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WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES No. 512

Viral Hepatitis

Report of a WHO Scientific Group

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

GENEVA
1973

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* * *

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Geneva, 25–30 September 1972

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VIRAL HEPATITIS

Report of a WHO Scientific Group

A WHO Scientific Group on Viral Hepatitis met in Geneva from 25 to 30 September 1972. Dr M. Takabe, Director of the Division of Communicable Diseases, opened the meeting on behalf of the Director-General.

INTRODUCTION

Hepatitis was recognized as a serious public health problem by the World Health Organization soon after its establishment and in 1951 the Third World Health Assembly requested that an Expert Committee be convened to consider epidemic and serum hepatitis and to make relevant recommendations. The report of this Committee was published in 1953.[1] A second Committee published a further report in 1964.[2]

To these reports the problems of what are now designated viral hepatitis A (epidemic or infectious hepatitis) and viral hepatitis B (serum hepatitis) were each considered in detail. However, because of the discovery of the Australia antigen in 1961 and its subsequent recognition as a specific marker of infection with the agent of viral hepatitis B, there has been great progress in the understanding of the clinical, epidemiological and immunological behaviour of this form of the disease. Relatively speaking there has been much less progress in the understanding of viral hepatitis A but there have been some advances, particularly in studies with nonhuman primates. These and other recent research work on viral hepatitis A are described here but for more detailed information on the epidemiology, public health importance, and control of viral hepatitis A the second report[2] should be consulted.

The main body of this report deals with viral hepatitis B, although the subject is not covered exhaustively, emphasis being placed on recent advances in knowledge of the disease. The epidemiology in different geographical, ethnic, and social groups and the techniques for measuring hepatitis B antigen and antibody are described. The present status of efforts to propagate the virus(es) in tissue culture and in nonhuman primates is recorded. The immunopathology and the possible influence of genetic factors are reviewed. The problems in blood transfusion services, special groups of patients, and hospital staffs are discussed and recommendations are made on the use of tests and the application of control measures, and on

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1953, No. 62.
[2] *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 285.

the areas to which further research might profitably be directed. An attempt is also made to simplify the terminology of hepatitis, which has been confused for many years.

Knowledge of hepatitis B is still increasing and new information is appearing almost daily. References have therefore been kept to a minimum and in the main restricted to reviews and papers giving practical information of current importance.

THE TERMINOLOGY OF HEPATITIS

As shown in Table 1 there are a multitude of terms for viral hepatitis. Until recently differences between the two main types ("infectious" and "serum") were dependent on epidemiological observations - the route of infection and the period of incubation—and on the results of studies of transmission in human volunteers.

TABLE 1. SYNONYMS FOR VIRAL HEPATITIS

Viral hepatitis A	Viral hepatitis B
Acute catarrhal jaundice	Arsenotherapy hepatitis
Acute viral hepatitis	Ad(1)-hepatitis
A-(B) hepatitis	Ad/BN hepatitis
Australia antigen-negative hepatitis	Australia antigen hepatitis
Botkin's disease	B-(H) hepatitis
Catarrhal jaundice	Hepatitis B (H. B.)
Common infective hepatic jaundice	Injury hepatitis
Common-source hepatitis	Homologous serum hepatitis (virus B)
Epidemic catarrhal jaundice	Homologous serum jaundice (HSJ)
Epidemic hepatitis	Inoculation hepatitis
Epidemic jaundice	Long-incubation hepatitis
Hepatitis A (H. A.)	MS-2 hepatitis
Icterus epidemicus	Parenteral hepatitis
Infectious hepatitis	Post-arsphenamine jaundice
Infectious jaundice	Post-transfusion hepatitis
Infective hepatitis (virus A)	Post-vaccinal jaundice
Jaundice des camps	Salvarsan jaundice
MS-1 hepatitis	Serum (MS-2) hepatitis
Short-incubation hepatitis	Serum hepatitis (SH)
Salaelongolesucht	Serum jaundice
Viral hepatitis type A	Syringe jaundice
	Syringe-transmitted hepatitis
	Tattoo jaundice
	Transfusion-associated hepatitis
	Transfusion hepatitis
	Viral hepatitis type B
	Yellow fever vaccine hepatitis

Terminology of the antigen

The discovery of the association between hepatitis and the Australia antigen has permitted the use of serological methods with which a proportion of patients and carriers of at least one of the hepatitis agents may be detected. Although at an earlier stage there were conflicting views, there is now general agreement that this antigen is related only to the so-called "serum" hepatitis. It has been suggested that hepatitis could be broadly classified under two headings: Australia antigen-positive hepatitis and Australia antigen-negative hepatitis. However, negative results could be due to a variety of unrelated factors including relatively insensitive methods, lack of good reagents, different antigenic determinants, and the testing of serum specimens at different times in the course of illness.

The priority of the term Australia antigen is acknowledged, but if its association with hepatitis is specific then the name Australia antigen could be misleading, implying as it does an unusual association with that country. An alternative proposal has been that the designation hepatitis-associated antigen be used, but, if other antigen-antibody systems are discovered that prove to be specific for other types of hepatitis, the term hepatitis-associated antigen will create great confusion.

The terms hepatitis A and B were introduced as long ago as 1947.[1] It is proposed, therefore, that the Australia antigen be referred to as:

hepatitis B antigen (HB Ag)

and the corresponding antibody as:

hepatitis B antibody (HB Ab)

Terminology of the disease

The terminology of the actual disease is more difficult. The general term viral hepatitis refers, by common usage, to hepatitis caused by two presumptive viruses, although it is recognized that other viruses may also be implicated.

It is proposed that the common forms of viral hepatitis be subdivided principally on epidemiological grounds, taking into consideration the presence of hepatitis B antigen, into:

viral hepatitis type A

and

viral hepatitis type B.

There is substantial historical, epidemiological, and experimental evidence to suggest that these two types of hepatitis are caused by antigenically

[1] *Lancet*, 1947, 2, 691–692.

distinct agents.[1-3] It is appreciated that it is not possible to allocate every patient with hepatitis to one of these two groups and that viral hepatitis infections exist that are due to other agents, only some of which have been recognized. This is a problem frequently confronting epidemiologists, clinicians, and pathologists that will only be resolved when the different aetiological agents of hepatitis have been identified.

VIRAL HEPATITIS TYPE A

Viral hepatitis type A is a contagious disease transmitted by the faecal-oral, parenteral, and possibly other routes. The virus is present in the blood during the early, acute phase of the infection and is excreted in faeces and perhaps other body fluids during the first 1-2 weeks of the disease; excretion for longer periods has not been established.

The disease is a major public health problem, occurring endemically in all parts of the world, with frequent reports of minor or major epidemics. Common source outbreaks are most frequently initiated by faecal contamination of water and food. However, spread is usually by person to person contact. Subclinical cases are common and a possible source of spread. The disease has a low mortality but occasionally patients may be incapacitated for weeks or months. There is no specific treatment.

Spread from the patient is reduced by appropriate precautions, which include good personal hygiene, the sanitary disposal of excreta, and the sterilization of eating utensils and body and bed linen after use. Pooled human immunoglobulin (a 16% solution at 0.02-0.12 ml/kg body weight) administered before exposure to the virus or early during the incubation period prevents or attenuates the clinical illness.[4] Immunoglobulin may not always prevent infection with hepatitis type A virus, and an inapparent or subclinical hepatitis may lead to prolonged immunity.

Attempts to identify hepatitis type A agent(s) by immunological methods

At present there are no specific tests for hepatitis type A. Recently the presence of an antigen designated "epidemic hepatitis-associated antigen" or "Milan antigen" in the sera of patients in the acute phase of hepatitis was described. Although it was first thought that this antigen might be specific, later studies showed it to be a lipoprotein, possibly abnormal, lacking specificity for hepatitis type A.

[1] *Wld Hlth Org. techn. Rep. Ser.*, 1953, No. 52.
[2] *Bull. Wld Hlth Org.*, 1970, 42, 957-992.
[3] *Wld Hlth Org. techn. Rep. Ser.*, 1964, No. 285.

In other studies, an antigen was found in extracts of faeces obtained from patients during the first 3 weeks of hepatitis type A disease. Antigenic activity was associated with 15-25-nm and 40-45-nm particles that morphologically resembled hepatitis B-antigen particles, but the tubular structures frequently found in preparations of hepatitis B antigen were not observed. One component cross-reacted with hepatitis B antigen but another was shown to be antigenically distinct. Antisera against this antigen were prepared in rabbits and guinea-pigs and yielded some encouraging results. However, antisera prepared later behaved erratically and an association with the faecal antigen could not be found in a large outbreak of hepatitis type A in a closed population. Studies of this antigen are continuing.

Tissue culture studies

All of the many attempts to isolate and propagate hepatitis type A agent(s) in cell and organ cultures have been unsuccessful.

Transmission of hepatitis type A to human volunteers

Studies of hepatitis in human volunteers demonstrated the routes of transmission of both types of viral hepatitis, helped to define their immunological differences, determined the efficacy of human immunoglobulin in preventing or attenuating hepatitis type A, and established some of the characteristics of the causative agents. Human volunteer studies were restricted in the past—and should continue to be so in the future—by the potential seriousness of the disease; the rules for conduct of human volunteer experiments should be adhered to strictly.

Animal studies

In the past all attempts to transmit hepatitis types A and B to many animal species, including nonhuman primates, failed or yielded inconsistent results. Occasionally chimpanzees developed a hepatitis-like illness after inoculation with human hepatitis material but because they seemed to acquire immunity rather rapidly after capture, their use was discontinued. Subsequently more than 40 small clusters of cases of viral hepatitis in human beings in close contact with nonhuman primates were reported. In most instances outbreaks occurred in association with recently imported young chimpanzees, but a gorilla, Celebes apes, gibbons, and woolly monkeys were also involved in a few of these episodes. It could not be determined whether the responsible agents were of human or nonhuman primate origin. As a result of these observations, interest in experimental transmission studies was revived and nonhuman primates of different species were again inoculated with human hepatitis materials, not identified as type A or B. Histological liver changes compatible with a diagnosis

of hepatitis were reported in chimpanzees (*Pan troglodytes*), young patas monkeys (*Erythrocebus patas*), mangabey monkeys (*Cercocebus torquatus torquatus*), mona monkeys (*Cercopithecus mona mona*) and patty-nosed monkeys (*Cercopithecus nictitans nictitans*). However, the disease was not transmitted consistently from animal to animal and interpretation was complicated by hepatitis occuring in animals that had not been inoculated.

Human hepatitis type A materials, inoculated into certain species of marmoset (*Saguinus*, spp.), induced viral hepatitis and the disease was passed in series from animal to animal.[1] This finding was confirmed by several laboratories. Marmosets that had had hepatitis once were shown to be resistant to reinfection with the same strain of hepatitis virus but they were not necessarily resistant to infection with another strain. Attempts to neutralize one of these strains with convalescent human or marmoset sera were unsuccessful. The transmission studies were challenged by one group of investigators,[2, 3] who reported that the disease observed in marmosets in their laboratory represented activation of a marmoset hepatitis agent rather than transmission of the human disease to the nonhuman primates.

In a series of studies not yet published, marmosets were inoculated either with the acute phase serum of a human volunteer previously infected with the Willowbrook MS-1 strain of hepatitis type A agent, with the convalescent serum of the same volunteer, or with a mixture of acute and convalescent sera. Of the 12 marmosets inoculated with the acute phase serum, 10 developed hepatitis. None of the 6 inoculated with the convalescent serum showed any signs of hepatitis, and of the 8 animals inoculated with the mixture of acute and convalescent sera, 7 remained normal and 1 developed mild hepatitis with a prolonged incubation period. These results suggest neutralization of hepatitis type A virus present in acute serum by the convalescent serum. If confirmed, these results may remove doubts about the nature of the hepatitis that follows the inoculation of human hepatitis materials into marmosets.

Electron microscopic studies

Attempts to demonstrate hepatitis type A by electron microscopy have failed. The significance of corona-like or para-myxovirus-like particles present in the sera of some hepatitis patients has recently been discussed but a causal relationship with hepatitis appears doubtful, since similar particles have also been found in the sera of patients with other diseases. It may be that such virus-like particles are fragments of cell organelles.

[1] Deinhardt, F., Wolfe, L., Jonge, U. & Holmes, A. W. (1972) *Canad. med. Ass. J.*, **106**, 468–472.

[2] Parks, W. P. & Melnick, J. L. (1969) *J. infect. Dis.*, **120**, 539–547.

[3] Parks, W. P., Melnick, J. L., Voss, W. R., Singer, D. B., Rosenberg, H. S., Alpatt, J. & Casarez, A. M. (1969) *J. infect. Dis.*, **120**, 548–559.

VIRAL HEPATITIS TYPE B

Distribution and prevalence

Until a few years ago it had become generally accepted, on the basis of long-held concepts of transmission, that viral hepatitis type B was limited in its distribution to populations in those areas of the world where there were modern medical services and parenteral therapy was frequently practised. Cases of hepatitis arising outside such settings were thought to be of the type A variety. The discovery of hepatitis B antigen, together with the demonstration of its persistence in the blood for prolonged periods, resulted in the re-examination of theories concerning the transmission and distribution of this infection. If it is accepted that, as seems probable, circulating hepatitis B antigen or its specific antibody is evidence of current or past infection with the virus of hepatitis B, this agent has a worldwide distribution similar to that previously attributed only to hepatitis type A. Early serological surveys using the relatively insensitive immunodiffusion technique demonstrated the presence of antigen in sera collected from remote and insular populations. These findings have subsequently been confirmed and extended.

Seroepidemiological surveys on selected groups have shown that the prevalence of hepatitis B antigen in apparently healthy individuals in North America and Western Europe is 0.1–0.6%, in comparison to 5–20% in tropical Africa, South-East Asia and the Far East. Little information is available from serial samples collected over a period of time, which would permit evaluation of the carrier rate of the antigen in defined population groups. In tropical countries, the antigen is detected in individuals of all ages, most frequently in children aged 5–15 years. It is rarely found in adults over the age of 60 years. The prevalence of the antigen in Caucasians living in some tropical countries is higher than in those in temperate zones but is considerably lower than in the indigenous population. In all regions the antigen is detected more frequently in males than in females and in urban than in rural communities.

Incubation period

In the past, type A and type B hepatitis were distinguished by their period of incubation, 20–40 days for type A and 60–180 days for type B. However, many recent studies in experimentally infected volunteers, recipients of blood, and others have revealed a much wider range of incubation periods for hepatitis type B, overlapping with that of hepatitis type A and extending up to 180 days.

Modes of transmission

In spite of the current interest in the possible nonparenteral transmission of hepatitis type B, the parenteral route is still of major importance in temperate zones, particularly with regard to control and prevention in medical and public health practice. Limited early attempts to demonstrate icteric illness experimentally by the transmission of hepatitis B by other than parenteral routes and with materials other than blood or blood products appeared to give negative or equivocal results. These studies, which were carried out before sensitive biochemical indicators of liver damage such as serum enzyme activity were available, led to apparent confirmation of the concept of parenteral transmission alone. This was not seriously challenged until 1967 when it was demonstrated that serum containing the MS-2 strain of hepatitis B virus was infective when given orally.

The results of tests for hepatitis B antigen soon suggested that parenteral transmission alone could not explain the origin of all hepatitis type B infections. During the past few years there have been several reports of the presence of hepatitis B antigen in saliva, urine, bile, faeces, and various body fluids. Most of these studies have been very limited in scope and some could not subsequently be confirmed. Since blood may contaminate any of these body fluids under certain pathological conditions, their potential importance as vehicles for transmission cannot be discounted, but evidence of this is still not available. Similarly, venereal transmission is a possibility that requires further study.

Transplacental transmission of hepatitis B infection has long been suspected as a means of maintaining the agent in the population. In a recent study, transmission of hepatitis B antigen from mother to infant was relatively common when the mother had a hepatitis type B infection between the eighth month of gestation and the end of the second month postpartum. Transmission was less common when maternal hepatitis occurred earlier in pregnancy. Although the mode of transmission was not clear, the transplacental route seemed the most likely in a few instances. There was evidence of long-term carriage of hepatitis B antigen in these infants, with biochemical but no clinical evidence of hepatitis.

It is nevertheless too early to assess with certainty the relative importance of transplacental and perinatal transmission of the hepatitis B agent from mother to infant. Transmission to infants by mothers who are asymptomatic carriers of the antigen appears to be infrequent.

Although some modes of transmission of hepatitis type B infection in the tropics are similar to those in other parts of the world, additional factors may be of importance in these regions. These include ritual circumcision, tattooing, scarification, and the bites of blood-sucking insects. The role of biting insects in the transmission of the antigen requires further investigation. No consistent association has been found between the notification of hepatitis or prevalence of hepatitis B antigen and seasonal rains in Africa. While this does not exclude the possibility that mosquitos may play a role in the spread of hepatitis, it suggests that those mosquitos that multiply during the rainy season may not be involved. In New Guinea, the frequency of hepatitis B antigen was not found to be correlated with either mosquito activity or altitude, whereas the frequency of arbovirus antibodies showed a strong correlation with mosquito activity and was inversely related to altitude. There is a further report, yet to be confirmed, that some species of mosquito may serve as biological vectors for type B hepatitis infection. In a laboratory study hepatitis B antigen was found by an immunofluorescent antibody technique in the lumen of the gut of culicine mosquitos immediately after they were fed on antigen-positive blood or serum and the antigen persisted in the lumen for 10 days. The antigen then disappeared but reappeared in the salivary glands after 3 weeks. Antigen was also detected in the lumen of the gut and the salivary glands of culicine mosquitos that died 8 weeks later. Recently, pools of mosquitos caught in the wild in East Africa were tested for hepatis B antigen by the solid-phase radioimmunoassay technique and in another study mosquitos caught in West Africa were examined by counter-immunoelectrophoresis. Antigen was detected in 28 pools of mosquitos from East Africa and in 18 pools from West Africa. The species of mosquito in which antigen was demonstrated included *Mansonia africana*, *Mansonia uniformis*, *Anopheles funestus*, *Anopheles coustani*, *Anopheles pulsalis*, *Mansonia fuscopennata*, *Culex tritaeniorhynchus*, *Culex sitiens*, and *Culex pipiens fatigans*. Antigen was also detected in *Aedes africanus*, which seldom bites man, and in *Hemimerus talpoides*, which does not bite man. In another study, *Aedes aegypti* mosquitos were fed on a chronic carrier of hepatitis B antigen and on a healthy subject. The rate of disappearance of detectable antigen in the mosquitos paralleled blood meal digestion.

Transmission of hepatitis B by blood-sucking arthropods, if confirmed, would have important epidemiological implications. Criteria for active transmission would be the demonstration of multiplication of the virus in the arthropod and ability to infect by biting. Such evidence is not yet available. Mechanical transmission by blood-sucking arthropods is a possibility for hepatitis B, as for many other diseases, but this also has not yet been definitely established.

Hepatitis type B and medical care

It is generally agreed that not all cases of post-transfusion hepatitis are caused by hepatitis type B infection. The proportion due to hepatitis B or other undesignated agents probably varies with the circumstances. However, as more hepatitis B carriers are eliminated from serving as blood donors, the proportion of cases due to other types of hepatitis will increase.

Hepatitis B infection and the subsequent development of a chronic antigen carrier state have been observed among patients on maintenance haemodialysis and among other medical and surgical patients—including those in institutions—with conditions complicated by temporary or permanent immunological deficiency, either as part of their underlying disease or as a result of immunosuppressive therapy. Presumably the source of many such infections has been the therapeutic use of blood transfusions, but contact with other internal or external patients or carriers cannot be ruled out.

Changing patterns of infection in certain developed countries

During the past decade marked shifts in the age- and sex-specific rates for hepatitis have been observed in the USA and some European countries. These changes were subsequently found to be due to an increase in the number of hepatitis B infections, particularly among males in the 15–29-year age group. The infections were not related to blood transfusion or other medical procedures. These features, together with the loss of seasonal peaks and the increasingly large proportion of urban cases, suggested a likely association with the illicit use of drugs. It is quite possible that in addition to the increased risk of parenteral transmission, the mode of life of drug abusers may increase the level of nonparenteral transmission.

Genetic factors

In order to explain the geographical variation in the prevalence of apparently healthy chronic carriers of hepatitis B antigen, it was postulated that persistence of the antigen was dependent not only on infection by the agent associated with hepatitis B antigen but also on the presence in the homozygous state (Au^1/Au^1) of an autosomal recessive gene that conferred the ability to maintain the antigen in an individual acquiring it. This gene was considered to be rare in temperate populations, but common in tropical areas. It has been further suggested that individuals with such an inherited susceptibility do not usually display overt manifestation of hepatitis but nevertheless remain carriers of the infectious agent.[1] The interpretation of previous genetic analyses was based upon the assumption of total exposure of the population to the infectious agent, since only then could the effect of the gene be demonstrated.

Although familial clustering has been demonstrated in a number of instances it is not necessarily genetic factors that are involved, since vertical transmission appears to occur and perinatal transmission from mother to child may also take place. The relative importance of each mode of transmission is as yet undetermined. It is, however, accepted that the genetic composition of the host may influence the response of the host to infection with a variety of agents.

[1] Blumberg, B. S., Friedlaender, J. S., Woodside, A., Sutnick, A. I. & London, W. T. (1969) *Proc. Nat. Acad. Sci.*, 62, 1108–1115.

Subtypes of hepatitis B antigen and their significance

The hepatitis B antigen is not a single entity. According to current terminology, the common antigenic determinant shared by hepatitis B antigen is *a* and the two major antigenic subspecificities are *d* and *y*. The latter two behave in a mutually exclusive manner and are carried on the same particles as *a*. These determinants appear to be dictated by the infecting agent and are found to breed true in instances of experimental transmission and in appropriately studied epidemiological settings.[2] The antigenic subspecificities exhibit differences in their geographical distribution. Antigenic subtypes may vary in association with other factors and in their relationship to different clinical expressions of infection.

Recently two additional subspecificities, *w* and *r*, have been described. These behave independently and are presumably found in association with either *d* or *y*. They appear to occur in a nonrandom geographical distribution. Subtypes with the following antigenic characteristics have been identified: *adr*, *adw*, and *ayw*.

Immunopathology

Immune reactions

The pathogenesis of liver cell damage in hepatitis type B remains unclear. The view that the infectious agent exerts its destructive effect on hepatocytes by a direct cytopathic action seems inconsistent with the apparently benign persistence of large amounts of antigen both in the serum and the cytoplasm of liver cells of some healthy carriers of the antigen. Alternatively it may be that detrimental immunological reactions initiated by the hepatitis agent cause liver cell injury.

Non-organ-specific antibodies to mitochondria, smooth muscle, and nuclear components are found in a high proportion of patients with primary biliary cirrhosis and active chronic hepatitis and in a lower proportion of patients with cryptogenic cirrhosis. These varieties of chronic liver disease are believed to have an autoimmune etiology. The transient appearance in the serum of low titres of autoimmune antibodies in acute hepatitis is also well known. Thus smooth muscle antibody is found in 60–80% of patients with acute type A or type B hepatitis. Antinuclear antibodies are found less frequently and mitochondrial antibodies occur in less than 2%.

[2] Le Bouvier, G. L. (1972) *Amer. J. Dis. Child.*, 123, 420–424.

The titres of these antibodies are correlated with the severity of liver cell damage, suggesting that they are produced in response to release of antigens from damaged cells. In contrast, non-organ-specific serum autoantibodies persist at a high titre in the chronic liver diseases outlined above. Although not directly responsible for the progression of liver cell or bile duct destruction such autoantibodies have nevertheless proved useful as "autoimmune markers".

Until recently, there was no evidence of organ-specific immune reactions, even in those liver diseases long accepted as autoimmune. However, the isolation of two organ-specific proteins from human liver has allowed the demonstration of organ-specific antibodies in the sera of a proportion of patients with active chronic hepatitis. The same antigens caused inhibition of leucocyte migration in tests on cells from many patients with active chronic hepatitis or primary biliary cirrhosis. The production of organ-specific antibodies and cellular hypersensitivity in these diseases supports the concept that they are autoimmune. Furthermore, experimental active chronic hepatitis has been induced in rabbits by immunization with extracts containing these liver-specific proteins. Studies of organ-specific antibodies in viral hepatitis have not yet been carried out in man.

A rise in total serum globulin and in the immunoglobulin fraction accompanies both acute type A and acute type B hepatitis. A rise in IgM levels has been reported in acute hepatitis A but not in acute hepatitis B. However, a number of conflicting reports have been published and these may well reflect differences in the patients investigated and the epidemiological settings. Serial immunoglobulin determinations have shown an unexplained fall in IgG levels in patients with hepatitis B antigen and a rise in IgG levels in patients in whom the antigen was not detected. A study of the IgG levels in asymptomatic carriers showed no difference from those observed in a normal population.

The pathogenic significance of immune complexes is now being increasingly recognized in some diseases of obscure etiology. The effects of immune complexes depend largely on the ratio of antigen to antibody. Complexes formed in the presence of antigen excess are readily soluble and tend to remain in the circulation, from which they may be deposited in certain sites such as the walls of small vessels. Complexes formed in antibody excess are larger and relatively insoluble. They may be harmlessly eliminated by cells of the reticuloendothelial system, but they are also thought to be responsible for the acute anaphylactoid reaction of the Arthus response. Immune-complex deposits have a high affinity for complement and, through activation of the complement system, have a number of pathological sequelae, including aggregation of granulocytes with release of their lysosomal enzymes, production of histamine and kinin, and aggregation of platelets with microthrombus formation. Intravascular coagulation has recently been described in acute liver cell necrosis.

Studies of serum complement levels in liver disease have yielded variable results. A study of several components of the complement system in serial samples of serum obtained during the course of acute hepatitis showed depressed levels of total haemolytic complement (CH_{50}), C4, and C3 during the prodromal stage of acute hepatitis type B. These changes were associated with symptoms of a type III hypersensitivity reaction. In contrast, normal or elevated levels were seen in antigen-negative patients who were free from the prodromal symptoms as well as in a group with acute nonviral hepatitis. The levels of C1q were widely variable in all groups and the C9 levels were normal.

Circulating complexes of hepatitis B antigen and antibody have been demonstrated by electron microscopy in the sera of a few antigen-positive patients.

A progressive change in the ratio of antigen to antibody during the course of type B hepatitis may result in immune complex disease of the antigen-excess type during the early phase of the illness. Such immune complexes may be responsible for the characteristic serum-sickness-like syndrome of the prodromal stage. Later a state of antibody-excess may occur and this has been suggested as the mechanism responsible for some cases of fulminant liver necrosis. However, it is not generally agreed that complexes have a significant role in liver disease.

Initially, studies of cell-mediated immunity in liver disease appeared to show depression of nonspecific responses. Skin-sensitizing agents failed to produce delayed skin reactions in some patients with primary biliary cirrhosis or active chronic hepatitis. Transient depression of lymphocyte transformation to phytohaemagglutinin has been described in these conditions as well as in acute hepatitis. However, varying responses were also noted. Transformation of lymphocytes was induced by serum containing antigen in a small number of patients who had recovered from hepatitis B infection but a patient who had persistent antigenaemia failed to respond. These findings are yet to be confirmed. More studies of *in vitro* cell-mediated responses to liver-specific and hepatitis antigens are needed to clarify the role of these responses in the production of hepatitis.

The antigen carrier state and chronic liver disease

On the basis of longitudinal studies of patients with hepatitis B, an arbitrary definition of the carrier state has emerged. For practical purposes it has been agreed that a persistent carrier state exists in individuals in whom antigen has been detected repeatedly for more than 3 months. Such a carrier state may be associated with liver damage.

An increased frequency of the carrier state has been described in patients with Down's syndrome, lepromatous leprosy, and chronic renal failure and in patients undergoing immunosuppressive therapy. A varying proportion of such carriers have been found on investigation to have abnormal-

lities in the liver ranging in severity from minor changes in the nucleus of the cell to severe hepatitis and cirrhosis. Two forms of the chronic disease can be distinguished, persistent and aggressive. Clinically, chronic persistent hepatitis is a mild, benign disease, while chronic aggressive hepatitis tends to conform to the clinical syndrome of chronic active hepatitis, in which liver cell dysfunction is often severe and the prognosis is poor. However, considerable overlap exists between the clinical categories and their pathological counterparts. Aggressive changes may be seen in the course of uncomplicated acute viral hepatitis, but the prognosis in these cases is usually excellent.

Chronic persistent hepatitis is characterized histologically by preserved lobular architecture, portal inflammatory infiltration, and slight or no fibrosis. It is not always preceded by a recognizable acute illness, and malaise, hepatomegaly, and minor abnormalities of liver function are the clinical features. There is no progression to cirrhosis and the prognosis is good. Hepatitis B antigen has been detected repeatedly in only a small proportion of such patients.

Chronic aggressive hepatitis is usually characterized by *parenchymal* necrosis and inflammatory cell infiltration in so-called "piece-meal" distribution, rosette formation, and a varying degree of hepatic cirrhosis. Young females are most often affected and the onset may be insidious or acute. Features of multisystem involvement are frequently seen. Autoantibodies are frequently present in the serum and immunoglobulin levels are usually elevated. Immunological abnormalities have been reported in a high proportion of the relatives of such patients.

The etiology of active chronic hepatitis remains obscure. The detection of hepatitis B antigen in the sera of some cases is of great interest—it has been found in 4–60% of patients with active chronic hepatitis in temperate countries. The association seems to reflect the prevalence of hepatitis B infection in the population under study. Active chronic hepatitis patients with hepatitis B antigen differ from those who lack the antigen in that they tend to be male and older, autoantibodies are usually absent from the serum, and multisystem involvement is not present.

Cryptogenic cirrhosis may represent a heterogeneous group of conditions and the presence of autoantibodies in some of these patients in temperate zones suggests that silently progressive autoimmune liver disease is responsible. The role of hepatitis B antigen in cryptogenic cirrhosis is not well documented.

Chronic liver disease in Africa and Asia

Hepatitis B antigen and antibody have been detected in the sera of patients with chronic liver disease in different tropical countries. The presence of this antigen in a proportion of patients with a history of hepatitis or in patients with progressive liver disease suggests the possibility of an etiological association between the two. The prevalence of hepatitis B antigen in the macronodular types of cirrhosis, which are the most frequent types encountered in the tropics, varies from 10–33% and lends support to the view that macronodular cirrhosis may be a sequel to viral hepatitis. An association has not been found between hepatitis B antigen and the micronodular cirrhosis that is usually seen in temperate climates.

Liver cell carcinoma

Liver cell carcinoma is one of the commonest types of cancer encountered in tropical Africa and South-East Asia and in some parts of Africa it is the commonest type of cancer in adult males. Although the macronodular type of cirrhosis is present in 75% of patients with liver cell cancer, there is no histological evidence of fibrosis in the remainder. Many factors may be of significance in the pathogenesis of liver cell cancer, and viral hepatitis may be one of them. However, progression of viral hepatitis to cirrhosis has not yet been established.

Different studies of the frequency of hepatitis B antigen in liver cell carcinoma have shown it to vary from 0 to 80%. Preliminary evidence suggests that the frequency of antibodies to hepatitis B antigen in patients with liver cancer is reduced. The wide variations in the observed frequencies of hepatitis B antigen in liver cell carcinoma in the tropics may be due to true geographical differences, differences in titres of antigen, or variation in techniques and quality of reagents employed for detection of the antigen. Sensitive methods with standardized reagents should be used in areas with high frequency of liver carcinoma in order to evaluate the significance of observed differences.

The association between hepatitis B antigen and liver cell cancer may be of etiological significance in some geographical situations but the contribution of cirrhosis, which frequently coexists with this cancer, may be of greater importance. It should be noted that in certain tropical countries there is no significant difference in the prevalence of hepatitis B antigen in cirrhosis patients with and without carcinoma. Liver cell cancer is probably the cumulative result of numerous factors and the roles of viral and parasitic infections, mycotoxins, chemical carcinogens, and other undiscovered environmental and nutritional factors still present a challenge for further investigations.

Extrahepatic lesions

Polyarteritis may be present in the early stage of hepatitis B infection in association with low complement levels and other features of serum-sickness. Glomerulonephritis following hepatitis B infection has been

described in one patient in whom the antigen was demonstrated in the glomeruli by immunofluorescence. There seems to be an association between some cases of polyarteritis nodosa and hepatitis B infection. It remains to be established, however, whether or not such lesions are related to circulating immune complexes.

DETECTION AND MEASUREMENT OF HEPATITIS B ANTIGEN AND ANTIBODY

The need for simple, easily performed tests for hepatitis *B* antigen and antibody for large-scale screening of blood donors, on the one hand, and the need for highly sensitive and specific research techniques, on the other, has led to the development of many techniques that differ greatly in sensitivity, specificity, simplicity, and cost. Each method has its advantages and disadvantages but it is clear that successful detection of hepatitis *B* antigen and antibody depends as much on the meticulous performance of the chosen test as on its relative sensitivity.

In 1970, the tests for hepatitis *B* antigen and antibody in use at that time were described, with details of the methods found to be suitable for their performance.[1] In the intervening period new tests have been developed and modifications of older tests have been reported.

Table 2 gives information on the sensitivity, feasibility, expense, and time required for completion of each of the tests in common use. Sensitivity can be measured either by the number of correct answers obtained when a panel of antigen-positive and antigen-negative sera are examined by a given technique, or by measuring the antigen or antibody titres and comparing these with the titres measured by other techniques using the same sera. It should be noted that a test that is 1000 times more sensitive than another test will not detect 1000 times as many positive sera when employed for routine screening; it may not even detect twice as many. Unlike sensitivity, specificity is less amenable to objective testing. Each of the techniques listed below has been shown to be sufficiently specific, provided appropriate controls are included in the test. Special problems of specificity are considered in the discussions of individual tests.

Immunodiffusion

Immunodiffusion, the first technique used for detecting antigen or antibody, is still frequently employed because it is simple and specific and provides a useful means of establishing identity. The principal disadvantages are that it is slow to complete and lacks sensitivity. Sensitivity can be

[1] *Bull. Wld Hlth Org.*, 1970, 42, 957-962.

TABLE 2. TECHNIQUES FOR MEASURING HEPATITIS B ANTIGEN AND ANTIBODY

Technique	Relative sensitivity for detecting [a] HB Ag	HB Ab	Ease of performance [b]	Relative cost	Time required for completion (hours)
Immunodiffusion [c]	1–5	1–10	simple	inexpensive	24–72
Counter-immunoelectrophoresis [d]	5–15	5–10	simple	moderate	2
Complement fixation [e]	15–20	5–10	moderate	inexpensive	2–24
Immune adherence [f]	20–2 000	50–150	moderate	inexpensive	2
Latex particle agglutination [g] (antibody-coated)	15–100	–	simple	inexpensive	0.1–0.2
Passive haemagglutination and inhibition [h]	15–20	10 000	moderate	expensive	2
Radioimmunoassay [i]	2 000–10 000	10 000–1 000 000	complex	expensive	24–120
Immune electron microscopy [k]	1 000–2 000	–	complex	expensive	2–4

[a] Approximate reciprocal titre of an antigen or antibody arbitrarily assigned a titre of 1 when tested by the two-dimensional micro-Ouchterlony immunodiffusion technique. Range of sensitivity values obtained by various modifications of this basic technique.

[b] Including preparation of reagents.

[c] By specific various modifications to improve sensitivity – use of templates, low concentration of agarose, radial diffusion, rheophoresis, staining of precipitin lines.

[d] Very useful for large-scale screening of sera but sensitivity is markedly influenced by quality of reagents and skill of technician in detecting faint precipitin lines.

[e] Usefulness of this method is limited by anticomplementary activity in a proportion of sera but it is useful for quantifying antigen and antibody. Prozones occur with high-titre sera and can result in false negative results unless sera are tested at several dilutions.

[f] Large reactions with high-titre antigen-positive sera can result in false negative results if serum is not tested at several dilutions. However, this technique combines sensitivity with speed for detecting antigen.

[g] Not fully automated but simplicity and speed make it potentially useful. Relatively high frequency of nonspecific agglutination makes it necessary to include appropriate controls.

[h] Comparable to complement fixation and counter-immunoelectrophoresis for detecting antigen. One of the most sensitive and useful techniques for detecting and quantifying antibody. High frequency of nonspecific agglutination makes it necessary to include appropriate controls.

[i] Several types of radioimmunoassay method are in use, including double-antibody and solid-phase. One of the most expensive and elaborate of techniques, but the most sensitive for detecting antigen and antibody.

[k] Not suitable for large-scale testing and requires special skills and expensive equipment, but very valuable for identifying morphological types of antigen and antibody specific to them.

improved by refilling of wells, use of templates instead of wells, use of very low concentrations of agarose, augmentation of reagent contact by controlled evaporation of buffer from the surface (rheophoresis), radial immunodiffusion in antibody-impregnated gel, and concentration of the samples under test. Despite these modifications, immunodiffusion remains no more sensitive than the more rapid electrophoretic techniques. After primary exposure to the antigen, hepatitis B antibody is not usually detected by precipitin methods, but secondary exposure often results in the transient development of such antibody.

Counter-immunoelectrophoresis

Counter-immunoelectrophoresis has replaced immunodiffusion as the most widely used technique in large-scale screening for hepatitis B antigen. When performed with carefully prepared reagents, immunoelectrophoresis is a relatively simple, sensitive, and specific technique for screening large numbers of sera. Low-voltage immunoelectrophoresis is preferred to the high-voltage method because of its greater sensitivity and safety. A discontinuous buffer system increases the sensitivity and ease of reading precipitin lines. The technique has been employed for the simultaneous detection of antigen and antibody by interposing the test sample between an antibody-containing well on one side and an antigen-containing well on the other. However, this may lead to crossing over of one of the reagents, resulting in the formation of a precipitin line between the two reagents. Such false positive reactions can be diminished by careful selection of reagents and positioning of the wells. Another source of false positive reactions in immunoelectrophoretic tests is the presence of other precipitating antigen-antibody systems. These include antihuman antibodies, which are found in the serum of up to 0.2% of persons tested, and red cell and lipoprotein isoprecipitins.

The sensitivity of the method is markedly diminished by failure to examine carefully for weak precipitin reactions. Weak precipitin lines may be seen more readily if oblique illumination is used in a dark room and protein stains are employed. Specificity should be confirmed by reactions of identity or by appropriate blocking experiments.

Complement fixation

Complement fixation is more sensitive than immunoelectrophoresis for detecting antigen but approximately equivalent for measuring antibody. Sera containing high titres of hepatitis B antigen may not react at low dilutions because of the prozone phenomenon and sera should therefore be tested at several dilutions. Antibody to hepatitis B antigen varies markedly in its suitability for detecting the antigen. Differences in reactivity of complement-fixing antibodies and antigens may reflect differences in the antigenic composition of the antigens as well as differences in the specificity of the antibody.

Complement-fixing antibody is usually detected only after secondary exposure to the antigen and it is present for a period of days to weeks, whereas antibody detectable by more sensitive methods is frequently present for years following primary or secondary exposure. The pattern of response of complement-fixing antibody roughly parallels that of antibody detected by immunodiffusion and immunoelectrophoresis, but a number of precipitating antibodies that are detectable by immunodiffusion or counter-immunoelectrophoresis do not fix complement. For this reason the former techniques have proved to be more useful for routine screening procedures.

Serial serum specimens from patients with acute hepatitis are sometimes anticomplementary. This is usually related to the stage of the disease at which the specimen was obtained and is thought to be caused by the development of antigen-antibody complexes. Anticomplementary activity has been observed following hepatitis A as well as hepatitis B infection. It may result from a number of other causes, such as repeated freezing and thawing of the specimen or prolonged storage in the liquid state, bacterial contamination, or any procedure that leads to aggregation of globulins. For this reason anticomplementary activity should not be interpreted as being specifically associated with hepatitis.

Immune adherence

The technique of immune adherence is based on the observation that complexes of antigen, antibody, and complement adhere to primate erythrocytes. This phenomenon, like complement fixation, can be used for the measurement of antigen or antibody. Immune-adherence assay for hepatitis B antigen and antibody has been developed and is a sensitive technique for the detection of antigen. It is less sensitive for the detection of antibody, being somewhat more sensitive than complement fixation but less so than passive haemagglutination or radioimmunoassay. Large prozones are observed when the antigen titre is high and, for this reason, it is necessary to test sera over a wide range of dilutions. Great care must be exercised in cleaning equipment and in selecting complement and erythrocytes. It seems that erythrocytes from only a small proportion of individuals are suitable for this test.

Latex agglutination

The technique of latex agglutination has recently been modified for the detection of hepatitis B antigen. Latex particles, coated with hepatitis B antibody prepared in animals, are rapidly agglutinated by serum or plasma containing the antigen. The test requires a minimum of equipment and time, is relatively easy to interpret, and appears to be slightly more sensitive than complement fixation for detecting antigen. A proportion of normal sera yield false positive results with the test and latex particles coated with normal animal globulin must be used as a control for the specificity of the reaction. The cause of false positive reactions is unclear but it may be partly related to the rheumatoid factor and other factors in the sera under test. Despite such false positive reactions the technique appears to be useful for preliminary screening purposes. Sera containing a high titre of antigen can yield false negative results through the formation of a prozone of

nonreaction and it is therefore necessary to test both diluted and undiluted sera. Hepatitis B antibody has been detected by its ability to inhibit latex-agglutination, but this procedure has not been fully evaluated. Although the antibody-coated latex particles are reported to be stable for many weeks at 4°C, different lots of the reagent have been found to vary greatly in stability and sensitivity and further evaluation of this method is needed.

Passive haemagglutination and passive-haemagglutination inhibition

Passive haemagglutination and passive-haemagglutination inhibition have been used extensively for the detection of hepatitis B antibody and antigen respectively. The method is very sensitive for the detection of antibody and has the advantages of rapid completion and easy quantification. It is less sensitive for detecting antigen, comparable only to the simpler complement-fixation technique. It is relatively easy to perform but the preparation of suitable antigen-coated erythrocytes with chromic chloride has proved particularly difficult. Different lots of cells vary considerably in their sensitivity and meticulous care must be exercised in the washing of glassware and the actual performance of the labelling procedure. Non-specific agglutination is frequently observed with low dilutions of sera, particularly when animal sera are tested, and sera must be tested against control erythrocytes to detect such false positive reactions. Nonspecific agglutinins can be removed by absorption of the serum with control erythrocytes before retesting and by heat inactivation. False negative results have been obtained with low dilutions of high-titre antibody because of the formation of prozones and sera should therefore be tested at several dilutions.

Radioimmunoassay

Several radioimmunoassay techniques for detecting hepatitis B antigen or antibody have been described. These include assays in which antigen-antibody complexes are separated from unbound reagents by chromatoelectrophoresis, precipitation with antibody, or attachment to a solid phase. Double-antibody and solid-phase systems have largely replaced other methods because of their usefulness for large-scale screening. Such tests, employing ^{125}I-labelled antigen are the most sensitive of all the techniques currently in use for detecting hepatitis B antibody. A solid-phase system employing ^{125}I-labelled hepatitis B antibody appears to be one of the most useful and sensitive methods for detecting the antigen.

Radioimmunoassay has three disadvantages, slowness, high cost, and the hazards associated with the handling of radioactive isotopes. False positive results are seldom observed if care is taken in selecting and preparing reagents and in carrying out the test. However, sera from persons with humoral antibody to guineapigs, such as those found in animal handlers, yield false positive results in a solid-phase test that employs ^{125}I-labelled guineapig antibody to hepatitis B antigen. False negative reactions for antibody may be observed with sera containing a high titre, and such sera should therefore be diluted.

Immune electron microscopy

Immune electron microscopy has been especially useful for characterizing the different morphological forms of hepatitis B antigen and for investigating antigen-antibody systems. Although sensitive and specific, the method is expensive and is not suitable for large-scale testing. Samples should be tested under code and appropriate positive and negative controls included. Immune complexes of antigen and antibody may occur in the serum of certain patients with hepatitis B infection. These can best be detected by centrifugation of the serum without addition of antiserum and examination of the resuspended pellet by electron microscopy.

Other techniques

Other techniques have been described for the detection of hepatitis B antigen or antibody, including platelet agglutination, which has not gained widespread acceptance, and reversed passive haemagglutination and charcoal particle-agglutination inhibition, which are being evaluated.

Immunofluorescence and thin-section electron microscopy have proved useful for the cellular localization of antigen. The full interpretation of results obtained with these methods must await a better understanding of hepatitis B antigen.

Methods for subtyping

The subspecificities of hepatitis B antigen are identified by the formation of spurs in the immunodiffusion test or by precipitation in counter immunoelectrophoresis with antisera rendered monospecific by absorption with appropriate heterotypic antigens. Preliminary evidence suggests that modifications of more sensitive techniques such as radioimmunoassay and passive haemagglutination will also be useful for this purpose.

Other antigen-antibody systems

The antigen associated with the internal component of the 42-nm particle was discovered by the technique of immune electron microscopy and this method remains the most suitable for its demonstration. Attempts to isolate and characterize the antigen are being carried out in many laboratories and it is anticipated that other techniques for its detection will soon be available.

Antibody to various specificities associated with hepatitis B antigen has been less well studied, partly because very sensitive assays for such antibody are not yet available. However, there is preliminary evidence that antibody to the internal component of the 42-nm particle develops more frequently than antibody to the outer coat following hepatitis B infection and that the former may be a better indicator of previous infection than the latter.

HEPATITIS B AND BLOOD TRANSFUSION SERVICES

Prevalence of hepatitis B antigen in blood donors

Great variations in the prevalence of hepatitis B antigen in apparently healthy blood donors have been found in different parts of the world. Prevalence also varies with such factors as the socioeconomic status and sex of the donor, whether he is a volunteer or paid, and whether he lives privately or in an institution. Antigen has been detected most frequently in males in the younger age-groups. Limited surveys have also shown that the prevalence of hepatitis B antigen is no higher amongst donors with a past history of jaundice than in those without such a history.

The sensitivity of the technique and specificity of the reagents used for screening obviously influence the rate of detection of the antigen. However, it seems that most apparently healthy carriers have a high titre of circulating antigen that is readily detected even by insensitive methods, such as counter-immunoelectrophoresis.

Hepatitis B antigen in blood and blood derivatives

In the past blood derivatives were classified according to the risk of hepatitis to recipients. Whole fresh blood and single donor plasma were regarded as "average-risk" materials, pooled plasma, fibrinogen, and antihaemophilic globulin were considered "high-risk" products, and pooled immunoglobulin, albumin treated by heat at 60° C for 10 hours, and the less purified heated albumin fraction (plasma protein solution) had been shown to be safe by virtue of extensive use. Hepatitis B antigen has now been found in all components of plasma that are derived by the Cohn method of fractionation from plasma known to contain the antigen. Antigen has not been detected in the immunoglobulin fraction prepared from such plasma, although this fraction cannot be examined by sensitive methods. It is important to exclude antigen-positive plasma from the pool to be used for preparing blood derivatives for clinical use.

Recent studies suggest that frozen red cells carry a lower risk of transmitting hepatitis, probably because they are repeatedly washed. Additional studies must be made before their safety can be established.

Use of donors with clinical evidence of prior hepatitis infection

Policy regarding the exclusion from blood donation of individuals with a clinical history of hepatitis varies from country to country. The rationale for such exclusion was based upon evidence that some of them remained infectious long after apparent resolution of their illness. In retrospect, it would seem that most of these carriers were former hepatitis B patients.

Studies of hepatitis B infection among volunteers and those naturally infected with the virus suggest that a greater proportion of individuals who have had a mild or inapparent infection become chronic carriers of the antigen than of those who have had a more severe illness. For this reason exclusion from blood donation of individuals with a clinical history of hepatitis B infection, but who do not have detectable antigen, may not materially reduce the frequency of hepatitis among recipients of blood.

Similarly the exclusion from blood donation of those with serological evidence of previous infection with hepatitis B, indicated by the presence of antibody, may not be justified. Such antibody has been found in 10-40% of adults when sensitive techniques have been used for its detection. Many individuals with hepatitis B antibody have repeatedly given blood without producing hepatitis in the recipients of their blood. Furthermore prospective studies of recipients of antibody-containing blood revealed that such recipients do not have a higher frequency of post-transfusion hepatitis than do recipients of blood free of detectable hepatitis B antigen or its antibody. However, in practice, blood containing antibody detectable by immunodiffusion or immunoelectrophoresis is used, if suitable, either as a reagent or for fractionation and preparation of specific hepatitis B immunoglobulin.

Persons with hepatitis B antigen who are subsequently found to be negative may constitute a group that is epidemiologically different from those in whom antibody is detected without other evidence of previous infection. The risk of transfusing blood from the former group is undetermined and such blood should not be used.

The existence of a chronic carrier state following hepatitis A infection has not been proved and many doubt that it exists. The viraemic phase of acute hepatitis type A infection is brief and for this reason exclusion of donors with a clinical history of hepatitis A infection may not materially diminish the frequency of hepatitis among blood recipients. Serological methods for identifying antibody specific for hepatitis A are not yet available.

Post-transfusion hepatitis

The reported frequency of hepatitis following blood transfusion varies according to the origin and amount of blood transfused and the immune status of the recipients. The increased risk of contracting hepatitis following the transfusion of blood containing hepatitis B antigen has been well

documented. In several studies, over 50% of recipients of antigen-positive blood had evidence of hepatitis and approximately half the cases were icteric. Hepatitis B antigen or hepatitis B antibody was detected in many of the remaining recipients who did not develop hepatitis. In contrast, less than 10% of recipients of blood free from hepatitis B antigen had any evidence of hepatitis.

The present widely employed techniques for detecting hepatitis B antigen in blood are thought to be capable of preventing approximately 30% of cases of post-transfusion hepatitis. The effect the introduction of more sensitive techniques will have on the rate of post-transfusion hepatitis is not yet clear, but preliminary evidence suggests that it will not be great. A further significant reduction in the rate of post-transfusion hepatitis may require the development of biological tests for the hepatitis B virus, as well as a better understanding of the complex etiology of this form of the disease. Cases not due to virus B are thought to be due to a variety of causes, including hepatitis A virus, cytomegalovirus, and other, as yet unidentified agents.

Management of blood donors positive for hepatitis B antigen

A donor whose blood is found positive for antigen on screening should be excluded from further blood donation and the blood concerned must not be transfused. A positive result should preferably be confirmed by a second technique, a reaction of identity should be demonstrated, and further confirmation obtained, wherever possible, by a reference laboratory. The donor should then be advised and the need for further medical supervision considered.[1]

Safety in blood transfusion laboratories

There is some evidence of transfer of hepatitis B antigen to members of staff in blood transfusion service laboratories but there is no clear evidence for the transmission of infection from members of the staff to blood or blood products. Blood and blood products are prepared in closed systems or by using strict aseptic techniques so that, theoretically at least, the products should not be contaminated even if an antigen-positive person has assisted in their preparation. Nevertheless, it is recommended that persons with hepatitis B antigen should be excluded from such work and transferred to work that does not involve an open process. It is therefore advisable to establish routine testing for hepatitis B antigen in members of the staff.

Precautions should also be instituted to minimize the risk of laboratory staff contracting hepatitis in the course of their work. A recommended code of practice has recently been suggested.[1] A modified version is outlined in Annex 2. These precautions have been found to be both practical and acceptable to blood transfusion laboratories.

[1] United Kingdom, Advisory Group on testing for the presence of Australia (hepatitis-associated) antigen and its antibody (1972) unpublished report (revised).

OTHER SPECIAL RISK GROUPS

The combination of circumstances that places certain groups of the population at a special risk of acquiring viral hepatitis differs for hepatitis types A and B. Exposure to anicteric or icteric cases, particularly in children, in the family, school, residential institution, or hospital, is an important factor in the spread of hepatitis type A. In addition there is an occupational risk of hepatitis, not apparently type B, to handlers of chimpanzees and other nonhuman primates. Hepatitis type B infection is also a risk to persons exposed to transfusion of blood or plasma, injection of blood products, frequent tissue penetration or a need for repeated access to the venous or arterial circulations, and the establishment of extra-corporeal circulation.

Maintenance haemodialysis

Few units that practise haemodialysis or transplantation on any scale have escaped outbreaks of viral hepatitis.

In Europe there has been a steady increase in the number of centres in operation since 1966 and 23–43% have endemic hepatitis at any one time. The proportion of patients suffering from clinical hepatitis has risen slowly —from 4.7% in 1966 to 9.2% in 1971. In 1971, 11.5% of patients had hepatitis B antigen. During this period the absolute number of staff cases has also increased, from 26 in 1966 to 402 in 1971, presumably in parallel with the increasing number of dialysis units. The hepatitis case mortality among patients varies from 6% to 28% and, with the exception of 1966, mortality appears to have been low among staff. Exceptional staff mortality (33%) was observed in the Edinburgh outbreak of 1969–1970.

In the USA a survey revealed that 52 (80%) of the units had cases of hepatitis in patients during the 5-year period 1966–1970. In a similar study during 1967–1968, some 10% of 1008 patients and 3% of 1070 staff had hepatitis. The rates among staff were highest in dialysis nurses (4.1%), followed by dialysis technicians (3.4%), and lowest in renal physicians (1.2%). The prevalence of infection in 49 home dialysis programmes in the USA was lower; the rate for patients was about 4% for an 11-month period and the proportion of staff, including relatives, clinically infected

[1] United Kingdom, Advisory Group 1970-1972 on hepatitis and the treatment of chronic renal failure (1972) unpublished report.

was about 0.4%. In Europe hepatitis was slightly more common (7.7%) in patients on home dialysis. The predominant agent in these outbreaks is hepatitis type B but antigen-negative hepatitis has also been reported.

Most dialysis-associated outbreaks of hepatitis investigated since the discovery of hepatitis B antigen have revealed antigen in the majority of patients and in a substantial proportion of the staff. In some outbreaks, interpretation is complicated by the finding that antigen is carried for long periods by renal patients, and is therefore easily demonstrated by the somewhat insensitive tests available to most workers, whereas staff infected from the patients may carry antigen for only a short period or they may even be negative for antigen but subsequently develop antibody.

Entry of the hepatitis virus into dialysis or transplant units

In theory, hepatitis might be introduced into a unit by (1) the administration to patients of infected blood, plasma, or blood products; (2) the admission to the dialysis or transplantation programme of a patient carrying the agent either transiently or chronically; (3) the infection of patients already on the programme while outside the unit. Such infections might be the result of parenteral exposure elsewhere in the hospital, at home, or at work, or they might be due to nonparenteral modes of infection, still largely unidentified; (4) transplantation of a kidney from a donor who is carrying the agent or who has, in the instance of cadaver kidneys, received infected blood or blood products during the terminal illness; (5) parenteral or nonparenteral spread to patients from the unit staff, who might be either chronic or short-term carriers of the infectious agent.

The numerous accounts of outbreaks of dialysis-associated hepatitis are not very helpful in determining which of these possibilities are important in practice. It is often stated that the use, in renal centres, of blood to correct anaemia or, formerly, to prime dialysis machines, or the use of plasma to treat ascites or protein deficiency is of major importance in introducing hepatitis B agent. However, authenticated episodes in which a known infected unit of blood has initiated an outbreak are rare, perhaps because the first episode of infection in the patient is trivial and the epidemiological trail is soon overlayed by the spread of infection from patient to patient. Nevertheless, it is obviously prudent to restrict the use of blood and blood products in dialysis and transplant units and to use only those that have been screened for hepatitis B antigen.

Infection may be introduced as a consequence of the practice of giving temporary accommodation to patients from other dialysis units, where infection may be present or unrecognized. Two-way transfer of patients between dialysis and transplantation units also allows the spread of infection from one to the other.

Infection of a patient from a grafted kidney is not well documented, as the concurrent dialysis and transfusion procedures complicate observations, but hepatitis in both recipients of kidneys from the same cadaver has been reported.

Spread of infection from staff or other contacts to haemodialysis patients would clearly be difficult to detect without special epidemiological surveillance. At present there is little evidence that medical or nursing staff in haemodialysis centres have infected their patients. A few episodes have been described in which medical or nursing staff in the late incubation period of acute hepatitis B infection have infected patients on the general wards of hospitals.

The importance of nonparenteral spread of hepatitis B in introducing or maintaining infection in dialysis and transplantation units is unclear at present and needs further investigation.

A sequence of cases of hepatitis in dialysis patients usually suggests that the infectious agent is spreading from one patient to another via the dialysis equipment. The possibility of multiple, separate introductions of hepatitis via blood transfusion or by other means should also be considered. Subtyping of hepatitis B antigens may be of value in differentiating a homogeneous outbreak, due to the spread of one strain among patients, from multiple, separate introductions of different strains that might simulate a small outbreak. Both *ad* and *dy* subtypes have been found in dialysis-associated outbreaks. In most of these a single subtype has been identified.

The subtle failures of aseptic or sterile technique that result in patient-to-patient spread of infection may vary from centre to centre, but particular attention is drawn to the venous pressure monitor and its associated line as an element common to most equipment that is often overlooked. Contamination by successive patients of this gauge and the associated non-disposable connecting segment and port of entry represents a method of cross-infection between patients analogous to that occurring between individuals in vaccination campaigns in which the needle, but not the syringe, is changed between inoculations.

Spread of infection from patients to staff

The clearest incidents leading to infection of staff are those in which e skin or mucous membrane of the staff member is penetrated with a nypodermic needle, a Pasteur pipette, or some other sharp point contaminated with the blood or tissue fluids from a patient with clinical or subclinical hepatitis or chronic asymptomatic carriage of the antigen. Thus surveys revealed that 59% of dialysis staff who developed hepatitis had had such a tissue penetration within the preceding 6 months. Other observed modes of infection include spilling blood on to skin already breached by cuts or

scratches, or abnormally permeable because of eczema, formalin dermatitis, or other lesions. Extensive contamination of unbroken skin and in particular mucous membrane with blood from an infected patient may also initiate infection.

In the laboratory, a technician's skin may be contaminated with blood or serum from leaking specimen containers, contaminated request forms, while specimens are being pipetted into centrifuge tubes or autoanalyser cups, or during the preparation of blood films or the filling of haematocrit tubes—to name only a few procedures. Inadvertant aspiration of infected blood into the mouth during pipetting may result in infection. By analogy it is presumed that plasma, lymph, ascitic, pleural, or synovial fluid, and cell suspensions from patients with hepatitis B antigen might cause infection if aspirated into the mouth.

The handling and testing of specimens of faeces and urine from patients with hepatitis A is certainly hazardous. The risk from handling faeces and urine specimens from patients infected with hepatitis B is less certain. Until more is known about the distribution and frequency of the agent in excreta it seems wise to regard them as potentially infectious.

The role of aerosols of infected blood or blood fractions in the infection of staff is also in need of definition. Infection by inhalation of dried plasma has been recorded. Airborne infection, in the sense familiar from knowledge of respiratory virus diseases, is probably rare. This is suggested by the differential incidence of hepatitis in unit staff; the rates are lowest in secretaries, ward maids, porters, and other persons working in the same general area and breathing the same air as the dialysis technicians, nurses, and physicians but not in close physical contact with patients, the equipment, or specimens from the patients. It may also be noted that clinical hepatitis in laboratory workers is of a sporadic rather than of an explosive nature. In contrast, spectacular laboratory outbreaks of infection have occurred with viruses and rickettsiae that are highly infectious by the respiratory route.

The number of cases in staff may be underestimated because of a failure to appreciate that the risk is not limited to those in the dialysis or transplant units. The admission of infected renal patients to the general wards of the same or other hospitals for treatment of intercurrent illness places general medical and nursing staff at risk. The performance of autopsies on undiagnosed or unnotified cases of hepatitis B places pathologists and autopsy room attendants at risk. Careless handling of specimens may constitute a hazard in laboratories that have to perform routine liver-function tests and to monitor blood of dialysis and transplant patients. It is less well appreciated that research laboratories and others apparently unconnected with renal medicine, may become involved in the network of infection when these receive leucocytes for typing, serum or lymphocytes for immunological investigations, macrophages from peritoneal dialysis for research, or the excised kidney from a transplant patient for histological or other studies.

Infection in home contacts of dialysis and transplant patients

The home contacts of infected dialysis patients are also at risk. Reports of outbreaks of dialysis-associated hepatitis mention some cases in home contacts but the total population at risk is rarely given. Modes of infection are not always clear but contacts such as ambulance attendants or other persons have sometimes been infected as a result of contamination with blood from an arteriovenous shunt. Infection appears to be more common in the spouses of haemodialysis patients and infected staff than in children and other adults in the same household. In general, however, the practice of home dialysis does not appear to have resulted in substantially increased infection in home contacts.

Hospital staff

Medical and ancillary hospital staff in general have a prevalence of hepatitis some 3–6 times that of workers in other occupations. The higher prevalence of both forms of hepatitis and of hepatitis B antigen in staff directly or indirectly associated with dialysis and transplant units has been discussed above. Staff caring for children in hospitals and institutions also have a higher prevalence.

The relative risks of infection to surgeons, dentists, physicians, nurses, and other hospital staff who deal with acute hepatitis due to type A or B or with conditions sometimes associated with hepatitis B antigen, such as chronic hepatitis, cirrhosis, liver cell carcinoma, immunosuppression, or the unrecognized carrier, is less well defined.

Chronic carriers in staff members

It should not be assumed that a staff member who is a chronic antigen carrier is necessarily a hazard. Careful studies of the professional and other contacts of known carriers to detect the transmission of apparent or inapparent infection are needed to resolve the matter. In the meantime, because of the special risk of spread of infection in dialysis and transplant units, it is prudent to exclude such carriers from these areas.

Control of hepatitis, with particular reference to dialysis and transplant units

Many of the precautions to be taken are based on common-sense grounds of general hygiene and from general experience of hospital cross-infection.

There are also some specific measures dictated by special situations, such as those to be taken in the face of an outbreak, which are outlined in two recent reports [1,2] and in Annex 1.

CURRENT RESEARCH

Hepatitis B in nonhuman primates

The many attempts to transmit hepatitis B virus to nonhuman primates have yielded, until recently, equivocal or negative results. The detection of hepatitis B antigen and antibody in the serum of a small proportion of chimpanzees, orangutans, and gibbons renewed interest in finding a suitable laboratory model. Recent studies, employing sensitive assay systems for hepatitis B antigen and antibody, have established the susceptibility of the chimpanzee to infection with the human hepatitis B virus and, furthermore, have provided evidence for the susceptibility of other nonhuman primate species.

Antigen and antibody studies in captive animals

Hepatitis B antigen was detected in 6–12% of captive chimpanzees when they were tested by relatively insensitive techniques. Most animals appear to be symptomless carriers of the antigen and, with rare exceptions, naturally-acquired infection is not associated with clinical hepatitis in the host. Hepatitis occurring among human beings exposed to chimpanzees and other nonhuman primates is rarely, if ever, type B hepatitis. The frequency of hepatitis B antigen in wild chimpanzees is not known. Hepatitis B antigen has been detected in approximately 6% of orangutans and 13% of gibbons, but neither of these species has been studied as extensively as has the chimpanzee. Carriage of the antigen in the orangutan and gibbon seems to be chronic and not associated with detectable hepatitis.

Hepatitis B antibody has been detected by radioimmunoassay in the captive chimpanzee, orangutan, gibbon, baboon, Celebes ape, patas monkey, vervet, several species of macaque, mangabey, and langur, and in a number of New World monkey species. Antibody was found in approximately 50% of chimpanzees examined but in less than 10% of most Old World and New World monkeys. The specificity of hepatitis B antibody detected in nonhuman primates has been demonstrated by appropriate blocking experiments with hepatitis B antigen. However, the possibility that such antibody is formed in response to a different but related antigen has not been excluded.

Exposure of nonhuman primates to hepatitis B antigen appears to be widespread and antibody has been detected in animals from many widely separated colonies. Natural infection of the chimpanzee has been documented in animals held captive for many years. Preliminary evidence suggests that natural infection of the chimpanzee is associated primarily, if not exclusively, with the *ad* subdeterminant of hepatitis B antigen. Similar studies of the antigen obtained from other nonhuman primate species have not been reported. It is not known whether strains of hepatitis B virus indigenous to these nonhuman primates are different from human strains. However, the antigen and antibody derived from chimpanzees do show reactions of identity with hepatitis B antigen and antibody of human origin.

Transmission studies

Two factors seem to have been responsible for the apparent failure of previous attempts to transmit hepatitis B to nonhuman primates. The first is the high frequency of naturally acquired antibody among the apes and the second is the relatively mild nature of the infection in nonhuman primates.

The infectivity of human hepatitis B virus for the chimpanzee has recently been established but, because of limited data, it is premature to draw firm conclusions. Infection of the chimpanzee appears to resemble infection in man in the general pattern of response, including the incubation period and histological changes in the liver.

Hepatitis B virus associated with both the *ad* and *ay* subspecificities of the antigen has been successfully transmitted to the chimpanzee and produced evidence of hepatitis. The antigen was detected by immunofluorescence in the cytoplasm of liver cells from infected chimpanzees. Virus-like particles and antigen thought to be associated with the internal component of the 42-nm particle have also been detected by electron microscopy and immunofluorescence respectively.

Acute-phase plasma taken from a chimpanzee infected with agent of human origin was infectious for other chimpanzees. Similarly serum containing hepatitis B antigen from a chimpanzee presumably infected naturally with the agent produced hepatitis in recipient chimpanzees. All these animals developed slight elevations of aminotransferase activity after inoculation of very large doses of plasma.

Transmission of hepatitis B virus to two infant vervet monkeys by inoculation of partially purified antigen has been reported. The antigen was present in the serum 24 hours after inoculation in quantities thought to exceed the amount of antigen injected and it was transmitted to an additional vervet monkey, but attempts to confirm these studies have not been successful.

[1] United Kingdom, Advisory Group 1970–1972 on hepatitis and the treatment of chronic renal failure (1972) unpublished report.

[2] United Kingdom, Advisory Group on testing for the presence of Australia (hepatitis-associated) antigen and its antibody (1972) unpublished report (revised).

Evidence for transmission of hepatitis B virus to the rhesus monkey is more convincing, since the agent has been passaged serially 6 times in this species. The antigen, or antibody, or both were detected at each passage level. Antigen was present in very small quantities, which were detectable only by radioimmunoassay. Antibody was detected by radioimmunoassay and passive haemagglutination. Hepatitis temporally related to infection was not detected in any of the animals but the period of incubation, measured by the production of antigen, was similar to that observed in man and chimpanzees.

Preliminary evidence suggests that the rhesus monkey is less susceptible than the chimpanzee to infection with the human hepatitis B virus. At present only the chimpanzee appears to approach man in susceptibility to infection with this virus and it is the only species known to develop hepatitis following exposure.

It is expected that progress in the use of animal models for the study of hepatitis B will be impeded by a shortage of suitable animals, particularly the apes. The world populations of orangutans and gibbons are already alarmingly small and these animals must be protected and used with discretion for hepatitis studies. Chimpanzees, although not as rare as the other apes, are being subjected to the combined threats of overhunting and encroachment of civilization. The same adverse pressures are being felt to varying degrees by all species of nonhuman primates. For these reasons, hepatitis studies utilizing nonhuman primates should not be undertaken without full cognizance of the problems involved in acquiring and maintaining seronegative animals and in documenting the often very mild and evanescent infection.

Tissue culture studies of hepatitis type B virus

The problem of growing hepatitis viruses in a readily available tissue culture system remains the major obstacle to further progress. The many attempts at isolation in a variety of cell and organ cultures of different origin have resulted in a large collection of "hepatitis-candidate" viruses, none of which have since been shown to be the causal agents of human hepatitis.

Tissue culture of human liver

Methods have been developed in recent years for obtaining primary cultures of differentiated hepatocytes from human embryo and adult livers. Cytoplasmic and nuclear fluorescence were detected in inoculated cultures of human embryo liver cells after staining with human serum containing hepatitis B antibody conjugated with fluorescein. Only cytoplasmic fluorescence was noted when an antibody prepared in guinea-pigs against purified hepatitis B antigen was employed. Similar fluorescent changes were observed in the cells of liver cultures inoculated with supernatant fluid that had been passaged in culture twice. The specificity of fluorescence was demonstrated by appropriate blocking experiments. Fluorescent changes were not observed in appropriate control cultures nor in a number of other cells examined. Cytopathic changes were not observed. Similar fluorescence was subsequently observed in liver cells cultured from biopsy material obtained from two patients with circulating hepatitis B antigen. In addition the antigen was detected by radioimmunoassay in the supernatant fluid of a few cultures.

Organ culture studies

The morphological and functional capacity of cells maintained in organ culture may offer a system for cultivating viruses that closely simulates conditions in the intact host. Examples of the specificity of effect on the target organ and differential susceptibility of different hosts are found within each class of virus, and organ cultures have reproduced many, but not yet all, of the phenomena of specificity observed in the intact animal. Furthermore organ culture techniques have proved valuable for the cultivation of viruses that are difficult to grow in conventional monolayer cell cultures. Several attempts to propagate the hepatitis B agent in organ cultures have now been reported.

Cultured fragments of human inguinal lymph nodes obtained from children at the time of herniorrhaphy were incubated with serum containing hepatitis B antigen. The antigen was detected by immunodiffusion and complement fixation in pooled 4- and 6-day fluids harvested from one of the groups of lymph node organ cultures. The 3 types of particle generally associated with the antigen were found by electron microscopy in the original serum and also in the pooled organ culture fluid. However, whereas the large double-shelled 36–44-nm particles were sparse in the original serum, the 3 types of particle—small spherical, tubular, and large spheroidal structures—were present in almost equal numbers in the harvested organ culture fluid. Ultra-thin sections of these explants revealed both intracellular and extracellular clusters of antigen-like particles.

More recently it has been reported that hepatitis B antigen can be produced in human embryonic liver organ cultures. A progressive rise in the titre of hepatitis B antigen, measured by several techniques and confirmed by immune electron microscopy, has been demonstrated with a limited number of sera. One successful passage of material harvested from cultures on day 8 has been accomplished with two specimens. Adaptation of the agent passaged in organ culture to growth in conventional cell cultures is most important and urgent, because of the difficulty of obtaining suitable fresh human fetal liver.

The susceptibility of rhesus monkeys to human type B hepatitis prompted studies on the feasibility of using liver cultures from newly killed nonhuman primates; findings similar to those obtained with human embryo liver organ cultures have been reported. Liver cultures from rhesus monkeys, however, appeared to be less efficient than preparations derived from human embryo livers. The full significance of these results is not yet known.

Intrinsic interference in WI-38 cells

WI-38 human diploid cells inoculated with sera containing hepatitis B antigen were reported to be resistant to infection by Newcastle disease virus 5–12 days later, as demonstrated by the haemadsorption-negative plaque test for intrinsic interference. No cytopathic changes were observed. The interference phenomenon was lost 3–5 days after its first appearance and normal haemagglutinin formation occurred in cells that had been inoculated with hepatitis type B sera and subsequently challenged with Newcastle disease virus. Similar results have been obtained with cultures inoculated with serum from patients with hepatitis type A. However, attempts to confirm these studies have been unsuccessful.

These recent reports on the attempted cultivation of hepatitis B agent using tissue and organ cultures derived from human and nonhuman primates are encouraging. However, further studies are required to determine whether replication of the agent associated with hepatitis B antigen can be readily established as a practical tool for investigating the biological characteristics of the associated infectious agent or whether the reported production of the antigen in culture is the result of abortive infection.

PROSPECTS OF IMMUNIZATION AND IMMUNOTHERAPY

Attempts at prevention of infection with hepatitis type B virus have followed recognized methods for the control of infectious diseases. As long as it was accepted that the infection was transmitted only from person to person by a parenteral route and that the only source of the agent was contaminated blood or blood fractions, it seemed reasonable to anticipate that identification and exclusion of carriers or physical or chemical inactivation of the virus would be effective and perhaps sufficient control measures. These approaches assumed even greater significance because of the seeming impossible task of isolating and growing the virus for vaccine production. However, attempts at prevention or prophylaxis through the use of pooled immunoglobulin were begun towards the end of the Second World War, immediately following the demonstration of the effectiveness of immunoglobulin in the suppression of type A hepatitis infection. Studies have continued intermittently for almost 30 years without clear resolution.

Passive immunization

Since the original publication of findings suggesting that pooled human immunoglobulin given soon after whole blood transfusion could significantly reduce the incidence of post-transfusion hepatitis, several major studies have been carried out. These studies varied in the dosage, timing and amount of immunoglobulin and in the levels of risk to the recipients. Their lack of uniformity may account for some of the discrepancies in the results and in their interpretation. Although few would now recommend the use of pooled immunoglobulin as a routine accompaniment to blood transfusion, opinion is not unanimous on this point. The addition of immunoglobulin to blood to be transfused has not been fully evaluated and interest in this type of approach has diminished following the development of methods for more effective screening of donors.

Most preparations of immunoglobulin appear to contain little or no hepatitis B antibody but immunoglobulin with a high titre of such antibody has been prepared from the plasma of selected donors. Passive immunization with specific hepatitis B immunoglobulin appeared to offer protection in some subjects but others developed evidence of hepatitis that could not be distinguished from infection in the controls. The frequency of the chronic carrier state of hepatitis B antigen was similar in the two groups. The results of limited clinical tests of other lots of hepatitis B immunoglobulin provided additional evidence for partial protection when administered within the first week of transfusion and possibly complete protection after accidental contamination with blood containing the antigen. However, there is insufficient information at present from these limited studies to provide a basis for recommending the use of either normal pooled or specific hepatitis B immunoglobulin.

Active immunization

For many years the prospects of either an attenuated or inactivated vaccine against hepatitis type B were assumed to be entirely dependent upon the isolation and laboratory adaptation of the infective agent. One approach to the problem resulted from the observation that heating the well documented MS-2 serum, which contains hepatitis B antigen, to 98°C for 1 minute destroyed infectivity but not antigenicity. This finding provided a hitherto untried approach to active immunization. Subsequent observations indicated that two or three inoculations of the heated serum conferred partial protection against challenge with infective MS-2 serum.

In other studies serum containing the antigen was heated at 60°C for 10 hours but such conditions failed to inactivate the agent completely, as was shown by the acquisition of hepatitis B antigen or the development of hepatitis in a proportion of the recipients. These results indicate that the

heating conditions were insufficient for adequate inactivation of the infectious agent. The limitations of such studies prevent any generalizations at this stage concerning the potential usefulness of this method of active immunization. However, the results of these and the hepatitis B immunoglobulin studies do offer some reassurance that modification of hepatitis B infection by either of these immunization procedures does not render the recipient more susceptible to severe hepatic or other disease or to persistence of the antigen. Additional studies are needed.

Therapeutic measures

On the basis of very limited observations it has been suggested that the use of human plasma or immunoglobulin containing hepatitis B antibody is worthy of controlled trials in treating hepatic coma associated with hepatitis B infection. However, the possibility of immune-complex disease arising from such treatment has to be seriously considered.

RECOMMENDATIONS

General recommendations

(1) Recent advances in the understanding of viral hepatitis B are such that it now justifies greater international attention and should find an important place in WHO's programme on virus diseases. The question of reagents should be given priority.

(2) WHO should facilitate and support the training of scientific and technical personnel, for example by organizing short courses. The establishment of an international reference centre and several regional reference centres would be desirable, particular provision being made for the identification of subtypes of hepatitis B antigen.

(3) Noting that, in its work on other groups of viruses, WHO has been particularly successful in developing collaborative studies of field and laboratory problems, both through the network of reference centres and by enlisting the cooperation of other laboratories and of epidemiologists, the Group recommends that similar studies be developed in the field of hepatitis ---particular attention being given to investigations in countries with warm climates.

(4) Hepatitis B should be included in the programme for the collection and dissemination of information on virus diseases diagnosed in laboratories. There is an urgent need to extend the free exchange of information and

close collaboration between the national and international bodies engaged in active research on hepatitis and its possible sequelae. WHO could play a leading part in developing such cooperation.

(5) The views expressed on terminology (see pp. 8-10) should be taken into account in the ninth revision of the International Classification of Diseases in so far as they are compatible with the envisaged overall arrangement of the classification and the provisions of other situations of a similar nature.

(6) It has been firmly established that blood containing hepatitis B antigen should not be used for transfusion. The method used for detection of hepatitis B antigen in blood donors should be simple, rapid, sensitive, and specific. At the present time a method having the sensitivity of counter-immunoelectrophoresis is recommended as a desired minimum for use throughout the world.

(7) At present there is no evidence that carriers of hepatitis B antigen belonging to medical or other professions coming into close contact with the general population present a hazard, nevertheless, such individuals should use precautions in their professional activities and studies of the professional and other contacts of these carriers should be made to detect whether transmission of infection occurs.

(8) The value of specific human hepatitis B immunoglobulin in passive protection should be determined, at least in circumstances of clear accidental exposure to infectious material.

Recommendations for future research

The discovery and application of serological techniques related to hepatitis B antigen have stimulated marked interest and intensive research efforts over the past few years. Although these have been highly productive, the findings have raised many new questions calling for a multidisciplinary approach crossing national boundaries. Collaboration, cooperation, and an unusually open sharing of information, opportunities, facilities, and materials have become commonly accepted features of what is now a worldwide research effort to answer the many remaining fundamental and practical questions. The Group made the following recommendations on the areas to which further research might profitably be directed:

(1) In spite of the many serological methods and reagents currently available for detecting hepatitis B antigen and antibodies there is still need for improvement, particularly in the development of improved screening tests and of methods that are of greater sensitivity and simplicity but that do not sacrifice specificity or economy. There is also a need for increased availability of improved standardized reference reagents of both antigen and antibody, including subtypes, so as to provide more reliable comparisons of results.

(2) The discovery of different subspecificities of hepatitis B antigen and preliminary impressions of their potential significance call for more extensive studies of their geographical distribution and possible associations with varying clinical expressions of infection.

(3) Well designed epidemiological studies are required to define more clearly the ecology of hepatitis type B in populations living in different areas under varied conditions. The relative importance of various potential modes of transmission, including haemophagous arthropods, requires further investigation. In those areas where it is feasible, studies of the prevalence of antigen and antibody among feral nonhuman primates may provide important information about possible animal reservoirs of infection. Such information may suggest more appropriate experimental laboratory models, hopefully for type A hepatitis as well as type B. These would open new approaches to studies of pathogenesis and provide a system for the assay of infectivity that is necessary for the development and testing of therapeutic and immunizing materials.

(4) There continues to be a serious need for finding and developing suitable cell and organ culture techniques for the isolation and propagation of both hepatitis A and hepatitis B virus.

Annex 1

OUTLINE OF PROCEDURES FOR CONTROL OF HEPATITIS IN DIALYSIS AND TRANSPLANTATION UNITS*

(1) Control of infection is most likely to be achieved by comprehensive measures based on well recognized principles.

(2) Blood transfusion should be minimized for patients with chronic renal failure and only blood negative for hepatitis B antigen should be used. Similar precautions should be taken for patients with progressive renal failure who may ultimately require dialysis. Pooled plasma carries a greater risk of infection than individual, tested units of blood. Frozen packed red cells may carry less risk of infection than whole blood but may be less readily available.

(3) Patients and staff in maintenance dialysis and renal transplantation units should be screened at regular intervals for the presence of hepatitis B antigen and abnormal levels of aminotransferase.

(4) Patients with chronic renal failure should be screened prior to admission to maintenance dialysis units. Those showing evidence of antigen or other signs of infection should not be admitted to the main unit. Whether or not they should be accepted for treatment in an isolation unit is a clinical decision to be taken by the director.

(5) Movement between units should be regulated to prevent inadvertent transfer of infection from one unit to another.

(6) Early discharge to home dialysis, where feasible, will minimize the risk of hepatitis to other patients.

(7) Wherever possible, patients in hospital should undertake their own dialysis, partly as a measure to protect staff against accidental skin penetration and other accidents while taking patients on and off the dialysers.

(8) In potentially infectious patients, transplantation may diminish the risk of hepatitis B for staff and other patients by reducing the need for frequent access to the circulation. There is a risk to the surgical team during the operation and the subsequent immunosuppression may prolong anti-enaemia.

(9) Isolation facilities must be available in maintenance dialysis and renal transplantation units. These facilities should be functionally separate

* Reproduced, with minor modifications, from: United Kingdom, Advisory Group 1970–1972 on hepatitis and the treatment of chronic renal failure (1972) unpublished report, by permission of the Controller, H. M. Stationery Office

yielding 10 000 parts per million (10 cm³/l) of available chlorine. The disinfectant for objects not known to be soiled with blood and other materials from patients is " weak hypochlorite ", which yields 1 000 p.p.m. (10 cm³/l) chlorine. These disinfectant solutions are made up freshly each day in carefully cleansed containers. Since hypochlorite corrodes metal, 2% glutaraldehyde is used for the disinfection of centrifuges and other equipment with metal components. The most reliable means of disinfection is by heat and contaminated equipment should therefore, where practicable, be autoclaved; if it is to be reused, it should be soaked in disinfectant before autoclaving to prevent " baking on " of blood, etc.

5. Mishaps

Cuts and pricks should at once be washed with soap and water. If the eyes are contaminated by splashing they should immediately be rinsed, while open, with tap water or physiological saline. If the mouth is contaminated, it should at once, before swallowing, be rinsed out with water. If the skin is soiled with blood, it should be rinsed with strong hypochlorite and then washed with soap and water. Spillages of blood or other material from patients should at once be swabbed with strong hypochlorite.

6. Reporting of mishaps

Significant mishaps, e.g., cuts and pricks with instruments possibly contaminated with blood, and soiling of broken skin, splashing of the eyes, or contamination of the mouth with blood, must be reported to the Safety Officer, who will inform the Head of the laboratory. Spillage of high-risk specimens such as hepatitis B antigen-positive blood, even if not associated with personal contamination, must also be reported.

7. Personal hygiene

Smoking, eating, and drinking are prohibited in the laboratories and passages. Labels must not be licked. Care should be taken not to put the fingers or other objects into the mouth. The mouth should never be used for pipetting. Hands should be washed after any procedure in which they may possibly have become contaminated with traces of blood or other material from patients. This should be done in the wash hand basin, not in laboratory sink. The hands should not be wiped on the coat or gown.

8. Protective clothing

All staff must wear a gown with a closed front or a coat with an overlapping front when in any working area and a plastic apron and disposable gloves when opening or processing specimens. Barrier cream should be applied to the hands before putting on gloves, which should not be worn for more than 2 hours at a time. Gown, apron, and gloves must be removed and the hands washed, before leaving the laboratory for any purpose or going to the staff room. Disposable gloves must be worn only once and then be placed in a disposable bag for incineration. The apron must be placed on the staff member's apron peg and the gown or coat on his gown peg. At the end of each day, the apron must be immersed for a few minutes in a pail of weak hypochlorite, then rinsed in warm water and hung up to dry before reuse. The gown or coat should be placed in the laundry bag at the end of each week or more frequently if necessary. If the gown or coat is accidentally soiled with blood or other material from patients, it should at once be wiped liberally with strong hypochlorite and within a few minutes be rinsed with water. A visor or safety spectacles must be worn when there is a danger of splashing of a specimen.

9. Care of work places

Each bench worker should ensure that a wash-bottle and a disposal jar containing strong hypochlorite, a supply of swabs, and a plastic disposal bag are provided at his work place. The hypochlorite should be renewed each day and should be tested several times a day with a starch-iodide paper to confirm by a dark blue reaction that it is still active. Any spillage of specimens must be swabbed at once with strong hypochlorite and the bench surface must be wiped with hypochlorite at the end of each day's work. Since accidents and errors are most likely to happen when the work place is crowded with equipment and materials, care should be taken to keep the work place tidy. Tubes and other containers should be placed only in the appropriate rack or tray, never directly on the bench. Equipment must be kept clean.

10. Receipt of specimens

Incoming specimens should be scrutinized to confirm that they have been properly closed and packed. Those from patients having, or suspected of having, hepatitis or hepatitis B antigen should bear " high risk " labels and be enclosed in plastic bags; the request form should not be enclosed in the same compartment of the bag as the specimen. Soiled and leaking containers should be shown to the Safety Officer, who may decide that they should be discarded without being removed from their bags. Soiled request forms must be incinerated. The receiving technician, wearing disposable gloves, should remove the specimen from the plastic bag and place the bag in a container for incineration. He should open the specimen container slowly to avoid producing droplet aerosol.

To deal with high-risk specimens, the following points are suggested for inclusion in safety codes for haematology and blood transfusion laboratories:

Cross-matching

Disposable gloves should be worn. Since the outsides of tubes readily become contaminated with dilute serum during the centrifugation of cell suspensions for the anti-human-globulin test, the tubes should be placed in metal racks that are afterwards autoclaved or in plastic racks that are afterwards placed in weak hypochlorite. Soiled areas of the centrifuge should be wiped with 2% glutaraldehyde. Standardized dropping pipettes should be used to distribute reagents for blood grouping and cross-matching, but a separate pipette should be used to distribute the serum and cells from each patient and this pipette should not be rinsed for reuse but should at once be discarded, together with its rubber teat, into strong hypochlorite or into a pail for autoclaving. When sedimented cells have been pipetted on to slides for microscopic examination, the pipettes should be rinsed in jars that are later autoclaved with their contents. The slides should be discarded into strong hypochlorite. Tiles and plates used for grouping and anti-human-globulin tests should be placed in strong hypochlorite overnight.

Haematological procedures

Disposable gloves should be worn. Containers of specimens should be checked for tightness of closure before placing them on the mechanical mixer or centrifuge. Pipetting of specimens and filling of ESR tubes must be done with a rubber teat, never by mouth. Swabs used to wipe the pipette should be thick enough to prevent contamination of the gloved fingers and soiled swabs should be placed in a container for autoclaving or incineration. The capillary tube used to place a drop of blood on a slide and the spreader used to make a film should be discarded into hypochlorite.

The film should be spread in such a way that it does not reach the edges of the slide, where it might contaminate the gloved fingers when the slide is handled.

Tissue typing

The supernatant fluid from centrifuged lymphocyte suspensions should be discarded into a container with strong hypochlorite. Great care should be taken to avoid pricking the fingers with the microsyringe needle and a thick rubber thimble should be worn on the index finger for protection during distribution and needle wiping. Microsyringes with detachable needles that can be autoclaved should be used. The metal plunger should be removed gently from the glass barrel of the syringe and the two parts and the needle should be put in a container for autoclaving. Test plates and trays should be autoclaved before disposal.

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