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P.L. MOLLISON

CBE, MD, FRCP, FRCPath, FRCOG, FRS

Emeritus Professor of Haematology in the University of London

Formerly Director, MRC Experimental Haematology Unit

St Mary's Hospital Medical School, London

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SIR ALAN N. DRURY

CBE, FRS

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POST-TRANSFUSION VIRAL HEPATITIS

Viral hepatitis, acquired from the donor, remains the commonest lethal complication of blood transfusion. The discovery, in 1968 (see below), that the viraemic phase of serum hepatitis (hepatitis B or HB) could be recognized, encouraged the hope that all infectious donors could be detected and post-transfusion hepatitis thus eliminated. Although the transmission of hepatitis B virus (HBV) is now largely preventible, it has emerged that other viruses, not yet characterized, can also cause post-transfusion hepatitis (PTH) and that it will not be possible to eliminate PTH until tests for these other viruses have been developed.

Hepatitis virus B (HBV)-associated antigens and antibodies

An enormously important step in the control of the spread of hepatitis virus B was taken with the discovery, in serum, of an antigen associated with the disease. Initially, the relationship was not appreciated and the antigen—first recognized in the serum of an Australian aborigine, hence the provisional name 'Australia antigen'—at first appeared to have an association with acute leukaemia (Blumberg *et al.* 1965). However, it was not long before the association with hepatitis was realized (Prince, 1968; Blumberg *et al.* 1968). Australia antigen is now known to be unassembled viral coat, or surface antigen, and is termed HBsAg. Electron microscopy of serum containing HBsAg reveals so-called Dane particles of diameter 42 nm which are now known to be the complete HB virus (HBV). These particles are made up of an inner protein core (HBcAg) containing double-stranded DNA and DNA polymerase, surrounded by an outer coat of HBsAg. Antibodies to the surface antigen and the core antigen are known respectively as anti-HBs and anti-HBc. HBeAg is a soluble antigen (though also particle-associated), found only in some sera containing HBsAg.

HBsAg carriers

In random volunteer blood donors in the U.K. and the U.S.A. the frequency of HBsAg is about 0.1% (Wallace *et al.* 1972; Cherubin and Prince, 1971). In commercial blood donors, for reasons discussed below, the frequency of positives is about 10 times greater (Walsh *et al.* 1970; Cherubin and Prince, 1971). The prevalence of HBsAg in the population varies considerably in different parts of the world and exceeds 5% in some countries.

The most sensitive test for HBsAg is radioimmunoassay (RIA), which can detect 1 ng HBsAg/ml serum. Taking the mol. wt of HBsAg as 3×10^6, 1 ng is equivalent to 2×10^8 particles/ml, so that a negative RIA test is compatible with the presence of as many as 10^8 particles/ml (L.R.Overby, personal communication).

Although some blood donors who are found to be HBsAg positive are only transiently positive it is commoner for positivity to persist. In North London about 85% of the HBsAg-positive donations detected by screening come from long-term

HBsAg carriers; the remainder come from donors experiencing acute HBV infections (Barbara and Briggs, 1981). One case has been reported in which a donor who was known to have transmitted serum hepatitis 19 years previously was found to be HBsAg-positive (Zuckerman and Taylor, 1969).

It appears that the majority (more than 70%) of apparently normal blood donors who are found to be HBsAg-positive have a normal liver function, as judged by the serum aspartate transaminase level (D.S.Dane and T.E.Cleghorn, personal communication). Similarly, abnormal liver function tests (LFTs) are found in 30% of cases by Reesink *et al.* (1980) who also found that when the LFTs remained abnormal over a period of time moderate to severe histological liver disease was present in eight out of nine carriers. All carriers were found to be either HBeAg-positive (21%) or anti-HBe-positive (79%). Abnormal LFTs were found significantly more often in HBeAg-positive carriers (seven out of nine) than in anti-HBe-positive carriers (six out of 34).

Anti-HBs

Anti-HBs develops in most people who recover from hepatitis B infections. The frequency of anti-HBs in blood donors in North London was found to be about 2% (Tedder *et al.* 1980a). Multiply-transfused patients, such as haemophiliacs, have a very high frequency of anti-HBs. The presence of anti-HBs in the plasma prevents further infection with HBV.

Anti-HBc

Anti-HBc is found in persons who have been infected with HBV. It can first be detected during the incubation period and persists thereafter. High titres are found in carriers. During the recovery phase of acute hepatitis B anti-HBc may be present in the absence of HBsAg and anti-HBs. Donations taken at this time can cause PTH (Hoofnagle *et al.* 1978).

HBeAg and anti-HBe

HBeAg is present during the incubation period of acute hepatitis B and anti-HBe develops during recovery. HBsAg carriers initially have HBeAg in their blood and a high level of HBsAg. This phase lasts for about a decade but the duration varies. It is followed by a second phase in which anti-HBe replaces HBeAg and HBsAg falls to lower levels. Although infectivity is greatly reduced during this second phase, the blood may still transmit PTH (D.S.Dane, personal communication).

Transmission of HBV

Very small amounts of infected serum or plasma given either intravenously or subcutaneously can transmit HBV. The minimum infective dose of plasma from a carrier was estimated by Murray (1955) to be 1×10^{-6} ml and Drake *et al.* (1952) found that 4×10^{-5} ml given by subcutaneous injection could transmit the disease. Recognized modes of transmission, apart from the transfusion of blood or

blood products, include the use of common syringes and needles among drug addicts; failure to sterilize dental equipment and tattooing needles; sharing of razors or toothbrushes and sexual contact. HBV infections are particularly common in male homosexuals. Spread by the faecal-oral route and by insects remain uncertain; see review by Mosley (1975).

Hepatitis transmitted by transfusion may or may not be associated with jaundice; the diagnosis of non-icteric PTH is usually based on a rise in liver enzymes during the known incubation period, e.g. a raised SGPT level (2–2.5 × upper limit of normal) in two samples taken 1 week apart, in the absence of any other obvious cause (Alter *et al.* 1975).

In PTH due to HBV the mean incubation period was found to be 73 d (range 39–107 d) by Prince (1975) and 63 d (30–150 d) by Gocke (1972).

All blood donations should normally be tested for HBsAg. Blood should not be released for transfusion until it has been shown that the test is negative. Where it is not possible to complete testing before a donation is issued the clinician should be told that the donation has not been tested for the presence of HBsAg and the donation should be so marked (Report of NHS Advisory Group, 1975).

Transmission by washed red cells

Experiments in chimpanzees have shown that when blood is relatively lightly contaminated with HBV and then frozen with glycerol, the deglycerolized red cell suspension, although HBsAg negative, can transmit HBV infection (Alter *et al.* 1978).

In an experimental study in human volunteers in which red cells from a donor who was later found to be incubating HBV were injected, the incidence of hepatitis in subjects who received washed red cells was actually higher, although not significantly higher, than in subjects who received unwashed cells (Rinker and Galambos, 1981).

Finally, in a follow-up of some 8000 patients who had received red cells, sometimes previously frozen and in all cases washed in an automatic centrifuge, there were 56 cases of hepatitis, about one-third of which were icteric; the true incidence was presumably even higher since only subjects who became ill were tested (Haugen, 1979).

HBV in plasma fractions

When a modification of the cold ethanol method of fractionating plasma is used, the fractions obtained at an early stage of the fractionation process, i.e. fibrinogen, Factor VIII and a concentrate of Factors II, VII, IX and X in which complete virions (i.e. Dane particles) are readily demonstrable, carry a high risk of transmitting hepatitis. Later fractions, i.e. immunoglobulin and plasma protein fraction (albumin), are either devoid of intact virus particles or depleted of most intact infectious HB particles (Trepo *et al.* 1978). In fact, the transmission of HBV by immunoglobulin or PPF is virtually unknown; in the case of PPF an added safety factor is the heating process to which it is submitted.

HBV transmitted by HBsAg-negative donors

HBV may be transmitted by donors who are in the early stages of the incubation period before HBsAg can be detected in the blood. Transmission in these circumstances was described by Rinker and Galambos (1981) in the incident referred to above. There was an outbreak of hepatitis among 32 volunteers who had received red cells from a single donor and it was then discovered that the donor's blood, which had previously been HBsAg negative, had become HBsAg positive. Transmission of HBV had occurred at least 36 and possibly 76 d before HBsAg became detectable in the donor's blood and it became apparent that the donor had become infected through sexual contact with a partner who later developed hepatitis. Of the 32 subjects who received the donor's blood at the time when it was HBsAg negative, 19 became HBsAg positive and 14 of these developed varying degrees of hepatitis. Nine other subjects showed a primary antibody response to HBV.

Management of the HBsAg-positive donor

It has been recommended that if a blood donor is found to give a positive test for HBsAg the serum should be sent to a reference laboratory and, if the positive result is confirmed, the subject should be permanently debarred from being a blood donor. It is further suggested that the subject should have liver function tests and that if these are positive he should be advised to consult a physician (Report of NHS Advisory Group, 1975).

A survey carried out by Alter (1975) indicated that carriers of HBsAg are not commonly a danger to those with whom they come into contact. However, a few instances have been reported where health-care workers who were carriers transmitted HBV to their patients (e.g. Collaborative Study, 1980). It has been recommended that HBsAg-positive staff of haemodialysis units should not deal with 'clean' patients and also that the staff of transfusion laboratories should be tested for HBsAg and that those who are found to be positive should not assist in the preparation by an open process of blood or blood products intended for clinical use (Report of NHS Advisory Group, 1975). Apart from these situations there seems no need to restrict the activities of known HBsAg carriers among health-care workers unless there is evidence that they have infected their patients (DHSS, 1981).

Serological findings in relation to an attack of hepatitis caused by HBV

In persons exposed to HBV, HBsAg appears early in the incubation period followed by anti-HBc. DNA polymerase and HBeAg also both appear during the incubation period. In acute hepatitis B, HBsAg reaches a peak late in the incubation period and then declines during the illness and convalescence, disappearing from the blood after a period which varies from a week to several months. The carrier state most commonly develops after asymptomatic infections (Tedder *et al.* 1980b). In the U.K., where HBV infections occur mainly in young adults, about 5% of those infected become carriers (J.A.J.Barbara, personal

communication). In the countries where infections are common in infancy and childhood the proportion of infected individuals who become carriers is higher (Sobeslavsky, 1978).

Protection against HBV by antibody

Subjects whose serum contains anti-HBs are protected from hepatitis due to HBV (Grady and Lee, 1975; Seeff *et al.* 1977). The administration of immunoglobulin prepared from subjects with relatively potent anti-HBs was found to reduce the risk of hepatitis in subjects accidentally exposed to HBV infection compared with a control group treated with standard immunoglobulin, i.e. prepared from random donors (Grady and Lee, 1975).

The major indication for giving hepatitis B immunoglobulin is following a single acute exposure to HBV, as when blood known or strongly suspected to contain HBsAg is accidentally inoculated, ingested orally or splashed onto mucous membranes. In such cases hepatitis B immunoglobulin with a high anti-HBs titre should be given in a dose of approximately 5 ml (for adults) as soon as possible after exposure (WHO, 1977a).

Standard immunoglobulin seems usually to contain too low a titre of anti-HBs to be of value in prophylaxis. In a double blind randomized trial in 2204 patients the administration of normal immunoglobulin failed to reduce the incidence of hepatitis B although it did reduce the incidence of icteric non-B (presumably also non-A) hepatitis (Seeff *et al.* 1977).

Effect of heat on HBV

Evidence that human serum albumin contaminated with HBV can be made non-infective by being heated to 60°C for 10 h was reviewed by Hoofnagle *et al.* (1976). The same authors referred to an outbreak of HBV amongst recipients of plasma protein fraction from one manufacturer and referred to the study of Soulier *et al.* (1972) showing that serum heated to 60°C for 10 h maintains infectivity. They discussed the possibility that serum may contain a substance that protects HBV, or alternatively that albumin prepared from infected serum may contain a relatively small amount of HBV. They concluded that, whereas albumin heated to 60°C for 10 h was usually incapable of transmitting HBV, serum, and possibly plasma protein solution, particularly when heavily contaminated with HBV, after being heated to 60°C for 10 h might still be capable of transmitting the virus.

HBV vaccine

The efficacy of a vaccine against HBV was demonstrated by Szumness *et al.* (1980). The vaccine used was prepared from plasma of HBsAg carriers; 20 nm spherical particles were purified from the plasma and then treated with formalin to kill any residual live virus. Vaccine or placebo were given in a randomized double blind trial to over 1000 homosexual men, known to be at high risk from

HBV infection. Anti-HBs developed in 96% of subjects receiving the vaccine. During an 18-month follow-up clinical or subclinical infections due to HBV developed in 1.4–3.4% of the vaccinated group but in 18–27% of the group receiving the placebo. In the vaccinated group, most of the infections occurred within 3 months of giving the first dose of vaccine; no clinical evidence of HBV infection was seen in subjects who had already developed a detectable immune response.

Non-A, non-B hepatitis

This rather clumsy term is used to describe hepatitis in which both HAV and HBV have been excluded. The term hepatitis C is not used because there is evidence that there is more than one kind of non-A, non-B virus and because no specific tests have yet been developed. The mode of transmission of non-A, non-B hepatitis may sometimes be similar to that of hepatitis B. Non-A, non-B hepatitis is prevalent following transfusion or other percutaneous exposure; it is commoner in populations of low socio-economic status and is probably spread by close person-to-person contact: it is associated with a chronic carrier state (Alter, 1980). Non-A, non-B PTH has a slightly shorter incubation period than hepatitis B, i.e. between 6 and 10 weeks with a peak of about 8 weeks (Alter, 1980), compared with a mean of 9–10 weeks in PTH due to HBV. As a rule, non-A, non-B hepatitis is symptomatically mild. Patients seldom need to be admitted to hospital. Nevertheless, up to 60% of cases have abnormal alanine aminotransferase (ALT) (previously called serum glutamic pyruvic transaminase (SGPT)) levels for more than 1 year; if a liver biopsy is taken, most of the cases show histological evidence of a significant chronic liver disease and approximately 10% show features of cirrhosis (Alter, 1980). A striking feature in non-A, non-B hepatitis is the tendency for serum hepatic enzyme levels to fluctuate markedly over a relatively short time. Although typical non-A, non-B hepatitis differs in several respects from typical B hepatitis there is substantial overlap and the two forms cannot be differentiated solely on clinical grounds.

The transmission of non-A, non-B hepatitis by a Factor IX concentrate was described by Wyke *et al.* (1979). Of 17 patients with chronic liver disease who received the concentrate, four developed hepatitis, with a mean incubation period of 65 d; there were three deaths. Chimpanzees injected with the concentrate developed hepatitis.

Raised serum concentrations of liver enzymes in carriers of non-A, non-B hepatitis

The incidence of non-A, non-B hepatitis in recipients of blood transfusion is directly related to the level of alanine aminotransferase (ALT) in the relevant blood donors (Aach *et al.* 1981). Although non-A, non-B hepatitis develops in some patients who have received only blood from donors with normal ALT levels, it can be deduced that at least 21% of cases of transfusion-associated hepatitis might be prevented by excluding only 3% of the present donor population, i.e.

those with an ALT level above 44 i.u. (Holland *et al.* 1981). Recipients of blood from donors with the lowest ALT levels (less than 15 i.u.), who received an average of 2.7 units of blood, had an incidence of non-A, non-B hepatitis of 5%. Although this finding implies that it is not possible to identify completely safe donors by carrying out tests for levels of serum liver enzymes, it seems clear that in research work involving the injection of donor blood into volunteers it is essential to use only donors whose serum liver enzymes are strictly within normal limits.

In an episode described by Guyer *et al.* (1979), seven out of nine recipients of small amounts of blood from a single donor developed non-A, non-B hepatitis 28–50 d later; the donor must have remained infectious for at least 34 d; tests on the donor 2 months after the earliest time of transmission of hepatitis showed raised serum concentrations of liver enzymes.

The minimum carrier rate of non-A, non-B virus in volunteer blood donors in the U.S.A. has been estimated to be 1.6% and in commercial blood donors to be 5.4% (Blum and Vyas, 1982).

The role of hepatitis A virus (HAV)

HAV, the infectious agent of epidemic jaundice, is an extremely unusual cause of PTH. In 14 published series, relating to transfusions given to about 9000 subjects, not a single case of PTH due to HAV was observed (see review by Blum and Vyas, 1982). However, hepatitis A has been seen following the transfusion of blood from a donor in the early stages of incubating the disease (Seeberg *et al.* 1981; Barbara *et al.* 1982).

Acute infection with HAV is most readily recognized by the finding of specific IgM antibodies to the virus.

Frequency of post-transfusion hepatitis (PTH)

Anicteric cases of PTH are commoner than icteric cases. For example, in a study reported from the U.S.A. in which 2204 patients were followed and in which PTH was diagnosed in 241 patients, the disease was icteric in less than one-fifth of the cases (Seeff *et al.* 1977). It follows that repeated sampling of recipients is necessary if all cases are to be detected and that only prospective studies are likely to give a true indication of the frequency of PTH. In 14 prospective studies reviewed by Blum and Vyas (1982), the overall frequency of hepatitis, in patients receiving HBsAg-negative blood from volunteer donors, varied from 4 to 13%, between 89 and 100% of cases were due to non-A, non-B virus.

In the U.K. no prospective survey, carried out exclusively with HBsAg-negative blood, has been reported. Nevertheless there is evidence that non-A, non-B viruses play a smaller part in the U.K. than in the U.S.A. Investigations on sporadic cases of PTH indicate that some are due to HBV and that these are generally caused by the use of donors in the early or late stages of HBV infections, at a time when tests for HBsAg were negative (D.S.Dane, personal communication).