are sero-

[a] PURCELL, R. H. ET AL. *American journal of the medical sciences*, **270** : 61 (1975).

logically indistinguishable, and infectivity has not been localized to one region of the density gradient. The heavy particles are relatively unstable in cesium chloride and their buoyant density tends to shift to approximately 1.34 g/ml after repeated banding. Based upon a density of 1.34 g/ml and a size of 27 nm, a sedimentation coefficient in sucrose of 110S has been estimated for these particles.

The biochemical nature of HAV has not been widely studied owing to the difficulty of obtaining virus particles. However, attempts have been made to determine the type of nucleic acid of this virus by studying its staining characteristics when exposed to acridine orange. While the quantities of material available were insufficient for definitive tests, these studies suggested that the nucleic acid of HAV may be either RNA or single-stranded DNA. These results, together with the finding of virus-like particles in the cytoplasm of hepatocytes obtained from marmosets and chimpanzees experimentally infected with HAV and the demonstration of a major band of particles with a buoyant density of 1.34 g/ml, have led to the suggestion that HAV is a member of the enterovirus subgroup of the picornaviruses. However, the remarkable heat stability of HAV and the existence of a multiplicity of populations with different buoyant densities and in particular one with a density of 1.4 g/ml suggest that HAV is similar to the parvoviruses. Additional studies of its nucleic acid and polypeptide composition must be carried out before it can be determined to which, if either, of these two classes of small viruses HAV belongs.

5. SEROLOGICAL TECHNIQUES FOR HEPATITIS A

Four methods have been developed for assaying hepatitis A virus antigen (HAV antigen) and antibody to HAV (anti-HAV). At present they are being used only in research laboratories, largely because of the difficulty of finding convenient sources of antigen.

5.1 Immune electron microscopy

This is a technique whereby the interaction between virus particles and specific antibody is visualized by electron microscopy. When immune electron microscopy is performed by an experienced investigator, as few as 10^4–10^6 particles per millilitre may be detected, a level that is approximately 1000 times more sensitive than that reported for routine electron microscopy.

Detection of HAV particles by immune electron microscopy is achieved by incubating specific anti-HAV serum with a filtrate of faeces. Immune aggregates of virus and antibody and single particles coated with antibody are concentrated by ultracentrifugation and negatively stained with tungstophosphoric acid. Each grid is then examined for typical HAV particles at a magnification of 40 000–60 000. For antibody quantification, a faecal extract containing HAV particles is mixed with an unknown serum specimen.

Problems associated with this technique include the presence in faecal specimens of a multitude of bacterial, bacteriophage, viral, and other particulate antigens whose differentiation is difficult for the inexperienced microscopist. In addition, antibodies specific for these other antigens may be present if human antisera are used for the examination of faeces for HAV by immune electron microscopy. It must be emphasized that this method is satisfactory only in the hands of an experienced microscopist.

Locating sources of reagents is another problem : large quantities of HAV particles are not yet readily available. This problem is mostly due to the fact that the initial shedding of particles by infected patients often precedes the earliest detectable rise in alanine aminotransferase (EC 2.6.1.2) or the onset of prodromal symptoms. Maximum shedding of virus particles occurs shortly thereafter, before the onset of jaundice; once jaundice develops, the amount of particle shedding falls abruptly. Thus, few or no HAV particles are found in faecal extracts by the time most patients are seen by physicians.

Despite the cumbersome, time-consuming aspects of immune electron microscopy, the technique has been essential for identifying human or nonhuman primate faecal preparations rich in HAV.

5.2 Complement fixation and immune adherence haemagglutination

The practical limitations of immune electron microscopy were recognized early, and the need for a rapid, more sensitive quantitative assay that could be applied to a large number of specimens became apparent. Using liver extracts obtained from *Saguinus mystax* marmosets infected with HAV, investigators developed a specific diagnostic complement fixation test [a] and an immune adherence haemagglutination assay [b] for

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[a] PROVOST, P. J. ET AL. *Proceedings of the Society for Experimental Biology and Medicine*, **148** : 962 (1975).

[b] MILLER, W. J. ET AL. *Proceedings of the Society for Experimental Biology and Medicine*, **149** : 254 (1975).

the detection of anti-HAV. Immune adherence haemagglutination is a specific immunological reaction in which microorganisms or other antigens complexed with antibody and complement become attached to the surface membrane of selected primate erythrocytes. The reaction is mediated through the first four components of complement, although bound C3 is the component primarily responsible for mediating the reaction. Immune adherence haemagglutination is simple to perform and is reported to be 10–100 times more sensitive than complement fixation for detecting anti-HAV. However, problems with specificity have occasionally occurred and some preparations of antigen have failed to work satisfactorily. In some cases, difficulties were traced to insufficient purity of the antigen or to its low concentration, or to haemolysis of cells by preparations containing detergents used in the purification procedure. The presence of cesium chloride in a concentration above 1.07 kg/l (1.07 g/ml) inhibits immune adherence haemagglutination.

5.3 Solid-phase radioimmunoassay

The radioimmunoassay procedure can be conveniently divided into 3 stages : (*i*) adsorption of unlabelled antibody to the poly(vinyl chloride) wells of a microtitre plate ; (*ii*) extraction of immunologically active antigen by the coupled antibody ; and (*iii*) detection of bound antigen by combination with labelled specific antibody. Test results are expressed frequently in terms of a positive/negative ratio. This is determined by dividing the counts per minute of the test sample by the mean counts per minute of the negative control samples. Any alterations in the test conditions that reduce nonspecific background counts in the negative control wells and simultaneously stabilize or enhance specific binding will result in a substantial increase in the positive/negative ratio.

The sensitivity of radioimmunoassay can be enhanced by increasing the quantity of antibody adsorbed to the microtitre wells. Recent studies have shown that binding of IgG to poly(vinyl chloride) is dependent on the initial concentration of antibody, time and temperature, and the quality of the adsorbing protein. Dilution of unlabelled antibody with a protein-containing diluent (such as phosphate buffer solution containing bovine serum albumin) decreases antibody adsorption, especially when use is made of IgG preparations or immunoglobulin.

A blocking test can also be used to measure anti-HAV.[a] Briefly, microtitre wells coated with anti-HAV are incubated with purified HAV

[a] PURCELL, R. H. ET AL. *Journal of immunology*, **116** : 349 (1976).

and then washed, and the residual fluid is removed. Decimal dilutions of the sera to be tested are added and incubation continues overnight at 4°C. After another washing step, labelled anti-HAV is added. A reduction in radioactivity of 40% or more as compared with control serum containing no anti-HAV is considered evidence for the presence of anti-HAV in the sample.

Another radioimmunoassay procedure[a] for measuring anti-HAV has been described in which microtitre wells are directly coated with the serum to be tested for anti-HAV content. After suitable incubation with purified HAV, labelled anti-HAV is added. After further incubation and washing, the wells are cut out and counted in a gamma counter. The test sample is considered positive for anti-HAV if the counts per minute of the test sample are more than twice the mean counts per minute of a set of negative control sera tested at the same time. Optimum sensitivity and specificity in this system are obtained when sera are screened at a dilution of 1 : 1000.

In contrast to immune adherence haemagglutination, which detects anti-HAV only in late convalescent phase sera, the other 3 techniques —immune electron microscopy, complement fixation, and radioimmunoassay—detect anti-HAV in sera collected during the acute and convalescent phase of illness. This may be due to their enhanced ability to detect IgM-specific anti-HAV.

Complement fixation is the least sensitive of the 4 techniques for the detection of anti-HAV. Immune electron microscopy and radioimmunoassay are apparently of equal sensitivity, while the sensitivity of immune adherence haemagglutination is intermediate between their level and that of complement fixation.

6. HEPATITIS A IN ANIMAL MODELS

The failure to find an *in vitro* cell culture system for the propagation of human HAV has prompted an extensive search for animal models to study the pathogenesis of the infection, to provide materials for biophysical characterization of the infective agent, and to produce reagents for serological tests. To date only nonhuman primates have been found useful in this respect. Most studies have involved chimpanzees and marmoset monkeys of the *Saguinus* species.[b]

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[a] HOLLINGER, F. B. ET AL. *American journal of clinical pathology*, **65** : 854 (1976).

[b] DEINHARDT, F. *Advances in virus research*, **20** : 113 (1976).

The serological techniques developed for the detection of HAV and anti-HAV make it possible to determine the susceptibility of chimpanzees to infection with HAV. With these techniques it has been shown that as many as 90% of chimpanzees captured from the wild possess anti-HAV when tested after importation and are therefore unsuitable for infectivity studies. The colony-born chimpanzee is much more likely to be susceptible to HAV infection. Inoculation of susceptible chimpanzees with HAV by either the oral or the intravenous route consistently produces hepatitis in these animals, accompanied by excretion of the virus in faeces during the incubation period and the early acute phase of the illness.

The interval between inoculation and the onset of elevation of serum enzymes in typical experimental infections ranges from 15 to 30 days. Although chimpanzees do not become icteric, the histopathological features seen in liver biopsy specimens obtained from these animals during the acute phase of illness are similar to those seen in man. In addition to the HAV excreted in faeces, virus particles are also found in bile and have been seen by immune electron microscopy in the cytoplasm of chimpanzee hepatocytes. Suspensions of HAV purified from chimpanzee faeces have been used successfully as reagents for the radioimmunoassay and immune adherence haemagglutination assay of anti-HAV.

The susceptibility of marmosets to infection with HAV has been confirmed in a number of studies conducted in several laboratories. Different species of marmosets are not, however, equally susceptible to HAV infection; *S. mystax* are the most uniformly susceptible. The MS-1 strain of HAV has been serially passaged in *S. mystax*. In all passages, animals have developed biochemical and histological evidence of hepatitis, and anti-HAV has been found in convalescent sera. However, in any given set of experimental inoculations, not all animals necessarily develop hepatitis or anti-HAV. The percentage of successful inductions of hepatitis may range from 30% to 100%. These findings suggest that experimental inoculations are best conducted in groups of at least 5 animals. In addition to the MS-1 strain, the CR-326 strain of HAV from Costa Rica has been studied extensively in *S. mystax*. The morphological and immunological characteristics of the CR-326 and MS-1 strains of HAV isolated from experimentally infected marmosets are similar. On the other hand, recent studies have shown that the GB agent, originally isolated in marmoset studies, is not related to HAV. Marmosets that develop hepatitis when inoculated with this agent do not produce anti-HAV, and after their convalescence they develop hepatitis again when inoculated with HAV.

The supply of nonhuman primates, especially marmosets, is at present severely limited. These primates are of importance for research in viral hepatitis, which requires at least small numbers of laboratory-bred animals. It is recognized that hepatitis studies using nonhuman primates should be undertaken only after due consideration is given to the need for preserving these species. Breeding colonies for chimpanzees and marmosets should be established wherever possible.

7. EPIDEMIOLOGY AND CONTROL OF HEPATITIS A

Geographical distribution. Hepatitis A has a worldwide distribution but the exact incidence is difficult to estimate because of differences in surveillance and differing patterns of disease. Epidemics have been described in many countries and the disease appears to be endemic in numerous tropical and subtropical areas.

Age distribution. In developed countries, infections occur at all ages with about 50% of clinical cases being seen in children less than 15 years old. In tropical and subtropical areas, most infections are probably acquired in childhood and many are subclinical.

Seasonal pattern. In temperate zones, hepatitis A occurs in epidemic waves with peaks every 5–20 years. In some but not all temperate areas, e.g., Scandinavia and the USA, the disease has a pronounced seasonal variation, with a peak in late autumn and early winter and low prevalence in midsummer. In India the disease tends to be associated with periods of heavy rainfall.

The long-term trend is not clear, but evidence from several developed countries suggests that the incidence of hepatitis A is declining and that a greater proportion of cases are occurring in adults. Whether the decline is a long-term trend or merely the low point of the current epidemic cycle is not known.

7.1 Modes of spread

Intestinal–oral

In the absence of a major human or animal reservoir of infection, it is probable that the hepatitis A virus is maintained by serial transfer probably by the intestinal–oral route. The commonest mode of spread is by close contact such as occurs in the home. Infection occurs readily under conditions of poor sanitation and overcrowding, such as may be seen in institutions for the mentally retarded and during wars.

Waterborne epidemics of hepatitis A have been documented on many occasions and this mode of spread is probably common in countries where standards of hygiene and sanitation are low. The largest outbreak to date occurred in New Delhi during December 1955 and January 1956 when 29 300 cases resulted from the contamination of a major water supply with human sewage. During the period 1971–74, 13 outbreaks involving 351 patients and resulting from contamination of private or recreational waters were documented in the USA. This represented 0.2% of the number of cases of hepatitis A and 2% of all waterborne infections reported over that period in the USA.

Numerous foodborne epidemics have been described and attributed to infected food handlers' shedding virus during the incubation period of the disease. Where careful epidemiological studies are performed it is often possible to detect the source of the outbreak, which is usually uncooked food or food that has been handled after cooking. Despite the dramatic quality of such outbreaks, there is little evidence that either foodborne or waterborne transmission is a major factor in the maintenance of infection in developed countries.

The ingestion of shellfish grown in polluted waters is attended by a risk of acquiring hepatitis A. The mode of preparation of the shellfish is important : frying appears to destroy the virus whereas steaming does not, probably because the shells open and the contents are consumed before virucidal temperatures are attained. Some outbreaks raise serious questions about monitoring programmes, because the waters in question have met current national requirements for shellfish growing.

Other modes of spread

The possibility that hepatitis A can be spread by the respiratory route, by infected urine, and by sexual contact has been considered but there is currently no convincing evidence that this occurs. Serum containing HAV has been shown to be infective upon inoculation and occasional cases of syringe-transmitted hepatitis A have been reported, but the fact that the disease is rarely transmitted by transfusion suggests that persistent viraemia is a rare event.

There is no evidence that a pregnant woman who develops hepatitis A transmits the infection to her fetus, and the suggestion that maternal infection during pregnancy may result in Down's syndrome in the baby has not been substantiated. In India, in contrast to the situation in other countries, a high mortality has been observed in women who develop hepatitis during the second or third trimester of pregnancy.

Epidemics of hepatitis among people who come into contact with jungle-caught chimpanzees and other nonhuman primates and the detection of anti-HAV in a large proportion of these animals have raised the possibility that they constitute an extrahuman reservoir of infection. Alternatively, it is possible that the animals are infected during the holding period after capture, and subsequently transmit the disease to their handlers.

Specific diagnostic tests for infection with HAV, based on the detection of the virus in the faeces or rising serum titres of anti-HAV, are now available for laboratory use. Infection with HAV has been confirmed in both experimentally induced and naturally occurring disease in most of the epidemiological situations in which this virus has classically been implicated, namely person-to-person spread, institutional outbreaks, and epidemics associated with the ingestion of contaminated food, water, and shellfish.

Recent sero-epidemiological studies in the USA have demonstrated an increasing prevalence of antibody with age and relationship with socioeconomic class (Table 1). Preliminary studies of volunteer blood donors show marked differences in antibody prevalence in different countries (Table 2), which presumably reflect differing epidemiological patterns.

7.2 Control

Spread from the patient with hepatitis A is reduced by appropriate precautions such as good personal hygiene, the sanitary disposal of

TABLE 1. PREVALENCE OF ANTIBODY TO HEPATITIS A VIRUS (ANTI-HAV) BY AGE AND SOCIOECONOMIC CLASS [a]

Age (years)	Socioeconomic class							
	Upper		Middle		Lower		Total	
	No. tested	% with anti-HAV	No. tested	% with anti-HAV	No. tested	% with anti HAV	No. tested	% with anti-HAV
0– 4	6	0	19	5	13	31	38	13
5–14	36	3	90	20	51	24	177	18
15–29	29	17	58	57	21	48	108	44
30–49	47	49	73	78	20	80	140	69
50 +	30	70	23	83	22	77	75	76
Total	148	34	263	49	127	46	538	36

[a] Based on unpublished data from a study in Corpus Christi, TX, USA.

TABLE 2. DETECTION OF ANTIBODY TO HEPATITIS A VIRUS (ANTI-HAV) IN VOLUNTEER BLOOD DONORS FROM VARIOUS COUNTRIES [a]

Country	No. tested	No. with anti-HAV	% with anti-HAV
Switzerland	98	23	23.5
USA	629	251	39.9
Senegal	102 [b]	76	74.5
Belgium	133	116	87.2
China (Province of Taiwan)	93	82	88.2
Israel	112	105	93.8
Yugoslavia	100	97	97.0

[a] Based on unpublished data.
[b] Patients admitted to hospital with diseases other than those affecting the liver.

excreta, and the sterilization of eating utensils and of body and bed linen after use.

The intramuscular administration of normal pooled human immunoglobulin (a 16% solution in a dose of 0.02–0.12 ml/kg of body weight) before exposure to the virus or early during the incubation period will prevent or attenuate a clinical illness, while not always preventing infection. Inapparent or subclinical hepatitis may develop. This may be followed by passive–active immunity, which could confer prolonged immunity on the individual.

The value of immunoglobulin in controlling outbreaks of infection in places such as nursery schools has been demonstrated repeatedly. However, the view has been expressed that the use of immunoglobulin on a very large scale is unwise for 3 reasons: (*i*) individuals with unrecognized anicteric or subclinical disease may disseminate the virus in the general community, (*ii*) the practice appears to be wasteful, and (*iii*) repeated injections of immunoglobulin may be undesirable in, for example, healthy children.

The newly developed methods for titrating anti-HAV make it possible to quantify specific antibody in pooled immunoglobulin. All batches of immunoglobulin should be titrated for anti-HAV as soon as suitable reagents become generally available and studies correlating antibody levels with protection are to be encouraged.

8. PROPERTIES OF HEPATITIS B VIRUS

The circulating particles associated with hepatitis B virus (HBV) fall into at least three distinct morphological categories: (*i*) small pleomorphic spherical particles with an average diameter of 22 nm; (*ii*) tubular

forms of varying length and shape; and (*iii*) the HBV itself—a large (42-nm) double-shelled spheroidal particle. The large HBV particles may be full, partially full, or empty, and they can be separated into a core and an outer coat or envelope.

8.1 Hepatitis B surface antigen

The presence of distinct surface antigenic determinants on particulate structures associated with HBV has facilitated the isolation of the surface antigen (HBsAg) from normal serum proteins for immunochemical analysis. The lipoprotein nature of the surface antigen permits its partial separation from the normal serum proteins by virtue of its characteristic buoyant density. Antigenic activity is found at a density within the range defining one of the two major subclasses of serum high-density lipoproteins (HDL_3: 1.08–1.21 g/ml). The exact buoyant density of HBsAg varies with the serum tested and with the chemical used to constitute the density gradient. Centrifugation of serum in buffered cesium chloride results in the separation of the surface antigen at an average density of 1.20 g/ml. Although the tubular forms are present in the same fraction, only some of the empty or partially full HBV particles are found at this density. Full or partially full HBV particles are recovered at the slightly higher density of 1.25 g/ml after equilibrium centrifugation in cesium chloride.

The purified small 22-nm particles, which comprise the bulk of the surface antigenic mass in most sera, have been the preparations most commonly used for serological and biochemical studies. All three major morphological forms may be resolved by rate zonal centrifugation, with the relatively slow-sedimenting small particles having a mean sedimentation coefficient (in water at 20°C) in the range 33–54S. The diffusion coefficient of the small particles in the analytical ultracentrifuge has been found to be 2.278×10^{-7} cm^2/s. This value is compatible with an estimated molecular weight of 2.4×10^6, which is in good agreement with another estimate of 2.5×10^6 based on gel filtration.

The lipid content of the purified small particles may account for up to 30% of their total weight. Analysis of a chloroform–methanol extract by thin-layer chromatography in silica gel revealed predominantly polar lipids as well as cholesterol and smaller quantities of non-polar lipids. Phosphatidyl choline, sphingomyelin, and lysophosphatidyl choline were the major phospholipids present. Phosphatidyl serine was notably absent and phosphatidyl ethanolamine could not be detected in one study.

The protein moiety of HBsAg-bearing particles has been extensively analysed. Examination of purified small spherical particles by ultraviolet absorption spectroscopy has shown an absorption spectrum typical of protein. A substantial tryptophan content of approximately 14% may account for the somewhat high absorbance (at 280 nm for a 1% solution) of 37.26, although other values in the 25–30 range have been reported. HBsAg is also rich in hydrophobic amino acids, particularly leucine. Studies by optical rotatory dispersion and circular dichroism indicate that 70–80% of the total protein content exists in the form of α-helix. A similar high α-helix content occurs in serum low-density lipoproteins and the filamentous bacteriophages, but other viruses in general have a 10–25% α-helix content. It should be noted that proline, which accounts for 11.6–13.6% of the total HBsAg amino acids, does not take part in α-helix formation.

The polypeptide composition of the surface antigen has been subjected to extensive analysis. Initially, 2 major polypeptide species with average molecular weights of 25 000 and 30 000 were described. Other components of higher molecular weight were present in variable amounts at certain stages of purification and were assumed to be contaminating serum proteins with a possible stabilizing role in preserving antigenic activity. However, further studies of HBsAg have demonstrated the presence of both larger and smaller polypeptide components. Reproducible differences between the polypeptide compositions of the *ad* and *ay* subtypes of HBsAg have been reported, additional minor components being found in the *ay* material. In another study, however, no fundamental qualitative difference was found between the protein of these subtypes. Periodic-acid-Schiff staining of acrylamide gels has been found in at least 3 polypeptides, indicating the presence of a carbohydrate moiety. In addition, 2 glycosphingolipids have been extracted that are structurally similar to the fucosylglycolipids, or blood group glycolipids.

Little information is available on the quaternary structure of HBsAg particles and its importance in maintaining antigenic integrity.

Studies involving the treatment of purified HBsAg with organic solvents and dissociating reagents showed that antigenic activity was remarkably stable in the presence of compounds promoting denaturation, in particular diethyl ether, chloroform–urea in the proportion of 1 : 1, sodium dodecyl sulfate, and various proteolytic enzymes. However, treatment with ethanol and butanol led to a complete loss of antigenic reactivity. It has also been shown that HBsAg remains stable after incubation at an acid pH for several hours. Treatment by 5-fold dilution

with 0.02N HCl, pH 2.3, containing 0.02% pepsin resulted in an antigen free of normal serum proteins. Such a preparation was found to be suitable for the immunization of both guineapigs and rabbits. It was also noted that pretreatment with sodium dodecyl sulfate or diethyl ether increased the susceptibility of the antigen to proteolytic enzymes. Superficial lipids may therefore exert a protective effect on antigenic determinants composed primarily of protein.

The reduction of disulfide bonds results in a complete loss of HBsAg reactivity, although considerable antigenic activity may be regained by the alkylation of free sulfhydryl groups with iodoacetamide. Immunodiffusion and haemagglutination-inhibition techniques have been used to define reduction-sensitive and reduction-resistant components of the antigen. The group determinant *a* was destroyed by exposure to dithiothreitol at concentrations below 0.01 mol/l. At higher concentrations of dithiothreitol, antigenic activity resistant to reduction and unrelated to the *d*, *y*, *w*, and *r* subdeterminants was present on the same antigen particles.

The reactivity of HBsAg is heat-stable ; no loss of antigenic activity occurs after purified antigen is heated for 10 h at 60°C, although heating for 5 min at 100°C completely abolishes its affinity for antibody. Total loss of antigenic activity has been reported after incubation of HBsAg for 60 min at 85°C. It has also been demonstrated that the common determinant *a* is stable at 60°C for periods of up to 21 h.

The stability of the surface antigen at high temperatures and its resistance to protease digestion suggest that it contains carbohydrate. The presence of carbohydrate as well as lipid and protein is also suggested by the precipitation of radiolabelled antigen by concanavalin A and a positive anthrone reaction. The possibility has been raised that carbohydrate may play a role in maintaining the serological activity of HBsAg. A 90% reduction in serological activity of purified surface antigen particles was found after treatment with sodium periodate (0.1 mol/l) for 4 h at 37°C. A significant proportion of carbohydrate relative to protein content was found in the same preparations by the phenolsulfuric acid method. The carbohydrate content has been estimated at 3.6–6.5%, and there is evidence that at least some of the carbohydrate moiety is present as glycolipid. However, it remains to be established whether carbohydrate helps to preserve the structural integrity of adjacent antigenic sites or carries a novel haptenic specificity.

Several attempts have recently been made in animals to raise specific antisera to individual polypeptides separated from the surface antigen

by acrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Three glycopeptides of molecular weights 19 000, 24 000, and 27 000 and 2 larger non-glycosylated polypeptides failed to elicit an antibody response in guineapigs. The non-glycosylated polypeptides derived from *adw* and *ayw* subtypes elicited antibodies that cross-reacted in a radioimmunoprecipitation assay with intact surface antigen particles. The polypeptides were therefore considered to contain at least the *a* group-specific determinant. The 24 000 molecular weight glycopeptide produced antibodies that reacted only with the homologous antigen subtype. Further studies of these polypeptides demonstrated a cell-mediated immune response to the 24 000 and 40 000 molecular weight components. Peritoneal exudate cells from guineapigs inoculated with the 40 000 molecular weight polypeptide showed a significant response when challenged with intact homologous and intact heterologous HBsAg particles. Exudate cells from animals immunized with the 24 000 molecular weight glycopeptide derived from subtype *adw* antigen responded to intact homologous antigen and its 24 000 and 40 000 molecular weight components. However, a poor response to the *ayw* surface antigen was observed in these animals. In another study, antisera to 7 polypeptides obtained by sodium dodecyl sulfate–acrylamide gel electrophoresis of surface antigen with the *adw* determinants were found to react with *adw*- and *ayw*-coated red blood cells on passive haemagglutination assay, which indicated that each of the 7 polypeptides possessed at least 1 common group-specific determinant. Competition inhibition experiments using intact *adw* particles as the competing antigen yielded parallel slopes for the antisera. The displacement of the linear portion of the inhibition curve reflected a difference in the binding affinity of these antisera for the intact HBsAg particle. Further characterization based on the passive haemagglutination assay for antibody subtype analysis showed that each polypeptide stimulated subtype-specific as well as group-specific antibodies.

Although the HBsAg preparations in these studies contained no demonstrable normal human serum proteins, a positive cell-mediated immune response was elicited in guineapigs immunized with normal human serum upon challenge with the 24 000 molecular weight glycopeptide mentioned above. This finding suggests that this particular glycopeptide contains at least 1 antigenic determinant related to certain constituents of normal human serum.

It has been suggested that HBsAg particles may contain small amounts of normal serum components or that the latter may be contaminants of purified HBsAg preparations. Recently, it was reported

that surface antigen was specifically adsorbed to immunoadsorbent columns containing sheep anti-human plasma immunoglobulins covalently linked to Sepharose 4B. Prior treatment of purified surface antigen with proteases and nonionic detergents, in the presence or absence of diethyl ether, did not prevent adsorption of the antigen to columns containing antisera to prealbumin, albumin, apolipoproteins C and D, and the γ chain of IgG, which suggests that antigenic determinants related to host proteins may be associated with HBsAg. Reduction and alkylation of the preparation abolished surface antigen reactivity but did not prevent its adsorption, indicating that the HBsAg-associated determinants related to plasma proteins are distinct from the group-specific and subtype-specific determinants of the surface antigen. The presence of additional antigenic determinants in close association with HBsAg particles has also been noted. Low-affinity immunoprecipitation reactions with antisera to a range of normal human serum components have been demonstrated. These determinants were not released by exposure to acid, Tween 80, or ether, but they were removed by exposure of the surface antigen to trypsin or bromelain under conditions that otherwise preserved the structure of the small particles. It should be noted that the degree of purity of such preparations varies from laboratory to laboratory.

8.2 Hepatitis B core antigen

HBV particles consist of a core of about 27 nm in diameter with a 2-nm thick shell and an outer coat or envelope approximately 7 nm thick. Detergent treatment of these particles results in their separation into an outer coat of HBsAg and an inner component about 27 nm in diameter. Antibody in convalescent hepatitis B serum reacts with the core to yield distinct immune aggregates, without involvement of the other morphological forms of hepatitis B antigen. Similar core particles have been found in liver homogenates obtained *post mortem* from patients with chronic hepatitis and experimentally infected chimpanzees, and they have also been demonstrated by thin-section electron microscopy in liver biopsy specimens from patients with HBsAg-associated chronic liver disease.

The hepatitis B core antigen (HBcAg) has been closely identified with HBV particles separated by equilibrium centrifugation from the small spherical and tubular forms of the surface antigen. The buoyant density of the core can be estimated after the removal of the outer

surface envelope by treatment with nonionic detergents, although the exact value may vary depending on the completeness of removal of the surface antigen. After treatment of HBV particles with 1% Nonidet P40, core particles have been recovered at a density of 1.31 g/ml. The cores appeared to be aggregated by small protein molecules that were considered to be either core antibody or a " matrix " protein situated between the core antigen and the surface antigen and not removed by the nonionic detergent. In another study, HBcAg was separated at a similar density but still contained traces of surface antigen, in contrast to a second heavier population of 1.35–1.36 g/ml density that contained no detectable surface antigen. In this context, it should be noted that HBcAg-reactive particles extracted from infected human liver were shown to have a buoyant density of 1.30 g/ml. In a similar investigation of particles obtained from the liver of an experimentally infected chimpanzee, HBcAg activity was found in fractions with densities of 1.30–1.33 g/ml.

Several studies indicate that the core possesses icosahedral symmetry. Treatment of HBV particles with Tween 80 released a structure consisting of an outer shell having capsomere-like units approximately 4 nm in diameter. This observation is consistent with the demonstration by optical rotation electron microscopy of an icosahedral symmetry of the core. Examination by electron microscopy of purified particles from the liver of an experimentally infected chimpanzee also suggested a subunit structure organized according to the principles of icosahedral symmetry.

An HBcAg-associated DNA-dependent DNA polymerase activity is closely related to the DNA template of HBV. In all the preparations examined, which had been selected on the basis of a high proportion of HBV particles, a moderate rate of incorporation of ^{3}H-TTP into an acid-insoluble product was found over a 6-h period of incubation at 37°C. No exogenous template was required. The reaction was stimulated by magnesium ions and had an optimum pH of 7.7. The inclusion of the detergent Nonidet P40 considerably enhanced the rate of incorporation of ^{3}H-TTP. The product remained firmly bound to the core, as was shown by the specific precipitation of trace label by core antibody. Detergent treatment therefore appears to be necessary for the activation of the polymerase reaction through removal of the outer surface antigen coat from the HBV particle. Exposure of the core antigen during this reaction results in labelled material that can be used in radioimmune procedures for the detection of core antibody. Centrifugation studies demonstrated that the radiolabel cosediments with a

rapidly sedimenting fraction of released core components possessing a sedimentation coefficient of 110S.

The nature of the product was investigated further after phenol extraction of cores treated with sodium dodecyl sulfate and mercaptoethanol. Approximately 20% of the acid-precipitable label was recovered in the aqueous phase and was subsequently found to possess a buoyant density typical of DNA, banding at 1.71 g/ml. The double-stranded nature of this material was shown by its resistance to degradation by single-stranded nuclease (S_1) and the demonstration that its sedimentation coefficient of 15S remained unchanged over a wide range of salt concentrations.

Inability to inhibit the synthesis of DNA *de novo* by either DNase or RNase has made it necessary to disrupt purified cores in order to characterize the endogenous template. Double-stranded DNA has been isolated from circulating HBV particles as well as from particles closely resembling cores obtained from the nuclei of infected hepatocytes. Core particles isolated from plasma were concentrated more than a thousandfold before activation of the polymerase reaction and disruption with sodium dodecyl sulfate. The exposed extraparticulate DNA was examined by shadow-casting electron microscopy and found to consist of circular nucleic acid molecules with a mean contour length of 0.79 ± 0.09 μm. No single structures were seen after this material was exposed to 40% formamide, which confirmed its double-stranded nature. The fact that omission of the polymerase reaction did not alter these findings indicated that the double-stranded circular form was not a product of or was not modified by the polymerase reaction. In neither instance were supercoiled structures seen. Thermal denaturation indicated a G + C content of 48–49%, a value somewhat lower than the 56% approximation that emerged from another study in which similar double-stranded DNA was found in core particles obtained from the nuclei of infected human hepatocytes. More recently, it was confirmed that the 15S DNA structure isolated from the HBV particles in serum is circular. However, fragmentation with the restriction enzyme endonuclease R. Hae III both before and after *in vitro* DNA replication suggested the existence of single-stranded gaps along 10–20% of the total molecule length. These single-stranded regions may have contributed to the wide range of molecular lengths observed in a previous electron microscopy study because the length of single-stranded DNA molecules depends greatly on ionic conditions. In that study, the endogenous polymerase reaction appeared to repair the single-stranded gap in the double-stranded circular DNA. Moreover, the ability of a

polymerase obtained from avian myeloblastosis virus to synthesize a DNA product indicates that the specificity observed in the endogenous reaction resides in the template DNA and not in the HBcAg-associated DNA polymerase. The latter may in fact be host-derived. This possibility is supported by the finding that this enzyme activity could not be detected in core particles present in the nuclei of infected hepatocytes, which suggests that it may be acquired during passage of core particles through the cytoplasm of the hepatocyte.

A model of replication that does not require an exogenous primer has recently been proposed. Open double-stranded circles were seen by electron microscopy of detergent-treated HBV particles obtained by centrifugation of several litres of antigen-containing plasma. These structures probably arise through a " nick " in one of the two strands that permits elongation of the strand to take place from the exposed 3'-hydroxyl terminal nucleotide. It would be expected that this " rolling circle " mode of nucleic acid replication would produce short linear strands attached to the circular forms and that these structures would be found in preparations where the polymerase reaction had occurred before nucleic acid extraction. The molecular weight of DNA extracted from the virus core to date is no higher than 2.3×10^6, and it appears unlikely that all the information required for intracellular replication of the virus and for expression of the various antigenic determinants is coded by this DNA.

9. SEROLOGICAL TECHNIQUES FOR HEPATITIS B

A variety of serological methods are available for detecting the antigens and antibodies associated with HBV infection. The major practical uses of these techniques are (*a*) to establish the diagnosis of viral hepatitis type B, (*b*) to study the epidemiology of hepatitis B, (*c*) to evaluate passive and active immunization for prevention of hepatitis B, and (*d*) to identify blood and plasma donors who are carriers.

In the following sections, consideration is given to the question of which techniques are best suited for various practical and research applications, as dictated by their sensitivity, specificity, and simplicity. Some methods that have been in common use for a number of years are not discussed here at length ; these have been examined in detail in a previous report.[a]

[a] WHO Technical Report Series, No. 512, 1973, pp. 22–28.

Table 3 summarizes the serological techniques now available for the detection of the various hepatitis B antigens and antibodies and gives an indication of their relative sensitivity.

9.1 Hepatitis B surface antigen and antibody

Patients with acute hepatitis B usually have detectable HBsAg in their blood for a period ranging from a few days to several months. The interval between exposure and appearance of detectable serum HBsAg is related to the infectivity titre of the inoculum ; it may be as short as 2–3 weeks with very high-titre inocula and as long as 3–4 months with low-titre inocula. Abnormal liver function tests and clinical signs and symptoms appear some days or weeks after the initial appearance of HBsAg, often near the time when HBsAg levels are at their peak. In most cases, the disappearance of HBsAg and subsequent appearance of anti-HBs signal recovery from HBV infection and the development of immunity to reinfection. In about 5–10% of adults with hepatitis B,

TABLE 3. SEROLOGICAL TECHNIQUES FOR DETECTING HEPATITIS B ANTIGENS AND ANTIBODIES

Technique	Relative sensitivity [a] for detecting :				
	HBsAg	Anti-HBs	HBcAg	Anti-HBc	HBeAg and anti-HBe
Agar gel immunodiffusion	+	+			+
Counter-immunoelectrophoresis	++	++	+	+	
Complement fixation	++	++	+	+	
Rheophoresis	++	++	+	+	+
Reversed passive latex agglutination	++				
Passive haemagglutination	++ [b]	+++			
Immune adherence haemagglutination	+++	++	++	+++	
Immune electron microscopy	+++	+++	++	++	
Immunofluorescence microscopy	++	++	++	++	
Reversed passive haemagglutination	+++				
Solid-phase radioimmunoassay	++++	++++	+++	++++	
Radioimmunoprecipitation	++++	++++		++++	
Enzyme immunoassay	++++				

[a] Estimated gradation from least sensitive (+) to most sensitive (++++). These gradations do not give any indication of the relative *specificity* of the techniques.
[b] HBsAg detected by inhibition of passive haemagglutination.

however, infection with HBV persists and HBsAg remains detectable for many months or years.

HBsAg was first identified by the immunodiffusion technique. This technique is simple, inexpensive, and specific, but insensitive and slow. It has been particularly useful for identifying the major antigenic subtypes (*adw*, *ayw*, *adr*, and *ayr*) and for further antigenic analysis of HBsAg. In addition, the low sensitivity of immunodiffusion for anti-HBs detection makes it a convenient method for screening sera to find those with high anti-HBs titres that would be valuable for reagent and immunoglobulin production.

Counter-immunoelectrophoresis, complement fixation, rheophoresis, and reversed passive latex agglutination can also be used for HBsAg detection. They are 2–10 times more sensitive than immunodiffusion and much faster, requiring from a few minutes to a few hours for completion. It should be emphasized, however, that all these methods, with the possible exception of immunodiffusion and rheophoresis, are likely to give a considerable proportion of false positive results when used for routine testing of large numbers of samples.

Immune electron microscopy and immunofluorescence microscopy are highly specialized methods for HBsAg detection that have predominantly research applications. Immunofluorescence microscopy is particularly valuable for studying HBsAg in liver tissue, where it is found in the cytoplasm of infected hepatocytes.

The most sensitive methods for HBsAg and anti-HBs detection are radioimmunoassays, including solid-phase radioimmunoassay and radioimmunoprecipitation. These two types of radioimmunoassay differ primarily in the techniques used for separating bound from free radiolabelled reagents. The high sensitivity and objectivity of these methods tend to minimize false negative results. Although nonspecific false positive results did cause problems early in the development of radioimmunoassay methods, these difficulties appear to have been largely eliminated. Nevertheless, because of the need to ensure the specificity of reactions obtained with these highly sensitive techniques, it is generally considered essential to confirm that the reactions are specific by means of neutralization or blocking with unlabelled anti-HBs or HBsAg as appropriate.

Enzyme-linked immunosorbent assay has been adapted for HBsAg detection. In a recent evaluation, this technique was shown to have a sensitivity similar to that of radioimmunoassay. The enzyme–antibody conjugates can be quantified by their ability to degrade a suitable substrate. The colour change can be read with the naked eye, or objective

readings may be made in a simple spectrophotometer. Enzyme immunoassay appears to be a reliable technique in the hands of trained workers and has the advantages of stability and long shelf-life of the reagents, simplicity of the equipment, and high sensitivity. Further developments of enzyme immunoassay are expected. This method deserves careful evaluation in view of its potential wide application in laboratory practice.

The agglutination of erythrocytes coated with anti-HBs, termed reversed passive haemagglutination, is another sensitive method for HBsAg detection provided that the reagent preparation and test conditions are optimal. Under these conditions its sensitivity approaches but does not quite reach that of radioimmunoassay. Human, turkey, and sheep erythrocytes have all been used successfully to prepare reversed passive haemagglutination reagents; the greatest sensitivity has been achieved with anti-HBs–coated human red cells.

Human erythrocytes treated with chromium(III) chloride and coated with purified HBsAg provide a highly sensitive reagent for detecting anti-HBs by passive haemagglutination. However, since radioimmunoassay methods are somewhat more sensitive for this purpose, they are the methods of choice when it is essential to detect very low levels of anti-HBs. On the other hand, the quantification of antibody titres is somewhat simpler with passive haemagglutination than with radioimmunoassay.

Almost all the less sensitive methods for detecting HBsAg may also be applied to anti-HBs. Because of their low sensitivity, however, these methods will give many false negative results when used for population studies and for testing serum samples from patients who are convalescing from type B hepatitis. Their greatest usefulness for anti-HBs detection is in finding valuable high-titre sera.

9.2 Subtyping of hepatitis B surface antigen and antibody

The 4 major subtypes of HBsAg (*adw*, *ayw*, *adr*, and *ayr*) have all been shown to breed true, and therefore it is clear that they are specified by distinct genotypes of HBV. The *d/y* and *w/r* determinant pairs were discovered by studying spur formation in the reactions between certain antisera and HBsAg samples in immunodiffusion. These subtypes do not appear to be helpful in predicting the clinical course or outcome of HBV infection but they are extremely useful for epidemiological studies and have been found to vary markedly in prevalence in different parts of the world. The immunodiffusion method has con-

tinued to be the mainstay of HBsAg subtype studies, and it has pointed to several further categories of the major subtypes, termed *adw2*, *adw4*, *ayw1*, *ayw2*, *ayw3*, and *ayw4*, as well as to a host of additional, incompletely studied determinants, such as *q*, *x*, *f*, *t*, *n*, *j*, and *g*. Work is still in progress to determine whether any of these specificities are truly distinct determinants of HBsAg that are determined by the viral genome or whether they are to some extent reflections of the host's antigenic and genetic constitution.

It is of considerable practical importance that both radioimmunoassay and passive haemagglutination methods have been adapted to provide highly sensitive subtyping techniques for HBsAg as well as anti-HBs. Thus it is now possible to determine at least the *d*/*y* specificity of virtually all HBsAg and anti-HBs reactions. One new insight that has come from the increased sensitivity of subtyping is that concurrent or hybrid *adyw* infections occur in a larger number of individuals exposed to both subtypes than was previously suspected. Subtyping has also provided evidence that antibody to one subtype can coexist with other HBsAg subtypes in individuals with persistent infections.

9.3 Hepatitis B core antigen, antibody, and DNA polymerase

HBcAg has been obtained in working quantities from HBV-rich plasma and from infected liver tissue. The demonstration of anti-HBc by complement fixation, counter-immunoelectrophoresis, and immunofluorescence microscopy reveals that this antibody is a useful marker of HBV replication, although anti-HBc undoubtedly persists long after the cessation of viral replication in many individuals. Anti-HBc often appears while HBsAg is still present during acute infections, and it is detectable, often in high titres, in almost every HBsAg carrier. In fact, anti-HBc appears so often in carriers that it is now being evaluated for possible use as an indicator of the presence of HBV in individuals with no detectable HBsAg. A number of sera from such individuals have been shown by highly sensitive assays to contain anti-HBc, although this antibody had not been detected by less sensitive methods. A major challenge now is to ascertain which, if any, of these anti-HBc–positive, HBsAg–negative sera contain infective HBV.

Several laboratories have demonstrated DNA polymerase activity associated with HBcAg. This enzyme activity is often found early in HBV infection when large numbers of virus particles are present, and it persists in some chronically infected individuals. However, many HBsAg–positive sera that are probably infective contain no detectable

DNA polymerase. DNA polymerase may provide an indication of the level of infectivity, but it does not appear to be as reliable a marker of infectivity as HBsAg itself. Two highly sensitive radioimmunoassays for anti-HBc have been developed based on the use of HBcAg particles containing the tritiated DNA product of the DNA polymerase reaction as the radiolabelled antigen. In the first of these techniques the polymerase reaction and the antigen–antibody reaction are run at the same time, after which the radiolabelled immune complexes are precipitated with an anti-globulin serum. The second method uses prelabelled HBcAg that is tritiated by the DNA polymerase reaction before the radioimmunoprecipitation test for anti-HBc is carried out. Both methods are several hundred times more sensitive than complement fixation and counter-immunoelectrophoresis.

9.4 Hepatitis B *e* antigen and antibody

Another marker of HBV infection that appears to correlate with the number of virus particles and the degree of infectivity of HBsAg-positive sera is hepatitis B *e* antigen (HBeAg). This is a soluble antigen that does not seem to be part of the virion but is apparently specific for hepatitis B, since it is found only in sera containing HBsAg. At present, immunodiffusion is the only method used for detecting HBeAg and anti-HBe; and only a relatively small proportion of HBsAg-positive sera are found to contain either HBeAg or anti-HBe. In addition to its postulated direct relationship to infectivity, the presence of HBeAg in HBsAg carriers is thought to be an unfavourable prognostic sign as regards the severity and course of the chronic liver disease. Finally, considerable evidence has been cited in support of the hypothesis that detectable serum anti-HBe in carriers indicates a relatively low infectivity of their sera. In view of the insensitivity of the immunodiffusion method, however, and the fact that many HBsAg-positive sera contain neither HBeAg nor anti-HBe when tested by immunodiffusion, it is still difficult to interpret the biological significance of these markers.

10. THE IMMUNE RESPONSE IN HEPATITIS B

The immune response to infection with HBV is elicited by at least 3 antigenic systems—HBsAg, HBcAg, and HBeAg—resulting from replication of the virus in the liver.

10.1 Humoral response

The surface antigen appears in the serum of most patients during the incubation period of the acute infection, as early as 4–6 weeks after infection and 2–8 weeks before biochemical evidence of liver damage or the onset of jaundice. HBsAg persists during the acute illness and is usually cleared from the bloodstream during convalescence. Free core antigen has not been detected in circulating blood. Next to appear in the circulation is the associated DNA polymerase activity, which is found immediately before or at the time of raised serum transaminase levels. The polymerase activity persists for days or weeks in acute cases and for months or years in a proportion of persistent HBsAg carriers. Antibody to the core is found in the serum 2–10 weeks after the appearance of HBsAg. It can frequently be detected during the acute infection and for some time after recovery has taken place, although in declining titres. In general, the highest titres of anti-HBc are found in persistent HBsAg carriers. Anti-HBc appears to be correlated with the amount and duration of replication of the virus. Antibody to the surface component is the last to appear. A primary type of anti-HBs response occurs in clinical cases of hepatitis B after the disappearance of HBsAg from the serum. In fulminant hepatitis there is some evidence to suggest that an unusually strong and rapid immune clearance of HBsAg, associated with the early appearance of anti-HBs during the peak of liver damage, may be involved in the pathogenesis of this severe form of infection.[a] The anti-HBs response in most individuals who apparently resist infection on re-exposure to HBV is of the secondary anamnestic type. The titre of anti-HBc, however, remains unchanged.

Surface antigen–antibody complexes have been found in the sera of some patients during the incubation period and during the acute phase of illness. There is also evidence suggesting that extrahepatic antigen–antibody complexes are of importance in the pathogenesis of syndromes characterized by severe damage of blood vessels such as periarteritis nodosa, some forms of chronic glomerulonephritis where the complexes are present in the renal glomeruli, and infantile papular acrodermatitis. HBsAg, anti-HBs, anti-HBc, and surface antigen–antibody immune complexes have been identified in some patients with virtually all the recognized chronic sequelae of acute hepatitis B. It is not clear, however, why circulating immune complexes are not found in more such patients and why only a small proportion of patients with circulating

[a] WOOLF, I. L. ET AL. *British medical journal*, 2 : 669 (1976).

complexes develop vasculitis or polyarteritis. It may be that complexes are critical pathogenic factors only if they are of a particular size and of a certain antigen/antibody ratio. The precise role of immune complexes in the pathogenesis of liver damage remains to be determined.

10.2 Cell-mediated responses

Cellular immune responses are known to be of particular importance in determining the clinical manifestations and course of viral infections in man and in animals. The occurrence of cell-mediated immunity to hepatitis B antigens has been demonstrated by lymphocyte transformation and more particularly by leucocyte migration inhibition. When partially purified HBsAg is used as the test antigen, leucocyte migration inhibition is found in most patients during the acute phase of hepatitis B. Inhibition becomes less pronounced during convalescence and disappears after recovery. Leucocyte migration inhibition has also been demonstrated in a significant proportion of HBsAg-positive patients with chronic active hepatitis.[a] However, lymphocyte transformation and leucocyte migration have invariably been negative in asymptomatic persistent HBsAg carriers. These observations suggest that cell-mediated immunity may be involved in terminating infection with HBV and, under certain circumstances, in causing hepatocellular damage and creating autoimmunity. Normal T-cell function may be a prerequisite for the self-limited course of hepatitis ; if the function is defective it may favour the development of chronic liver damage, and if it is absent altogether the result may be the asymptomatic carrier state.

The hypothesis has been put forward that both HBsAg-positive and a proportion of the antigen-negative cases of chronic active hepatitis are initiated by exposure to HBV. According to this hypothesis, the virus penetrates some of the hepatocytes and, towards the end of the incubation period, virus-associated antigens appear on the surface of infected cells. T cells recognizing these new determinants destroy the infected hepatocytes. Virus is released and in turn infects other hepatocytes. This is normally prevented by the production of anti-HBs. But those patients who progress to antigen-positive active chronic hepatitis either fail to produce sufficient antibody or produce low-affinity antibody, and under these circumstances infection persists.

Another effect of T-cell stimulation by damaged hepatocytes is the activation of B cells responsive to existing hepatocyte cell surface antigens, including the liver-specific lipoprotein. Antibodies to this macro-

[a] LEE, W. M. ET AL. *British medical journal*, **1** : 705 (1975).

lipoprotein, which is thought to be a normal constituent of the normal hepatocyte membrane, are produced and, on entering the liver, bind to the surface of the periportal hepatocytes. While fixation of complement is one possible mechanism leading to necrosis, it is likely that K cells are also activated. These cells have receptors on their surface for the Fc portion of antibody molecules and are cytotoxic when bound to antibody-coated target cells.

The synthesis and release of damaging autoantibody would normally be subject to control by suppressor T cells. However, in antigen-positive active chronic hepatitis control is not achieved—despite normal suppressor T-cell function—because of the continuous activation of helper T cells. In hepatitis B antigen-negative cases, suppressor T-cell function is defective and, although the helper T-cell effect is only transient, the autoimmune response persists.

The isolation of two organ-specific proteins from human liver was followed by the demonstration of organ-specific antibodies in the sera of a proportion of patients with active chronic hepatitis. In addition, active chronic hepatitis has been induced in rabbits by repeated immunization with extracts containing these liver-specific proteins. Cellular hypersensitivity to these proteins, as measured by leucocyte migration inhibition, was present in a significant number of patients with active chronic hepatitis and patients with primary biliary cirrhosis. More recently, evidence of lymphocyte sensitization to HBsAg was found in all patients with acute hepatitis B tested, and transitory sensitization to liver-specific lipoprotein was detected in many of the patients.

These findings are consistent with the suggestion that a cell-mediated immune response to hepatitis B antigen, present at an early stage in acute hepatitis, causes acute liver damage through a cytotoxic effect on virus-infected cells. If the response to liver-specific lipoproteins persisted, it could be responsible for progression to chronic liver damage. It is also postulated that the progressive liver damage of active chronic hepatitis is due to an autoimmune reaction directed against a hepatocyte surface lipoprotein that is initiated in many cases by infection with HBV. Evidence of cell-mediated immunity to HBsAg has been found in many patients with antigen-negative chronic hepatitis, which suggests a high frequency of previous infection with HBV. The majority of HBsAg-positive patients also show a cellular response to the antigen. Evidence of sensitization to liver-specific lipoprotein was found in more than half the antigen-positive patients, with a similar frequency in the antigen-negative group. Although intercurrent infection with hepatitis B cannot be excluded, these results are in agreement with the hypothesis that

infection with hepatitis B virus is important in initiating the disease in many HBsAg-negative patients with active chronic hepatitis. Sensitization to the liver cell membrane antigen may be the autoimmune process common to all varieties of the disease and could be responsible for the perpetuation of the liver injury.[a] In one study, lymphocytes from 20 out of 22 patients with untreated active chronic hepatitis were shown to be cytotoxic when incubated with isolated rabbit hepatocytes. Blocking experiments strongly suggest that the cytotoxicity is due to an immunological reaction directed at this cell surface antigen.

Studies of cell-mediated immunity are more complex and difficult to carry out than those involving the humoral immune response, and reservations remain as to the interpretation of the results. Further studies of cell-mediated responses in viral hepatitis are needed to clarify their role in the pathogenesis of the disease.

11. HEPATITIS B IN ANIMAL MODELS

The difficulty of propagating hepatitis A and B viruses serially in tissue culture continues. HBsAg is infrequently produced in inoculated human liver organ cultures but serial passage has not been achieved and the replication of HBV appears to be abortive.[b] Similar results have been reported with fragments of human embryo liver cultivated on the chorioallantoic membrane of the developing chick embryo. Intranuclear virus-like particles have been localized in hepatocytes of cultured explants of liver biopsy specimens obtained from infants with persistent hepatitis B antigenaemia. The extensive literature on this subject has recently been reviewed.[b] Considerable further research is required on basic mechanisms governing the attachment of HBV to cell membranes, cell penetration, and the synthesis and assembly of the viral components in cell culture systems.

Because of the difficulties of finding suitable tissue cultures for HBV replication, studies of the virus have relied mainly on animal models. At present, the chimpanzee provides the only reliable animal model for

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[a] EDDLESTON, A. L. W. F. Etiological factors in immune mediated liver disease. In: Ferguson, A. & MacSween, R. N. M., ed. *Immunological aspects of the liver and gastrointestinal tract*. Lancaster, MTP Press, 1976, pp. 291–317.

[b] ZUCKERMAN, A. J. *Human viral hepatitis*. Amsterdam, North Holland/American Elsevier, 1975, pp. 386–394.

in vivo studies of HBV.[a,b] The rhesus monkey model has now been shown to be unsuitable for such studies, and there are too few gibbons available to permit extensive evaluation.

The chimpanzee model has been used to obtain important new data on the infectivity of material containing HBV, some properties of the virus, immunopathology, inactivation of HBV and safety testing of experimental vaccines, the efficacy of passive and active immunization, and attempts to interrupt the carrier state of HBV. Following are examples of significant new information gained from chimpanzee studies:

— evidence that active *in vivo* replication of HBV occurs only in hepatocytes and not in other tissues

— evidence that HBV is transmitted if the virus is placed in the mouth but not if it is placed in the intestine

— proof that HBV infectivity may persist in the presence of HBeAg

— evidence of the antigenicity and efficacy of experimental hepatitis B vaccines.

12. EPIDEMIOLOGY OF HEPATITIS B

12.1 The carrier state

On the basis of longitudinal studies of patients with hepatitis B, the persistent carrier state has been defined as the presence of HBsAg for more than 6 months. Such a carrier state may be associated with liver damage. The survival of HBV in man is ensured by the existence of a reservoir of persistent carriers, estimated to number 120 million. The prevalence of HBsAg in apparently healthy adults varies from 0.1% in parts of Europe, North America, and Australia to 15% in several tropical countries. Within each country, considerable differences in prevalence may exist between different ethnic and socioeconomic groups.[c]

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[a] WHO Technical Report Series, No. 570, 1975.

[b] BARKER, L. F. ET AL. *American journal of the medical sciences*, **270** : 189 (1975).

[c] SZMUNESS, W. *American journal of pathology*, **81** : 629 (1975).

A number of risk factors have been identified in relation to development of the carrier state. It is more common in males, more likely to follow infections acquired in childhood than those acquired in adult life, and more likely to occur in patients with natural or acquired immune deficiencies. An association with HL-A specificity has been reported but not confirmed.

In countries in which infection with HBV is relatively uncommon, the highest prevalence of HBsAg is observed in the 20–40-year age group. On the other hand, the prevalence of anti-HBs increases steadily with age. In countries in which HBV infection is common, the highest prevalence of HBsAg is found in children 4–8 years old, with steadily declining rates among older age groups. The decline in HBsAg carriage rates with age suggests that the carrier state is not lifelong. HBeAg has been found more commonly in young than in adult carriers, while the prevalence of anti-HBe appears to increase with age. These findings suggest that young carriers may be the most infective.

Subtypes of HBsAg. Studies in many laboratories [a] have shown that the major subtypes have differing geographical distributions (Fig. 1). For example, *ayw* occurs in a broad zone that extends from northern and western Africa through the eastern Mediterranean to the Indian subcontinent. In northern Europe, the Americas, and Australia *adw* predominates. Both *adw* and *adr* are found in Indonesia, Malaysia, Papua New Guinea, and Thailand, while the *adr* subtype is predominant in other parts of Asia and the Pacific.

In some countries, the subtype most frequently detected among patients with acute hepatitis B differs from that found in persistent carriers, presumably because the distribution of subtypes within the latter group is determined by past rather than current patterns of infection.

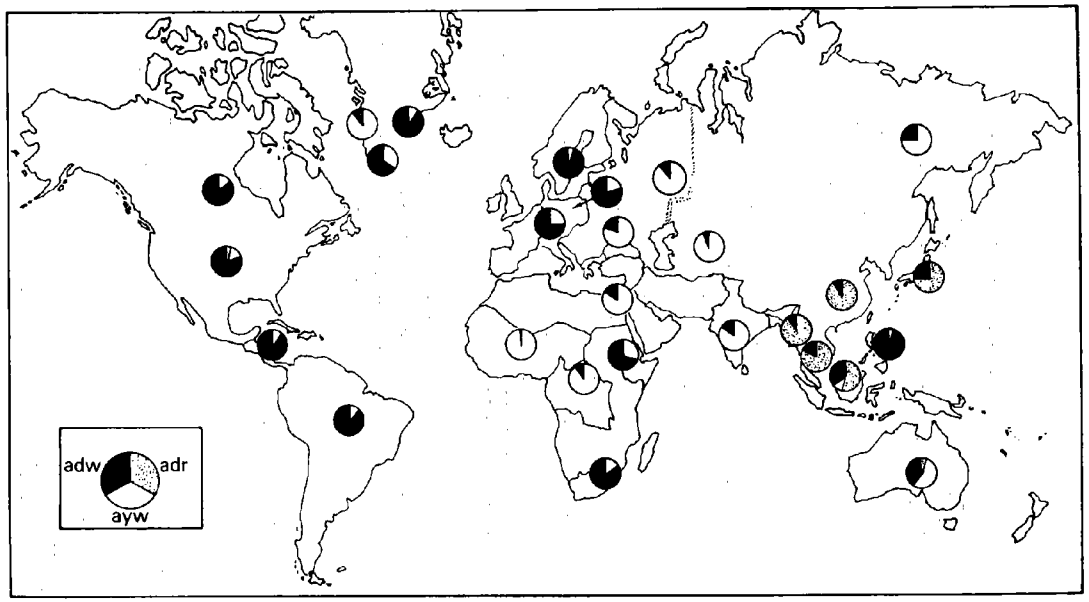
12.2 Modes of spread

Blood

In low prevalence areas, a major mode of spread continues to be the inoculation of blood and some blood products. Hepatis B may be transmitted as a result of transfusion or by accidental inoculation of

[a] LE BOUVIER, G. L. & WILLIAMS, A. *American journal of the medical sciences*, **270** : 165 (1975) ; NISHIOKA, K. ET AL. *Bulletin of the World Health Organization*, **52** : 293 (1975) ; COUROUCÉ, A. M. ET AL., ed. *HB_s antigen subtypes: Proceedings of the International Workshop on HB_s Antigen Subtypes, Paris, April 1975*. Basle, Karger, 1976 (*Bibliotheca haematologica*, No. 42), pp. 42–59.

FIG. 1. RELATIVE PREVALENCE OF MAJOR SUBTYPES *(adw, ayw, adr)* OF HEPATITIS B ANTIGEN IN THE WORLD [a]



[a] The *ayr* subtype has been identified in 2 of 53 (3.8%) sera from Papua New Guinea and 10 of 2431 (0.4%) sera from Japan ; otherwise it appears to be very rare. Data primarily obtained from : Couroucé, A. M. et al., ed. *HBs antigen subtypes : Proceedings of the International Workshop on HBs Antigen Subtypes, Paris, April 1975.* Basle, Karger, 1976 (*Bibliotheca haematologica*, No. 42) ; and Nishioka, K. et al. *Bulletin of the World Health Organization*, **52** : 293 (1975).

minute quantities of blood such as may occur during surgical and dental procedures, intravenous drug abuse, mass immunization, tattooing, acupuncture, and laboratory accidents. Accidental percutaneous inoculations by communally used razors, toothbrushes, bath brushes, and similar objects have been implicated as occasional causes of hepatitis B.

Mosquitos and other bloodsucking insects have been suggested as possible vectors of the disease. However, while HBsAg has been detected in bed bugs as well as in several species of mosquito, which have been either trapped in the wild or fed on infected blood, no convincing evidence of the replication of the virus in insects has been obtained. Although mechanical transmission of infection may occur, several studies have failed to demonstrate an association between mosquito activity and the prevalence of HBsAg and anti-HBs in man.

Perinatal transmission

Transmission of infection from carrier mothers to their babies can occur during the perinatal period and appears to be an important factor in determining the prevalence of HBV infection in some regions. The risk of infection varies from country to country and may reach 40%. In general it is higher for babies born to carrier mothers in countries with high carrier rates than for similar babies in countries with low carrier rates. Maternal factors that appear to be important in determining whether infection will occur are a history of transmission to previous children, a high titre of HBsAg, and/or the presence in the blood of HBeAg.

Acute hepatitis B is an unusual complication of pregnancy and may result in premature labour. If the disease occurs in the second or third trimester or within 2 months after delivery, there is a substantial risk that the baby will be infected.

Infection of the baby following acute or chronic infection of the mother is usually anicteric and is recognized by the appearance of HBsAg between 60 and 120 days after birth. Most children infected at this time become persistent carriers of the virus. Their long-term prognosis is unknown.

The mechanism of perinatal infection is uncertain. Although HBV can infect the fetus *in utero*, this would appear to happen rarely. Infection probably occurs during or shortly after birth as a result of a leak of maternal blood into the baby's circulation or its ingestion or inadvertent inoculation. Preliminary studies suggest that administration of hepati-

tis B immunoglobulin shortly after birth may provide the baby with some protection against infection during the perinatal period.

Other modes of spread

Higher rates of infection with HBV are observed in the families of persistent carriers than in the rest of the community. The mechanism of such contact spread is unknown, although mouth-to-mouth transmission may be important. HBsAg has been detected in the saliva of patients with acute hepatitis B and in chronic carriers, and preliminary studies suggest that saliva containing HBsAg is infective for gibbons and chimpanzees.

Cohabitation with an acutely or chronically infected person is attended by a significant risk of infection. Sexually promiscuous persons also have a higher than expected range of seropositivity. As HBsAg has been detected in semen, vaginal secretions, and menstrual blood, it is possible that it can cross mucosal surfaces exposed to these fluids during intercourse. HBsAg has also been detected in the serous exudates from skin ulcers. Contact with such material may play a role in the transmission of the infection, particularly in tropical countries.

Human transmission studies conducted many years ago, in which faecal extracts were administered orally or percutaneously, failed to demonstrate the infectivity of faeces, and no hepatitis B epidemics due to contaminated food or water have been observed.

13. HEPATITIS ASSOCIATED WITH TRANSFUSION

13.1 Assessment of the impact of testing for hepatitis B surface antigen

The application of sensitive tests for HBsAg to blood for transfusion and plasma for fractionation has become standard practice in many countries. The impact of HBsAg testing on the incidence of post-transfusion hepatitis is difficult to measure and varies from one location to another depending on factors such as the prevalence of HBV carriers in the donor population and the level of immunity to HBV in the recipients. For example, in areas of the world where the prevalence of HBsAg carriage is quite high, such as certain parts of Asia and Africa, there is also a very high prevalence of anti-HBs, which may protect recipients of blood transfusions from disease caused by HBV or at least reduce its severity. Furthermore, accurate assessment of the impact of HBsAg testing requires costly and time-consuming prospective studies

of transfused patients. In the absence of such studies, many cases of post-transfusion hepatitis will not be recognized because of their mild or subclinical nature. Even those that are recognized as overt cases may not always be reported to blood transfusion services or to health authorities. Despite these difficulties, the closer study of post-transfusion hepatitis in recent years has provided a number of important insights into the epidemiology of this infection and suggested methods for its control.

13.2 Major risk factors in post-transfusion hepatitis

It has long been recognized that the risk of post-transfusion hepatitis in patients increases with the number of units transfused, which means that it is preferable to compare risk in terms of units transfused rather than in terms of patients transfused. Studies in the USA have consistently shown that the risk of post-transfusion hepatitis is also higher with purchased blood than with blood from voluntary donors. Declining post-transfusion hepatitis rates have been documented in longitudinal studies over the past 5 years in a number of centres in the USA, but in many instances the decline was clearly due to the combined effect of eliminating the use of blood from paid donors and of screening out HBsAg-positive blood.

13.3 Post-transfusion hepatitis not caused by hepatitis A or B virus

Although HBsAg testing has not altogether eliminated the risk of hepatitis B associated with transfusion of blood bank products from single donors, the reduction in this infection documented in several prospective studies has been so marked that the predominant form of post-transfusion hepatitis is now associated with hepatitis viruses other than HAV and HBV. Whereas about 30% of cases of post-transfusion hepatitis, including most of the severe cases, used to be caused by HBV before the introduction of HBsAg testing, that virus now causes only about 10% of cases following transfusion of blood in which HBsAg is not detectable by a sensitive method. As for the remaining 90% of cases, since there are no specific serological tests for infection caused by hepatitis viruses other than A and B, only a diagnosis of exclusion is yet possible. So far, it has not been possible to implicate HAV, Epstein-Barr virus, or cytomegalovirus in this form of post-transfusion hepatitis.[a]

[a] ALTER, H. J. ET AL. *Lancet*, **2**: 838 (1975).

Although this syndrome cannot be readily distinguished from hepatitis B on clinical grounds, including incubation period and the duration and degree of bilirubin and transaminase elevation, there are indications that patients infected with HBV may be more likely to have severe acute disease and fulminant fatal disease.

13.4 Future prospects for the control of post-transfusion hepatitis

In countries where payment of donors attracts individuals whose blood carries an increased risk of causing post-transfusion hepatitis in the recipients, switching to an entirely voluntary donor system is clearly one of the most effective approaches available for reducing the incidence of post-transfusion hepatitis. In the USA the high hepatitis risk associated with purchased blood has been found to apply to infection caused not only by HBV but by other hepatitis viruses as well. Although a number of studies have shown that blood containing anti-HBs carries no increased risk of causing post-transfusion hepatitis, this may not be the case for blood containing anti-HBc. Evaluation of anti-HBc as an additional marker of HBV-contaminated blood is in progress.

While there are formidable obstacles to the physical removal or inactivation of HBV in blood, red blood cells that have been washed, with or without freezing and thawing before the washing, may possibly be less apt to transmit hepatitis because of the physical removal of the infective agent. This approach is under active investigation and may, of course, prove useful for the prevention of infection caused by other hepatitis viruses. However, the expense and the risk of bacterial contamination during processing constitute major practical impediments to the widespread use of frozen and washed red blood cells.

13.5 Hepatitis risk from plasma derivatives

Certain plasma derivatives prepared from large pools of plasma have been known to carry a very high risk of contamination with HBV. Although the hepatitis incidence associated with transfusion of these products has not been well quantified, in the past most batches of fibrinogen, antihaemophilic factor, and factor IX complex were believed likely to contain HBV. Testing of the source material has almost eliminated HBsAg-positive batches of these products, but there has not been sufficient experience with batches of plasma derivatives made from plasma tested by sensitive methods to determine the impact of such testing on the hepatitis risk to recipients of these derivatives. There is also no information available on the relative hepatitis risk associated

with the transfusion of plasma derivatives prepared from voluntary donor plasma versus paid donor plasma, although the prevalence of HBsAg has been found to be higher in the latter.

There are many documented episodes of hepatitis B caused by high-risk plasma derivatives but it is not yet clear whether these products can produce other forms of hepatitis, although one report suggests that other hepatitis infections may be caused by antihaemophilic factor concentrates. Recent studies have shown a high prevalence of elevated serum enzymes suggestive of chronic persistent hepatitis in patients with haemophilia who required repeated treatment with antihaemophilic factor over many years, but the relationship of these biochemical abnormalities to viral contamination of antihaemophilic factor is unclear and needs further study.

Immunoglobulin prepared by the cold ethanol fractionation method of Cohn and albumin products prepared by the same method and heated for 10 h at 60°C have a well-established reputation of being free from contamination with infective HBV. This may not be true for immunoglobulin prepared by other methods, such as ammonium sulfate fractionation. There was a recent episode of hepatitis B transmission by an albumin product, but the most likely explanation seemed to be that a manufacturing defect resulted in inadequate mixing during pasteurization.

Possible ways of improving the safety of the high-risk plasma derivatives include the use of small plasma pools from voluntary donors, the elimination of all HBsAg-reactive units after testing by a sensitive method in order to minimize contamination of the starting material with hepatitis virus, and perhaps new methods for fractionation and treatment of the final products. For the moment these products, which include antihaemophilic factor, factor IX complex, and fibrinogen, cannot be considered to be uniformly free from contamination with HBV, although there may well be individual batches that are safe. Until manufacturing methods are developed that will yield uniformly safe batches of these plasma derivatives, the potential benefit of their clinical use should be carefully weighed against the risk on an individual basis.

14. HEPATITIS AS AN OCCUPATIONAL HAZARD

Viral hepatitis is an occupational risk among health workers. Where specific studies have been undertaken, it has been shown that the major risk is from HBV and not from HAV. Rare instances of transmission of hepatitis B from health care workers to their patients have

been reported, but the available information shows that health care personnel are at greater risk of contracting hepatitis B from their patients than *vice versa* and that such personnel do not usually transmit the disease to patients.[a] Certain occupational categories and work areas have been identified as associated with an excess risk of hepatitis B infection. The occupational categories include physicians, dentists, nurses, and laboratory and dialysis technicians; work areas include haemodialysis and oncology units, hospital laboratories, blood banks, and surgical intensive-care units.[b]

The hepatitis risk is generally associated with the handling of blood or other contaminated secretions from patients, and the presumed source of exposure to HBV among health care personnel is the patients whom they serve. In one study of 6743 consecutive admissions to a large metropolitan hospital in an area of low HBV prevalence (0.4% HBsAg seropositivity in the general population), serum HBsAg was detected in 1.0% (66 cases) of the patients admitted, and the frequency was even higher (2.0%) among the male patients.[b] In only 12 of the HBsAg-positive cases (18%) was a diagnosis of viral hepatitis made on admission or a separate request made for determination of HBsAg. Thus the HBsAg seropositivity of the remaining 54 patients (84%) would have gone unsuspected if the special serological survey had not been undertaken. These findings indicate that hospital employees who come in contact with blood or other secretions of patients have ample opportunity to be repeatedly exposed to HBV. The risk of recurrent exposure is higher for employees working in hospitals located in areas of high HBV prevalence.

Physicians and dentists have an increased risk of contracting hepatitis B in the course of their work. A recent serological study of both physicians and dentists showed that the frequency of hepatitis B infection among both categories was 4 times greater than among comparable individuals working outside the health care field. Moreover, physicians and dentists in surgical specialties have an additional risk of contracting infection with HBV compared with their nonsurgical colleagues.

The prevention of occupational hepatitis B is extremely important. An appropriate environmental hygiene measure is to minimize the frequency of contact of susceptible persons with blood and other potentially infective secretions from patients. The implementation of this measure has eliminated the transmission of HBV without the need

[a] ALTER, H. J. ET AL. *New England journal of medicine*, **292**: 454 (1975).

[b] PATTISON, C. P. ET AL. *American journal of epidemiology*, **101**: 59 (1975).

for immunoprophylaxis, even in haemodialysis units where hepatitis B infection is endemic.

Health workers outside haemodialysis and oncology units who routinely handle blood and other secretions should adhere scrupulously to sound hygienic measures for all specimen handling. They should, for example, avoid touching their mouth or eyes with their hands. For further guidelines the model code of laboratory practice outlined in a previous report should be consulted.[a]

There is no evidence that the identification of patients who are HBsAg-positive so that their specimens can be specially labelled and handled would lead to any greater reduction of the occupational risk of hepatitis B than would result from the meticulous attention to procedures for the safe handling of all clinical specimens.

Haemodialysis and oncology units

An unusually high incidence of hepatitis B has been noted both among patients and among medical staff in haemodialysis and oncology units. While the disease tends to be self-limited—although sometimes severe—in the personnel, HBsAg frequently persists in the patients, presumably because of compromised host defences associated with their diseases and treatment. The response of patients to treatment, including kidney transplantation for those in dialysis units, does not appear to be influenced either favourably or adversely by the persistent HBsAg carrier state.[b] Exacerbation of liver disease manifested by massive necrosis of the hepatocytes followed by a fulminant fatal course has, however, been observed in chronically infected patients after the cessation of immunosuppressive therapy.[c]

The same subtype of HBsAg is generally found in both patients and staff of individual dialysis and oncology units, as would be expected in view of the epidemiological circumstances. A high prevalence of HBsAg has been described in patients with chronic renal disease, which presumably indicates the high infectivity of their blood.

In haemodialysis and oncology units the transmission of HBV may be reduced or eliminated by implementing the following measures :

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[a] WHO Technical Report Series, No. 512, 1973, pp. 47–52.

[b] SOULIER, J. P. ET AL. *Hépatite à virus B et hémodialyse*. Paris, Flammarion, 1975.

[c] GALBRAITH, R. M. ET AL. *Lancet*, **2** : 528 (1975).

— continuous screening of patients and staff for HBsAg from time of entry to the units, and the avoidance of contact between HBsAg-positive staff and susceptible patients
— segregation of HBsAg-negative patients from susceptible patients
— employment of staff with anti-HBs for care of HBsAg-positive patients
— segregation of all dialysing equipment used for HBsAg-positive patients.

Further guidelines can be found in a previous report.[a]

In circumstances where demonstrable HBV transmission is occurring and where hygienic measures cannot be applied, prophylaxis with immunoglobulin containing anti-HBs may be considered as suggested in the following section on passive immunization.

15. PASSIVE IMMUNIZATION AGAINST HEPATITIS B

15.1 Clinical trials

Although passive immunization with various immunoglobulin preparations used to have a poor reputation as a prophylactic measure against hepatitis B, this reputation was based on attempts to prevent post-transfusion hepatitis at a time when type B cases could not be distinguished from other types and before the specific antibody content (anti-HBs) of the globulin preparations could be determined. The availability of tests for anti-HBs has made it possible to select high-titre plasma for the manufacture of hepatitis B immunoglobulin and to conduct new clinical trials to determine the safety and effectiveness of passive immunization against hepatitis B. These trials have made use of hepatitis B immunoglobulin from many different batches in a variety of circumstances where the product might be valuable.

The trials have had some defects, particularly the absence of randomized control groups given true placebo preparations. Many of the studies resorted to ordinary immunoglobulin with a low anti-HBs titre as the control preparation, but this design makes interpretation of the results somewhat problematic because of the possible effectiveness of ordinary immunoglobulin for hepatitis B prophylaxis in some situations.[b] For example, just before the initiation of hepatitis B immuno-

[a] WHO Technical Report Series, No. 512, 1973, pp. 45–46.

[b] ALTER, H. J. ET AL. *New England journal of medicine*, **293** : 1093 (1975).

globulin trials, a study comparing ordinary immunoglobulin having a low anti-HBs titre with a true placebo in an endemic situation provided evidence that immunoglobulin conferred some protection against hepatitis B transmission by close personal contact. Two subsequent studies of hepatitis B immunoglobulin and ordinary immunoglobulin in which there were nonrandomized untreated control groups (one a group of retarded children in a custodial institution and the other a group of hospital employees) also suggested that ordinary immunoglobulin as well as hepatitis B immunoglobulin gave some protection to the subjects in these endemic hepatitis B settings. An early study of individuals directly inoculated with HBV demonstrated that hepatitis B immunoglobulin had a greater protective effect than ordinary immunoglobulin, but it also showed that subjects given ordinary immunoglobulin had partial protection as compared with an untreated nonrandomized control group. It is against this background of evidence suggesting some effectiveness of ordinary immunoglobulin but a possibly greater effectiveness of hepatitis B immunoglobulin that further clinical trials were undertaken in an attempt to draw firmer conclusions concerning effectiveness and to make recommendations for the use of passive immunization against hepatitis B.

There are many possible clinical applications of hepatitis B immunoglobulin or ordinary immunoglobulin against hepatitis B, but they fall into three major categories: (1) post-exposure prophylaxis, (2) pre-exposure or chronic exposure prophylaxis, and (3) therapy. From existing data it seems that hepatitis B immunoglobulin is most clearly indicated for post-exposure prophylaxis of a single acute exposure to HBV, as can occur in accidental inoculation or oral ingestion of HBsAg-positive clinical materials. In an early study, which simulated accidental exposure, and in more recent field studies, three batches of hepatitis B immunoglobulin prepared by three different manufacturers proved to be more effective than ordinary immunoglobulin in conferring passive immunity. The early study was too small to achieve statistical significance, although the trend towards effectiveness was clear. In one of the field studies, hepatitis B immunoglobulin was significantly more effective than ordinary immunoglobulin when attack rates were compared after 4–6 months, but no difference between the two types of immunoglobulin was found when attack rates were compared after 8–12 months because of a number of late cases in the recipients of hepatitis B immunoglobulin.[a] Subtype analysis and questioning of the subjects did not help to determine whether the late

[a] GRADY, G. F. ET AL. *New England journal of medicine*, **293**: 1067 (1975).

cases were due to delayed onset or to re-exposure, although many of the subjects were working in endemic settings such as dialysis units where re-exposure was a distinct possibility. In the other field study there was a significant lowering of the attack rate in recipients treated with hepatitis B immunoglobulin as compared with the group given the ordinary immunoglobulin, and these results were not altered by the appearance of late cases.[a]

In all the trials carried out so far there has been no evidence of infectivity of hepatitis B immunoglobulin or of an increased prevalence of HBsAg carriers among infected individuals given hepatitis B immunoglobulin, although the possibility that a carrier state could result from antibody-mediated immunosuppression had caused some apprehension before the trials were undertaken. However, in the large field studies of individuals exposed to HBV, either acutely or in endemic environments, there was a decrease in the prevalence of anti-HBs among recipients of hepatitis B immunoglobulin compared with recipients of ordinary immunoglobulin. This has been attributed either to antibody-mediated immunosuppression despite subclinical viral infection or to complete protection with inadequate viral replication to evoke an active immune response. Although the latter may be the more likely explanation, concern has been voiced that treatment with hepatitis B immunoglobulin may prolong the period of susceptibility of individuals to HBV by preventing subclinical infection and hence the development of passive–active immunity, an occurrence that was more common in the immunoglobulin recipients. In view of this possibility, the widespread use of hepatitis B immunoglobulin might not be advisable in endemic situations where repeated or chronic exposure is the rule. In these situations it may be possible to achieve passive–active immunity with immunoglobulin containing a relatively low anti-HBs titre. Ultimately, of course, the preferred approach to prevention of hepatitis B in endemic situations will be active immunization, if a safe and effective hepatitis B vaccine becomes available.

Immunoglobulin with a relatively low titre of anti-HBs may be suitable for passive immunization in some settings involving chronic exposure, such as dialysis units ; several studies comparing the effectiveness of hepatitis B immunoglobulin with that of ordinary immunoglobulin in such settings have shown differences that ranged from marginal to significant. Because the protective effect of passive immunization by any immunoglobulin preparation is only temporary, however,

[a] SEEFF, L. B. ET AL. *Lancet*, **2** : 939 (1975).

it is questionable whether it should be used at all in chronic exposure situations, since repeated injections would be needed until either active immunity develops or the individual leaves the endemic situation. In the case of dialysis units, in particular, other preventive approaches based on sound hygienic measures and epidemiological principles have been used to control HBV with considerable success. In such units, passive immunization should therefore probably be reserved for situations in which control cannot be achieved by other measures.

In the case of massive single exposure, such as the accidental transfusion of HBsAg-positive blood or infusion of high-risk plasma derivatives, there are no data from controlled studies indicating the effectiveness of either hepatitis B immunoglobulin or ordinary immunoglobulin. In fact, post-transfusion hepatitis B has become so uncommon with the testing of blood by sensitive methods that it would be difficult to obtain sufficient data on the prophylactic efficacy of passive immunization.

Studies of the effectiveness of hepatitis immunoglobulin for the treatment of acute fulminant hepatitis B and of the chronic HBsAg carrier state gave discouraging results, although no apparent safety problems were encountered. A final post-exposure use of hepatitis B immunoglobulin, which is still being evaluated and for which there is preliminary evidence of effectiveness, is for the protection of infants whose mothers are infected with HBV.

In the manufacture of hepatitis B immunoglobulin, to judge from the various clinical trials cited above, it would appear desirable to establish the potency requirement as an anti-HBs titre equal to or greater than 1 : 100 000 by radioimmunoassay or by passive haemagglutination. A reference preparation for comparative testing is clearly desirable in order to control the potency of manufactured batches of this preparation.

15.2 Guidelines for passive immunization against hepatitis B

1. As noted above, the major indication for hepatitis B immunoglobulin is post-exposure prophylaxis for a single acute exposure to HBV, such as when blood known or strongly suspected to contain HBsAg is accidentally inoculated (" needle stick "), ingested orally (as in a pipetting accident), or splashed on to mucous membranes. Hepatitis B immunoglobulin with a high anti-HBs titre, standardized against a reference preparation, should be administered in a dose of approximately 5 ml for adults as soon as possible after such exposure.

2. In endemic settings such as haemodialysis units where HBV transmission is known to occur and where preventive hygienic measures

cannot be implemented, prophylaxis with immunoglobulin containing anti-HBs for susceptible staff may be considered on a continuing basis until HBV transmission can be abolished. There is currently some controversy whether low-titre or high-titre immunoglobulin is preferable and what the dosage and frequency of administration should be under these circumstances.

3. Individuals with a significant titre of anti-HBs are generally resistant to HBV infection and usually require no passive immunization against it.

4. Passive immunization with hepatitis B immunoglobulin does not appear to be indicated after blood transfusion, provided that HBsAg-positive blood has been excluded by sensitive methods, because under such circumstances most cases of post-transfusion hepatitis are not due to HBV infection.

16. ACTIVE IMMUNIZATION AGAINST HEPATITIS B

16.1 Preliminary studies

The high rate of infection with HBV in certain defined populations in the developed countries and among the general population in many developing countries points to the urgent need for a hepatitis B vaccine. Among the groups that might benefit from such a vaccine are health care and laboratory personnel, patients and staff of haemodialysis units, residents and staff of institutions for the mentally retarded, patients who must undergo elective surgery requiring transfusion, and individuals living in regions where HBV infection is prevalent and especially where hepatocellular carcinoma is common.

Although most virus vaccines currently in use are derived from viruses or viral antigens grown in tissue cultures, HBV has not been isolated and serially passaged *in vitro*. It is therefore necessary to rely on other sources of viral antigen for the development of hepatitis B vaccines. Patients with chronic HBV infection provide a readily available source of the viral antigen since their plasma contains large quantities of HBsAg. The tubular and the 22-nm spherical forms of the surface antigen are thought to consist of excess viral coat material that is synthesized by the infected liver cell but not assembled into complete virus; they lack the HBcAg and the DNA-dependent DNA polymerase. These apparently noninfective forms of HBV can be separated from the infective particle by biophysical means, and the spherical form can be highly purified in large quantities. There thus exists the potential for

developing a hepatitis B vaccine from viral components obtained by plasmapheresis from persistent carriers of HBsAg.

The feasibility of such an approach to vaccine development was suggested by preliminary studies in which human serum or plasma containing HBsAg was heated at 98°C for 1 min or at 60°C for 10 h and administered to volunteers. The vaccine preparation heated at 98°C was demonstrated to be both safe and partly effective, but the vaccine prepared by heating at 60°C was found to contain residual live HBV. Although these preparations demonstrated that immunization against hepatitis B was feasible, they probably contained all of the antigenic components of HBV—including whole virions—as well as extraneous normal and possibly abnormal serum or plasma components. Heating of partially purified plasma derivatives, such as albumin and plasma protein fractions, has been used for many years to inactivate HBV in these therapeutic materials, but the residual infective HBV in the plasma preparation that was treated for 10 h at 60°C augured ill for this approach to making an inactivated hepatitis B vaccine. For these reasons current efforts to develop hepatitis B vaccines are directed mostly toward the purification and characterization of the 22-nm spherical form of HBsAg and its constituent proteins for use as immunogens.

Preliminary studies have been reported of experimental hepatitis B vaccines prepared from the 22-nm particles of HBsAg.[a,b] These subunit vaccines were prepared primarily by repeated isopycnic banding in cesium chloride and rate zonal centrifugation in sucrose. In order to ensure that no residual live HBV remained in the preparations, they were treated with formaldehyde solution under conditions that had been used successfully for the inactivation of other virus vaccines. The resulting vaccine preparations were found to be safe, immunogenic, and effective in preventing hepatitis B in chimpanzees. Initial small-scale safety tests of such experimental subunit vaccines are currently being conducted in volunteer subjects.

In addition to the development of experimental hepatitis B vaccines composed of the intact 22-nm spherical forms of HBsAg, attempts are being made to prepare vaccines from the constituent polypeptides.[c,d] These studies are aimed at the isolation and characterization of the seven

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[a] PURCELL, R. H. & GERIN, J. L. *American journal of the medical sciences*, **270**: 395 (1975).

[b] BUYNAK, E. B. ET AL. *Proceedings of the Society for Experimental Biology and Medicine*, **151**: 694 (1976).

[c] ZUCKERMAN, A. J. *Nature*, **255**: 104 (1975).

[d] MELNICK, J. L. ET AL. *Journal of infectious diseases*, **133**: 210 (1976).

polypeptides that can be identified in HBsAg preparations. Vaccines prepared from such polypeptides would have an added margin of safety since they would be even less likely than the subunit vaccines to contain live HBV or contaminating host proteins that might, at least theoretically, lead to untoward reactions of an autoimmune nature in some individuals. The major drawbacks to polypeptide vaccines at present are the difficulties encountered in obtaining large quantities of the peptides in pure form, the relatively poor immunogenicity of the polypeptides as compared with the 22-nm particle, and the contamination of some of the peptides with human serum components. However, if these technical problems can be overcome or if the antigenic component of one or more polypeptides can be synthesized *in vitro* and coupled to a suitable carrier protein, polypeptide preparations may become useful hepatitis B vaccines.

Recently, solid immunoadsorbent columns containing, respectively, anti-HBs of human origin and antibodies of animal origin to human serum have been used for the partial purification of an experimental hepatitis B vaccine based on the 22-nm particle.[a] After testing of the safety of this material in chimpanzees, it was administered to the patients and staff of a haemodialysis unit. Although the study did not include a true control group, a marked difference in the incidence of HBV infection was noted between the immunized group and a group of patients and staff who had not received the vaccine.

As interest in hepatitis B vaccines increases it is expected that there will be a growing number of experimental vaccines prepared in different laboratories by a variety of techniques. Certain minimum criteria should be established for such preparations before they are widely used in man. The following criteria and guidelines are proposed.

16.2 Guidelines for the preparation of experimental hepatitis B vaccines

1. Vaccines should be prepared only in laboratories with the expertise to ensure that they have a high degree of purity. It is essential that adequate tests of the safety and efficacy of such preparations should be conducted.

2. Consideration should be given to the selection of HBsAg-positive plasma that is devoid of HBV particles, DNA polymerase, and hepatitis B *e* antigen as the starting material from which the experimental

[a] MAUPAS, P. ET AL. *Lancet*, **2** : 1367 (1976).

vaccine is to be prepared, since these components are associated with relatively greater infectivity of HBV. However, this association is not absolute, and the absence of these indicators does not guarantee lack of infectivity. A further problem is that these markers of infectivity are usually found in plasma samples with the highest titres of HBsAg, which are precisely those that are potentially the most useful for preparation of vaccine.

3. The vaccine should be prepared in such a way as to remove most, if not all, possible contaminants such as liver antigens, nucleic acids, and other host proteins.

4. To minimize the risk of introducing other agents into the vaccine pool, consideration should be given to preparing experimental vaccines from a small number of carefully selected donors who have undergone repeated plasmapheresis. At present there are no tests for possible carriers of hepatitis viruses other than HAV and HBV.

5. Foreign antigens must not be introduced into the vaccine during the purification procedure. Experimental vaccines prepared by methods involving this risk must be rigorously examined for the presence of foreign proteins by the most sensitive techniques available.

6. The vaccine must be subjected to inactivation methods that are (*a*) most likely to inactivate HBV and (*b*) reproducible and acceptable for use with a virus vaccine. Both heating (e.g., treatment at 60°C for 10 h) and formaldehyde solution at a concentration of up to 0.5 ml/l appear to fulfil these criteria, but heating has failed under some circumstances to inactivate HBV completely and exposure to formaldehyde solution is ineffective when viruses are aggregated or when contaminating proteins are present in high concentrations.

7. The method of vaccine preparation must be (*a*) demonstrated consistently to inactivate or remove HBV by appropriate safety tests in seronegative chimpanzees, and (*b*) shown to yield a product free of other viruses, bacteria, and mycoplasmas by appropriate *in vitro* and *in vivo* safety tests.

8. The preparation method should consistently yield a vaccine that is immunogenic and capable of affording protection against challenge with live HBV in chimpanzees. Eventually an appropriate immunogenicity extinction test in nonhuman primates may be substituted for this criterion if a good correlation between the results of such a test and evidence of protection in chimpanzees can be demonstrated.

9. Initial tests in human volunteers of any new experimental hepatitis B vaccine should be performed in a stepwise manner, starting with a very small number of healthy adult volunteers capable of giving informed consent; the safety of the vaccine must be demonstrated in this initial group before it is administered to a larger group. Although there is merit in performing initial safety tests in persons at high risk of acquiring viral hepatitis B under natural conditions, the difficulty of differentiating between naturally acquired infection and possible vaccine-induced infection in such a group makes the use of a low-risk population preferable.

10. Recipients of experimental hepatitis B vaccines should be carefully monitored at frequent intervals for evidence of immune response to the vaccine, immune response to contaminating antigens, development of hepatitis, presence of autoimmune markers, and other untoward reactions. Particularly in initial tests in man, provisions should be made for long-term evaluation of vaccine safety.

11. Because the initial safety testing of hepatitis B vaccines first in chimpanzees and then in man entails a degree of risk and of expense, in terms of utilization of scarce resources, it is desirable to prepare large enough batches of experimental vaccine to suffice for several studies, the results of which can then be validly compared.

It is not yet clear whether hepatitis B vaccines must contain multiple subtypes of HBsAg to confer maximum protection or whether vaccine prepared from a single subtype will uniformly protect against all subtypes of the virus. Most epidemiological data and data obtained from cross-challenge experiments in chimpanzees suggest that infection with one subtype of HBV affords at least partial protection against other subtypes, but evidence of occasional reinfection among patients indicates that cross-protection may not be complete. Additional information about the degree and duration of cross-protection must be obtained before the definitive formulation of hepatitis B vaccines can be proposed.

17. INTERFERON THERAPY OF HEPATITIS B

Attempts to modify the course of HBV infection have generally been unsuccessful. However, the recent demonstration of an apparent inhibitory effect of interferon on the synthesis and/or maintenance of hepatitis B viral components in chronically infected patients and chimpanzees

offers new hope of more effective methods of treatment.[a,b,c] Both exogenous interferon derived from human leucocytes or prepared in cultures of human diploid fibroblasts and endogenous interferon induced by a synthetic nucleic acid derivative were effective. During treatment, the level of virus-associated DNA polymerase activity, serum HBsAg and HBeAg concentrations, and the quantity of HBsAg and HBcAg in the liver diminished in one or more of the studies. These changes were for the most part reversible, but in at least one patient improvement persisted well beyond cessation of treatment.

While these preliminary findings are encouraging, all the currently available preparations of human interferon and most of the potentially useful interferon inducers have been associated with one or more toxic manifestations, including bone marrow depression, hepatotoxicity, and fever.

A number of possible indications for interferon therapy are likely to be envisaged, including treatment of patients with acute and chronic hepatitis B, termination of persistent carriage of HBsAg, therapy of hepatocellular carcinoma, and interruption of perinatal transmission of hepatitis B infection by treatment of either the pregnant woman or the newborn. In the latter context it should be noted that interferon produces a severe glomerulonephritic syndrome in newborn mice but not in older mice.[d] Extensive safety studies in pregnant and newborn primates are required before human trials can be considered. Caution must be exercised in all trials with interferon and interferon inducers.

Interferon is not readily available at present and it is exceedingly expensive. However, if its safety and therapeutic efficacy can be established, expanded production will probably resolve these difficulties.

18. HEPATITIS B AND HEPATOCELLULAR CARCINOMA

Studies in many countries, mostly in Asia, Africa, and the Pacific and Mediterranean areas, have demonstrated an excess prevalence of markers of active hepatitis B infection—particularly HBsAg and anti-HBs—in patients with primary hepatocellular carcinoma as compared with matched controls or with the general population.[e]

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[a] GREENBERG, H. B. ET AL. *New England journal of medicine*, **259** : 517 (1976).

[b] DESMYTER, J. ET AL. *Lancet*, **2** : 645 (1976).

[c] PURCELL, R. H. ET AL. *Lancet*, **2** : 757 (1976).

[d] GRESSER, I. ET AL. *Nature*, **263** : 420 (1976).

[e] NISHIOKA, K. ET AL. *Bulletin of the World Health Organization*, **52** : 293 (1975).

There are several possible interpretations of this phenomenon. One is that patients with established hepatocellular carcinoma may be unusually susceptible to acute HBV infection and to the development of the persistent carrier state. In areas where the prevalence of postnecrotic cirrhosis and hepatocellular carcinoma is high, however, HBV infections followed by the acquisition of the persistent carrier state occur most frequently in infants and children. It seems likely, therefore, that persistent HBV infection occurs before the onset of chronic liver disease and not after.

A second possibility is that chronic infection with HBV causes postnecrotic cirrhosis and that hepatocellular carcinoma then arises from regenerative nodules by mechanisms in which HBV is not involved. The development of hepatocellular carcinoma from regenerative nodules may account for cases of this cancer that occur in patients with alcoholic cirrhosis. However, this sequence is not consistent with the fact that a number of cases of hepatocellular carcinoma associated with persistent HBV infection occur in patients with chronic active hepatitis who do not have cirrhosis.

Finally, it is possible that HBV acts either as a carcinogen or as a cocarcinogen in the persistently infected hepatocytes. Other cocarcinogenic influences, including genetic, hormonal, immunological, and environmental factors, may be necessary for the induction of hepatocellular carcinoma. However, if persistent HBV infection is a significant oncogenic factor in hepatocellular carcinoma, then prevention of HBV infection in childhood should reduce the incidence of this neoplasm. It may be possible by this indirect approach to test the hypothesis that HBV is capable of inducing hepatocellular carcinoma in man, and this may be done even without a basic understanding of the pathogenesis of chronic liver disease associated with persistent HBV infection. Although hypotheses abound to explain the pathogenic role of HBV in hepatocellular carcinoma, the actual mechanisms involved have so far eluded direct experimentation.

19. RECOMMENDATIONS

1. Reference preparations of the following reagents are urgently needed to serve as working standards for hepatitis A and B and should be established :

— HBsAg, including major subtypes
— anti-HBs, including major subtypes
— HBcAg

— anti-HBc
— HBeAg
— anti-HBe
— HAV
— anti-HAV.

2. One or more reference laboratories should be available for every WHO Region. These laboratories should provide advice and training in the application of different methods of testing for hepatitis antigens, antibodies, and viruses.

3. A technique at least as sensitive as reversed passive haemagglutination should be used to screen blood donors for HBsAg, and blood found to contain HBsAg should not be used for transfusion. Screening techniques should be under constant review in the light of developments in this field. Although radioimmunoassay is more objective and more sensitive than reversed passive haemagglutination, the reagents for reversed passive haemagglutination are more stable and less expensive and no special equipment is required. The enzyme immunoassay techniques now being developed show promise of providing the advantages of both radioimmunoassay and reversed passive haemagglutination without their respective disadvantages.

4. Because of the urgent need to ensure the use of sensitive and specific screening procedures for HBsAg on a worldwide basis, it is recommended that training courses in hepatitis B diagnostic techniques be conducted in several areas of the world under the auspices of WHO.

5. Volunteer blood donors need not be excluded on the basis of a previous history of hepatitis alone or on the finding of anti-HBs *provided that* (*a*) they have had no attack of hepatitis during at least the previous year and (*b*) their blood has been found negative for HBsAg by a very sensitive test.

6. High-risk blood donor groups cannot be categorically designated since the situation probably varies from country to country and from time to time, as well as within countries. Many studies have shown, however, that paid donors constitute a particularly high-risk group for transmitting hepatitis, and every effort should be made to introduce an entirely voluntary blood donor system.

7. Sensitive tests should be applied to ensure that only plasma units that are nonreactive for HBsAg are used for the preparation of plasma protein fractions.

8. The Committee concurred in the conclusion of previous WHO meetings [a] that at present there is no evidence that HBsAg carriers belonging to the medical or other professions in close contact with the general population routinely present a hazard, provided that they take general hygienic precautions in their professional activities. The previously recommended studies of their contacts [a] should be pursued to determine under what specific conditions transmission of infection may occur.

9. Government health services should attempt to ensure the reporting of all acute hepatitis cases by age and sex and to institute the differential reporting of type A, type B, and other types.

10. Experimental hepatitis B vaccines are currently being developed, some of them based on the use of HBsAg or one of its polypeptides. It is emphasized that, since the starting material for the preparation of these vaccines is human plasma, extreme caution must be exercised to ensure their safety and efficacy. They should be evaluated in animals before human studies are undertaken. Guidelines for the preparation of such experimental vaccines, including safety criteria, are outlined on pages 54–56 of this report.

11. National control authorities should make use of a reference preparation of hepatitis B immunoglobulin to ensure adequate potency of batches intended for clinical use.

12. A reference preparation of hepatitis B immunoglobulin should be subjected to an international collaborative study in order to determine its suitability as an international standard.

13. Clinical trials to establish the safety and effectiveness of new therapeutic or prophylactic regimens must often be conducted under unpredictable and changing environmental conditions, which makes objective evaluation of the results difficult. For this reason it is recommended that a double-blind design including a true placebo should be followed where possible.

14. In order to make the best use of expertise and of material and monetary resources, all WHO activities aimed at promoting knowledge of and eventual control of viral hepatitis should be closely coordinated.

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[a] WHO Technical Report Series, No. 512, 1973, p. 43 ; No. 570, 1975, p. 49.

Recommendations for future research

15. Further studies should be encouraged to obtain additional information about the effectiveness of pre-existing anti-HBs in preventing post-transfusion hepatitis caused by HBV in donor blood.

16. Priority should be given to the development of suitable cell and organ culture techniques for the isolation and propagation of hepatitis viruses.

17. More *in vitro* studies of cell-mediated responses to hepatitis antigens and liver-specific antigens should be carried out to clarify the role of these responses in the pathogenesis of liver damage.

18. Virus particles have been demonstrated in the faeces of patients with hepatitis A during the early acute phase of infection, and this material is currently the main source of the antigens needed for the development of serological tests. Interested investigators should therefore make arrangements to collect large amounts of early acute-phase faecal samples as well as acute and convalescent sera in well documented outbreaks of hepatitis A.

19. Since nonhuman primates, and especially marmosets, are important for research in viral hepatitis and their supply is at present severely limited, health administrators in countries where these animals are found should seek to have them made available in adequate numbers for the justified needs of medical research. The establishment of breeding colonies should be encouraged to help provide sufficient numbers of animals for research purposes.

20. Further efforts should be made to identify and characterize HBeAg and anti-HBe in view of the importance of this system for infectivity and vaccine studies.

21. The incidence of and factors favouring perinatal transmission of hepatitis B should be determined in different areas of the world. Measures to interrupt perinatal transmission of the disease, such as the administration of hepatitis B immunoglobulin, merit further evaluation.

22. The long-term outcome of hepatitis B infection acquired early in life should be studied, with particular attention to the role of HBV in hepatocellular carcinoma.

23. Mechanisms leading to the high hepatitis B carrier rate in endemic areas need to be defined, as does the relationship of subtypes to the clinical expression of the disease.

24. The possible role of arthropods in hepatitis transmission deserves further study.

25. International collaboration marked by an unusually open sharing of materials has become an established feature of the worldwide research efforts in the field of viral hepatitis. It is urged that such collaboration and sharing should continue, with due regard for national and international regulations concerning the safe dispatch of pathological material and specimens.