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,

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-

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]

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[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