Australia antigen and hepatitis: A review

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The description of Australia antigen by Blumberg, Alter, and Visnich in 1965, and the subsequent discovery of its association with hepatitis (Blumberg, Sutnick, and London, 1968) have stimulated renewed interest in the disease. The antigen occurs in the serum during the acute phase of illness and seems to be closely associated with infectivity. In this review our knowledge of its properties will be discussed first, then the various techniques for detecting it will be assessed, and lastly an attempt will be made to define the significance of the antigen in the pathogenesis and natural history of hepatitis.

Nomenclature

'Australia (Au) antigen', 'serum hepatitis (SH) antigen' and 'hepatitis associated antigen (HAA)' are all synonyms, and all are in common use. The non-committal term' Australia antigen' seems preferable not only because it alludes to the original description, but also because SH antigen misleadingly implies restriction of the antigen to cases of classical serum hepatitis, and 'hepatitis associated antigen' already needs further specifying following the description of the 'epidemic hepatitis associated antigen' (del Prete, Constantino, Doglia, Grazina, Ajdukiewicz, Dudley, Fox, and Sherlock, 1970).

Properties of Australia Antigen

Great difficulty has been encountered in obtaining antigen in a sufficiently pure state for chemical analysis. However, it seems that it consists mainly of protein (Gerin, Purcell, Hoggan, Holland, and Chanock, 1969). Some lipid is found in most preparations and may be incorporated into the structure of the antigen since treatment with mild detergents slightly alters its density, a small 'heavy' fraction being released (Barker, Smith, Gehle, and Shulman, 1969). The buoyant density of the antigen is approximately 1·20 g/cm³.

Treatment of the antigen with ether, desoxycholic acid, enzymes such as trypsin and pronase, and heating to 56°C overnight do not destroy its reactivity in serological tests (Barker *et al*, 1969; Gerin *et al*,

1969; Millman, Loeb, Bayer, and Blumberg, 1970a). However it is destroyed by 1% sodium dodecyl sulphate and by boiling. Krugman, Giles, and Hammond (1970) have reported that carefully controlled heating of diluted serum to 98°C for one minute destroys the infectivity, but not its antigenicity either *in vivo* or *in vitro*.

The most striking property of the antigen is its morphology which is sufficiently characteristic to be used as a diagnostic feature. Three types of particle are seen (Fig. 1). The most numerous are the small round forms about 16 to 18 nm diameter with an indefinite substructure which suggests they may be built up from a number of subunits (Bayer, Blumberg, and Werner, 1968). Long forms are almost invariably present. They are about 20 nm diameter with greatly variable length. They may be bent and are often expanded at one or both ends (Almeida, Zuckerman, Taylor, and Waterson, 1969). Horizontal striations with a periodicity of 3·5 nm are often seen. Dane, Cameron, and Briggs (1970) have described a third type of particle which is present in about one third of positive specimens (Cossart and Field, 1970). It is a double-shelled or 'doughnut' form about 45 nm diameter. The internal shell is about 20 nm diameter and when penetrated by stain it appears to be constructed from a number of identical subunits. All three particle types are included in immune complexes with specific antisera so are possibly constructed of the same subunits.

Despite the variation in size of the spherical particles, which is considerably greater than that expected for small isometric viruses, the morphology of Australia antigen strongly suggests that it may be a virus or derived from aberrations of viral multiplication. Much interest has therefore been shown in analyses for nucleic acid. The earliest reports (Gerin *et al*, 1969) showed that the concentration of nucleic acid must be very low, if indeed, there was any at all. This was not unexpected in view of the very low buoyant density of the antigen. However it may be that only a very small proportion of the particles are 'complete'. The suggestion that the double-shelled particles contain nucleic acid is based on evidence of their very slightly greater density compared with that of the other particles (Dane *et*
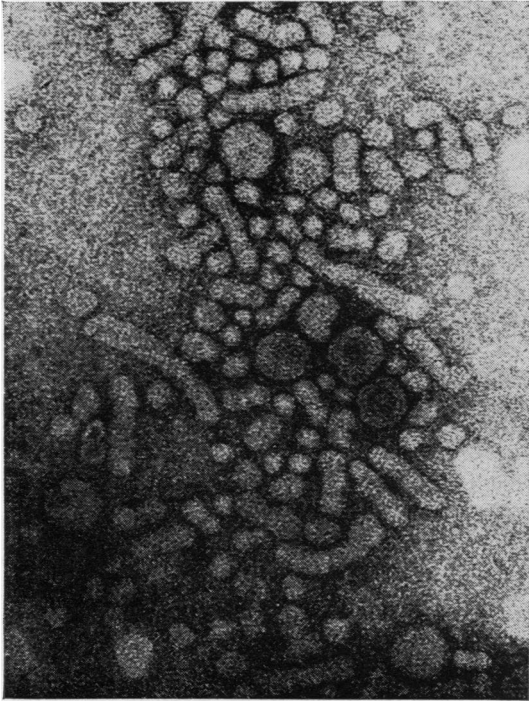
Fig. 1 *All three types of Australia antigen particles included in a clump made by the addition of specific antibody. Electron micrograph (magnification × 165,000) kindly supplied by Dr Anne M. Field.*

al, 1970) and on apparent staining of the core with uranyl acetate (Krohn, Finlayson, Jokelainen, Anderson, and Prince, 1970). Recently more direct evidence of a small RNA component has been obtained by Józwiak, Kościelak, Madaliński, Brzosko, Nowosławski, and Kłoczewiak (1971), but no attempt was made by this group to see whether this is confined to one particle type. It will be of great importance if these findings can be substantiated.

The concentration of Australia antigen in serum is often very high and has been estimated at about 10^{13} particles per millilitre (Almeida *et al*, 1969).

Detection

The antigen may be identified either by serological techniques or by visualization of the particles by electron microscopy.

Serological Methods

SOURCE OF ANTISERUM
The original discovery and almost all subsequent work employed antisera from human subjects who have been exposed repeatedly to the antigen, usually by receiving many blood transfusions. In England the proportion of such patients with anti-Australia antibody is low (about 1 % of haemophiliacs) when compared with about 30 % of haemophiliacs tested in the United States (Shulman and Barker, 1969). For this reason it is proving difficult to obtain sufficient serum for diagnostic purposes.

Animal sera have been produced in small amounts. Melartin and Blumberg (1966) described a method using an alum precipitate of antigen-positive serum to immunize rabbits. A more sophisticated technique was used by Purcell, Holland, Walsh, Wong, Morrow, and Chanock (1969) who used antigen purified by several cycles of density gradient centrifugation to immunize guinea pigs. Millman, Ziegenfuss, Raunio, London, Sutnick, and Blumberg (1970b) described the production of antibody in mouse ascitic fluids. Although in some instances very high titres have been obtained, these animal antisera have also contained antibodies to normal human serum proteins and require adsorption before use.

TECHNIQUES OF TESTING
Gel diffusion, complement fixation, and immune electrophoresis are the three methods in general use. The Australia antigen-antibody reaction can, however, be demonstrated by many other techniques, some of which, such as immune adherence and radioimmune assay, are of interest because of their great sensitivity. Many variations of each method have been described and the most appropriate choice will depend on the source of material to be tested as well as on the resources available and personal preference.

The gel diffusion test
This was the method used initially by Blumberg *et al* (1965) but it is usually performed by the technique of Prince (1968) using 0·9 % agarose in a Tris buffer for the gel as this yields more positives than Noble agar in phosphate-buffered saline. A conventional micro-Ouchterlony system is used with a pattern of six wells surrounding a centre well. Antigen controls are placed at 12 and 6 o'clock and antibody in the centre well. This pattern provides a check on the identity of a reaction between the antibody and a test serum and reinforcement for weak reactions. Using this arrangement of wells each specimen is tested simultaneously for antigen and antibody. From the technical viewpoint advantages of the gel test are its simplicity combined with great economy of antiserum and the automatic check on the identity of the reaction. Disadvantages are that it is

relatively insensitive since large amounts of antigen are required to produce a visible line, it is rather slow, and overnight incubation is needed to reveal strong reactions while weak reactions may not appear for several days. Clumps of antigen sometimes occur in the serum during the acute phase of hepatitis (Almeida and Waterson, 1969) and may be too large to migrate through the gel (Cossart, Field, Hargreaves, and Porter, 1971). False positive lines are sometimes produced by non-specific precipitates of lipoproteins in agarose (Gardner and Rosenberg, 1969) and may sometimes be difficult to interpret.

The test can be modified to increase the sensitivity either by concentrating the sera before testing or by using large wells (Dane *et al*, 1970), but this makes the system uneconomic both of labour and reagents.

The complement fixation test

This is best performed by a micro-adaptation of a standard virological technique with overnight fixation at $+4°C$. Satisfactory results are obtained with the methods suggested by Shulman and Barker (1969) and Purcell *et al* (1969), but the method of Bradstreet and Taylor (1962), using either micro-titre equipment or 0·025 ml droppers, is more robust for routine use.

The complement-fixation test is much more sensitive than the gel test, antigen titres being 50-100 times higher and antibody four to eight times higher. It is, however, technically more demanding and presents three intrinsic problems. First, there is no check on the specificity of positive reactions which is a serious difficulty so long as human antisera must be used. Secondly, strongly positive sera often exhibit a prozone so screening must be arranged to include a range of dilutions which means substantial quantities of antibody are required. Thirdly, anti-complementary activity is common in jaundiced sera. This may be due to non-specific factors or to the presence of complexes of Australia antigen and its antibody (Shulman and Barker, 1969). Sera containing complexes usually give clearly positive results on titration.

Immunoelectrophoresis

Many variants of this technique have been described (Gocke and Howe, 1970; Pesendorfer, Krassnitzky, and Wewalka, 1970; Prince and Burke, 1970; White, Lasheen, and Turner, 1970) and synonyms include 'countercurrent electrophoresis', 'crossover electrophoresis', and 'immunoelectro-osmorphoresis'. They depend on the observation by Alter and Blumberg (1966) that Australia antigen has an electrophoretic mobility close to that of α_2 globulin, and moves in the same direction as the current. In an agar gel antibody of the IgG class moves in the

opposite direction because of 'endosmosis', thus the antigen and antibody are forced together and a precipitin line is quickly produced.

In general the higher the voltage applied the quicker and more sensitive the results, but over-heating limits the use of high voltage techniques unless special equipment is available. For most purposes the method described by Pesendorfer *et al* (1970) is satisfactory and results are available in about two hours.

By placing test sera between control antigen and antibody both properties can be tested simultaneously.

Immunoelectrophoresis is an attractive technique because answers can be obtained so rapidly. Its sensitivity for detecting antigen is only slightly greater than that of the gel diffusion test, but for antibody the advantage is much greater. The test as usually performed, however, provides no check on the specificity of the reaction.

OTHER SEROLOGICAL TESTS

Immune adherence

Antigen/antibody complexes plus attached complement under certain conditions cause aggregation of both platelets (Melartin, Myllylä, and Penttinen, 1970) and red blood cells (Okochi, Mayumi, Haguino, and Saito, 1970). This very sensitive but difficult technique can be adapted to measure the Australia antigen/antibody reaction.

Radioimmunoassay

Either antigen or antibody can be labelled and the activity detected measures the concentration of the labelled material which reacts with a test serum. This is an extremely sensitive technique, but besides being elaborate and expensive is very difficult to standardize when purified reagents are not available. It has been used experimentally to measure Australia antigen and antibody (Walsh, Yalow, and Benen, 1970) but more experience is needed before its value is established.

A 'membrane CF test' (Saravis and Bonacker, 1970) and a 'chromed' red cell agglutination test (Vyas and Shulman, 1970) have also been described.

Electron microscopy

The serological tests as a whole suffer because the human antisera are in very short supply, are of low titre, are unstandardized, and contain many antibodies besides the one specific for Australia antigen. It is therefore very useful to have a non-serological method for detecting Australia antigen.

The antigen is present in serum in large amounts so ultracentrifugation is sufficient as a preparative procedure, and the simple negative staining tech-

nique described by Almeida *et al* (1969) is extremely satisfactory. If the particles are scanty or if long and double-shelled forms are difficult to find, the test may be elaborated by using antiserum to clump the particles before centrifugation. In this form the test is both highly specific and very sensitive.

COMPARISON OF THE TESTS

Sensitivity

The amount of antigen needed to produce a positive result varies considerably in the different test systems. For example, chequerboard titration with the same two sera by various methods gave the results shown in Table I.

	Endpoints Obtained with Same Sera by Different Tests			
	Method of Testing			
	Gel	CFT	Electrophoresis	EM
Antigen	1/16	1/1024	1/64	1/20
Antibody	1/4	1/16	1/8	1/10

Table I   *Comparison of Australia antigen tests*

While this comparison represents a rough order of sensitivity it must be pointed out that antibody in different sera may have such widely different optima against the same antigen even by the same test that in practice the results of screening tests may be affected (Schmidt and Lennette, 1970).

In choosing a method for testing it is necessary to know the concentration of antigen which is to be expected in clinical specimens, and about this point there is no certainty, as the amount seems to differ in different groups of patients. In testing 51,000 blood donors, G. R. Milne, J. Wallace, and A. Barr (personal communication) have detected Australia antigen in 40 and antibody in 30 by electrophoresis. In 5,574 sera tested in parallel by gel diffusion and electrophoresis, electrophoresis detected 10 positives, nine of which were positive by the gel test. Antibody was detected by electrophoresis in 13 donors, but only one of these was found by gel diffusion. Similarly amongst patients undergoing maintenance haemodialysis we have found that the use of the complement-fixation test reveals very few additional positive results compared with the gel test. On the other hand, when patients with hepatitis are tested on admission to hospital the proportion positive may be almost doubled if the complement-fixation test is used rather than the gel test (Cossart and Vahrman, 1970). In chronic liver disease, where the concentration of antigen might be expected to be low, the more sensitive tests are desirable although the claims made for the 'high

voltage' electrophoresis in this respect (Prince and Burke, 1970) remain to be confirmed in other centres.

Specificity

All the serological tests are liable to produce false positive results when human antisera are used since these contain numerous antibodies. These may be revealed when such 'positive' sera fail to react with all of a panel of different anti-Australia antisera, or by the failure of the false positive precipitin lines to fuse with control lines. Demonstration of reactions of identity may be difficult since in some cases lines produced against red cell components are sometimes similar in character and position to the Australia antigen lines and may be obtained even when the test sera are not obviously haemolysed.

False negative results may be due to low titres or to the presence of some anticoagulants such as acid-citrate dextrose which weakens the line obtained in the gel test. Sometimes the gel diffusion and electrophoresis tests may be negative despite high complement fixation titres, and in these instances the antigen is found in clumps when the specimen is examined in the electron microscope (Cossart *et al*, 1971). This situation may arise in acute hepatitis and probably also in chronic liver disease.

Antigenic variation in Australia antigen seems to be minor, although some human antisera seem to have a broader spectrum than others (Sutnick, Raunio, London, Millman, and Blumberg, 1970). Using rabbit antisera, Levene and Blumberg (1969) described Au_1 and Au_2 which showed partial identity in the gel test. It has, however, proved difficult to separate the effects of variation in optimum titre of both human and animal sera from true antigenic variation.

Speed

The time taken to obtain a result by electrophoresis is about two hours, and of the other tests, only electron microscopy can produce a result so quickly. The complement-fixation test requires overnight fixation for maximum sensitivity, while the gel test must be examined daily for two or three days, during which time new positives continue to appear.

Economy

The gel test uses the least antiserum and is also the least demanding technically. There is little to choose between electrophoresis and the complement-fixation test either in the requirement for antiserum (which is about four times that needed for the gel diffusion test) or in the laboratory facilities needed. Electron microscopy requires elaborate facilities and uses large amounts of antiserum, so is reserved for special projects.

The Role of Australia Antigen in Hepatitis

The original description of the 'SH antigen' by Prince (1968) associated it with post-transfusion hepatitis and this was quickly confirmed by others (Okochi and Murakami, 1968; Gocke and Kavey, 1969). Blumberg *et al* (1968), however, had reported that the antigen could be found in 'infectious hepatitis'.

Reports of the occurrence of the antigen are rapidly appearing from many laboratories, and although there is some difficulty in reconciling all these findings with the traditional concepts of 'infectious' and 'serum' hepatitis, an overall picture is emerging at least as far as the clinical and epidemiological features are concerned.

HEALTHY CARRIERS
Population surveys using the gel diffusion test show considerable geographical variation in incidence. This ranges from about 0·1 % in the United States and western Europe, to 1·0 % in Japan and even as high as 10 % in some of the Pacific Island populations (Blumberg *et al*, 1965; Prince, 1970). These differences are unlikely to be genetically determined since minority racial groups living in the United States have the same rate of antigen carriage as the rest of the population. Very little is known about the dynamics of this situation. Many of the carriers have never been jaundiced, and although some instances are known where an individual has remained a carrier of antigen for many years (Zuckerman and Taylor, 1969) there is also evidence that healthy people gain and lose the antigen thereby maintaining the carrier rate in the population as a whole (Soulier, Courouce-Pauty, and Benamon-Djiane, 1970).

There is a suggestion that some apparently healthy carriers may be experiencing subclinical attacks of hepatitis because their serum transaminase levels are on average slightly higher than the average for antigen-negative healthy persons (Okochi *et al*, 1970).

From this it seems that there must be a considerable circulation of this type of hepatitis in the community and this presupposes either a sufficient degree of infectivity by the faecal/oral or some other 'natural' route or that parenteral inoculation with serum-contaminated needles is relatively frequent. Certainly this high incidence of the antigen in people living in primitive conditions might result from transmission by insect vectors or by tattooing, ear piercing, etc.

It is difficult to interpret the findings about the age incidence of antigen carriage. Some series show little age or sex variation, but others (Krech, Sonnabend, and Jung, 1970; Okochi *et al*, 1970) show a peak

incidence in young adults and a predominance of males. There are as yet no large studies of normal persons who are not blood donors and all the available evidence may be biased by selection. However, Soulier *et al* (1970) reported the same incidence of carriers in healthy people with and without previous jaundice.

As an interesting theory to account for the establishment of both the genetic and infective modes of the carrier state, it may be proposed that vertical transmission might occur and that an individual acquiring the antigen during foetal life might thus be made immunologically tolerant and carry the antigen throughout life. Antigen-positive hepatitis in pregnancy has a variable outcome in this respect. When it occurs in the first trimester the foetus seems to escape (London, DiFiglia, and Rogers, 1969a) but if it occurs near term, some of the infants become antigen positive during the first two months of life, although the cord blood has so far always been negative when tested. In some instances (Wright, Perkins, Bower, and Jerrome, 1970) chronic hepatitis in the infant has developed; in others long-term carriage may follow (Schweitzer and Spears, 1970).

IMMUNOLOGICAL DEFICIENCY AND ANTIGEN CARRIAGE
Patients with Down's syndrome, lepromatous leprosy, chronic renal failure, or receiving immunosuppressive treatment, all have a predisposition to become long-term carriers of Australia antigen. Although they often have no clinical evidence of hepatitis, laboratory investigation usually reveals some elevation of the serum transaminase levels (Turner and White, 1969), and, in some patients with relatively minor biochemical abnormality, the liver biopsy shows clear evidence of hepatitis.

The relatively benign type of illness seen in these patients contrasts strikingly with their great susceptibility to other virus infections such as herpes simplex, cytomegalovirus, and varicella which tend to be mild in normal individuals.

Blumberg, Friedlander, Woodside, Sutnick, and London (1969) have suggested that the healthy carrier state may be due to a minor immunological deficiency in the individuals involved, and that this deficiency may be an inherited character. They advance family studies in support of this idea, but it is not so far possible to assess the relative importance of inheritance and contact in these reports.

POST-TRANSFUSION HEPATITIS
Prince (1968) showed very clearly that transfusion of blood containing Australia antigen is likely to be followed by antigenaemia in the recipient after an interval of some weeks, and that clinical hepatitis

usually follows soon after. By the time symptoms develop the concentration of antigen in the blood is already declining and it usually disappears completely during convalescence. Much effort has since been devoted to delineating this picture further.

The proportion of cases of post-transfusion hepatitis in which antigen can be detected during the acute phase of illness varies in different reports from 13% (Okochi and Murakami, 1968) to 75% (Hirschman, Shulman, Barker, and Smith, 1969). While some of this variation is undoubtedly due to the use of different methods for detecting the antigen there does seem to be real variation in different situations, perhaps reflecting a balance between the carrier rate in the community and the prevalence of 'infectious' hepatitis.

The sequence of events after transfusion of a unit of antigen-positive blood generally follows the course described above. The interval between the transfusion and detection of antigen in the recipient may be as short as three to four weeks or as long as 13 weeks. About one-quarter or two-thirds of patients develop antigenaemia after this type of exposure, and in these, most subsequently develop hepatitis (Gocke and Kavey, 1969). A smaller number develop transient antibody usually after two or three weeks, but there is as yet no information about the correlation of this with previous hepatitis or blood transfusion. So far it appears there are rare instances of a patient developing antigenaemia after transfusion of a unit of blood in which Australia antigen could not be found. This seems almost always to be due to inadequate techniques for testing, but in enquiries into the origin of a case of post-transfusion hepatitis the donor involved will not necessarily still be antigen positive by the time the recipient develops symptoms.

The extent of antigen-negative hepatitis resulting from blood transfusion seems to have been underestimated in the past, but this aspect has become more obvious as the Australia antigen situation has been defined more precisely.

OTHER TYPES OF 'SERUM HEPATITIS'
Many reports have now appeared describing outbreaks of Australia antigen-positive hepatitis in groups of narcotic addicts (Cherubin, Hargrove, and Prince, 1969). In most instances these have resulted from the sharing of needles or syringes, but occasionally close contacts have been affected although they deny parenteral use of drugs (Nordenfelt, Kaij, and Ursing, 1970).

Fractionation of serum containing Au antigen by the Cohn method appears to produce albumin and immunoglobin fractions free of antigen, whereas fibrinogen and cryoprecipitate are strongly positive

for antigen (Andrassy, Ritz, and Sanwald, 1970). This corresponds with clinical observations of the incidence of hepatitis after administration of these products.

EPIDEMIC HEPATITIS
Comparatively few outbreaks of hepatitis in schools, camps, or institutions have been investigated for the presence of Australia antigen, but those which have been studied have all been negative (Chang and O'Brien, 1970; Mosley, Barker, Shulman, and Hatch, 1970). This also seems to be the case for family outbreaks and probably reflects the low attack rate observed under experimental conditions for the antigen-positive type of hepatitis (Krugman, Giles, and Hammond, 1967). A new antigen 'epidemic hepatitis-associated antigen' (EHAA) has recently been described by del Prete *et al* (1970). This has been found in the acute phase serum of patients in some outbreaks and the only specific antibody detected so far is contained in serum from a single multiply transfused patient. This serum also contains anti-Australia antibody. The EHAA is labile on storage at $-20°C$; it does not seem to be particulate and its nature and significance are as yet unknown.

SPORADIC HEPATITIS
Most cases of hepatitis come into this category. Few have any clear history of contact with another case, or of parenteral inoculation. The proportion of these patients whose serum contains antigen varies considerably in different centres. The highest rates seem to be in large cities, with young adult males predominating (Cossart and Vahrman, 1970; Prince, Hargrove, Szmuness, Cherubin, Fontana, and Jeffries, 1970). Illicit parenteral use of drugs may be an important factor in the incidence (Dismukes, Karchmer, Johnson, and Dougherty, 1968) and if this is the case the problems of controlling hepatitis are likely to increase.

Antigen-positive hepatitis in childhood is very rare, despite the evidence that children are susceptible when inoculated parenterally or orally (Krugman *et al*, 1967).

CHRONIC LIVER DISEASE
The role of viruses in the aetiology of chronic liver disease remains undefined. Chronic active hepatitis and biliary cirrhosis in the United States (Wright, McCollum, and Klatskin, 1969; Prince, 1970) and in some European countries (Krassnitzky, Pesendorfer, and Wewalka, 1970) are not infrequently associated with the presence of Australia antigen, but in England and Australia this is not the case

(Fox, Niazi, and Sherlock, 1969; Matthews and Mackay, 1970). Wright *et al* (1969) demonstrated an inverse relationship between the presence of smooth muscle antibody and Australia antigen in chronic active hepatitis and this gives support to the belief that there are multiple factors involved in the aetiology of this disorder which may vary in different parts of the world.

In some countries, including the United States (Wright *et al*, 1969), Britain (Fox *et al*, 1969), and Australia (Matthews and Mackay, 1970), Australia antigen is seldom found in patients with portal cirrhosis. In others such as Spain (Guardia, 1970) it is not uncommon. Longitudinal studies of patients with antigen-positive and -negative hepatitis will be needed to clarify the relation between acute viral infection of the liver and subsequent cirrhosis.

HEPATOMA

Vogel, Anthony, Mody, and Barker (1970) have reported that a substantial proportion of their patients in East Africa with hepatoma are also Australia antigen positive and Sherlock, Fox, Niazi, and Scheuer (1970) have also found some patients positive. Further investigations will be needed comparing countries where the incidence of this tumour is both high and low.

A 'non-hepatic' role for Australia antigen has recently been suggested by Gocke, Hsu, Morgan, Bombadieri, Lockshin, and Christian (1970) who report its occurrence in patients with periarteritis nodose.

Pathogenesis

A coherent picture of the pathogenesis of antigen-positive hepatitis has not yet emerged, but the following points must be taken into account when attempting to suggest a mechanism for its production.

From the studies at Willowbrook State School it seems that Australia-antigen-positive hepatitis can spread naturally from person to person and it has been shown that serum containing the antigen is infectious when given by mouth (Krugman *et al*, 1967; Giles, McCollum, Berndston, and Krugman, 1969). However, the natural route of exit from the body is unknown. Excretion in faeces and urine seem the most likely, but no critical transmission experiments have been performed to test their infectivity. The antigen has been sought unsuccessfully in urine and faeces (Cossart and Vahrman, 1970), but other groups using similar techniques report its presence (Grob and Jemelka, 1971) during, and for some time after, the stage of antigenaemia. It is difficult to decide how to interpret this situation since infectivity

and Australia antigen activity may vary independently.

The site of production of antigen is unknown. There are reports of its detection by immunofluorescent methods in the nuclei of liver parenchymal cells in biopsies obtained during the acute phase of hepatitis (Nowosławski, Brzosko, Madaliński, and Krawczyński, 1970). Millman, Zavatone, Gerstley, and Blumberg (1969) also claim to have found antigen in bone marrow cells by similar methods. On the other hand, Almeida, Waterson, Trowell, and Neal (1970) have demonstrated enterovirus-like particles in homogenates of liver from patients dying of acute yellow atrophy of the liver and with high concentration of antigen in the blood. Electron microscopy has not revealed either virus particles or changes suggesting virus infections in thin sections of liver biopsy specimens obtained from patients with acute viral hepatitis (Wills, 1968).

The 'allergic' symptoms (skin rash and arthralgia) often experienced late in the incubation period of 'serum hepatitis' has suggested that the liver damage in hepatitis may be caused by an autoimmune mechanism. Some support for this idea may be derived from the way in which the appearance of the antigen alters during the progress of the disease (Almeida and Waterson, 1969). At first the particles are evenly dispersed, but they then become clumped, presumably by antibody, until the particles are contained in large aggregates. These clumps are then rapidly cleared from the serum and the antigenaemia is terminated. In the serum of long-term carriers the antigen particles are not clumped.

In chronic active hepatitis and biliary cirrhosis clumps seem to persist for long periods (Wright *et al*, 1970). No antibody response to Australia antigen following hepatitis is usually demonstrable, but immunity to challenge with the same agent is conferred on the individual (Krugman, Giles, and Hammond, 1967). As yet no test of previous infection has been devised.

Epidemiology of Antigen-positive Hepatitis

Initially it was thought that Australia antigen might be specific for 'serum hepatitis' as conventionally defined. In the light of more recent work it seems that antigen-positive hepatitis is indeed one specific type of hepatitis, but that either the features of 'serum hepatitis' must be considered to include the natural transmission of the agent, or, perhaps better, hepatitis be reclassified. Antigen-positive cases could then be contrasted with the antigen-negative cases which may or may not form a homogeneous aetiological group.

If the latter approach is adopted the features of

Feature	Au-positive	Au-negative
Incubation period	6 weeks–3 months (? or more)	3–6 weeks
Case of transmission	Parenteral + + +	Parenteral + + +
	Oral +	Oral + + +
Infectivity		
Faeces	? –	+ + +
Urine	? –	? +
Blood	+ + +	+ + +
Clinical features	Often prolonged or severe. Rash and arthralgia common	Usually mild. No allergic symptoms
Epidemiological pattern		
Sporadic cases	Mainly young adults—males predominating	Sporadic cases and family outbreaks mainly affect children
Outbreaks	Arise from exposure to icterogenic blood, eg, in renal dialysis units	Arise from contamination of food or water
Post-transfusion or injection	Any age according to exposure	Any age according to exposure
Long-term carriers	0·1 % healthy adults (England) but much higher in immunosuppressed persons	? Not recognized
Host range	Man ?Chimpanzee	Man ?Marmoset

Table II *Comparison of Australia antigen-positive and -negative hepatitis*

the two groups may be contrasted as shown in Table II.

The epidemiological situation and the relative importance of antigen-positive and -negative hepatitis vary considerably from country to country. The overall picture is of a low incidence of Australia antigen in healthy persons, with, however a previously unsuspected turnover in the membership of this pool which probably forms the ultimate reservoir of infection in the community. No animal reservoir has been identified although some primates, especially chimpanzees in captivity, may be antigen positive (Rivers and Keeling, 1970).

In England and Wales the great majority of cases of hepatitis notified to the Ministry of Health are in children in whom it is exceptional to find the antigen. In young adults, sporadic hepatitis is not infrequently antigen positive, and in the United States, hepatitis is now commonest in this age group, and it will be interesting to see if this upward trend in age incidence develops in other countries. Considerably increased opportunities for the spread of Australia-antigen-positive hepatitis have arisen with the development of intensive methods of treatment which often requires many blood transfusions, and with the widespread use of adrenal cortical steroids and immunosuppressive drugs. Immunosuppressed patients acquiring antigen tend to carry it for long periods while exhibiting little liver damage. When they are treated in hospital and when surgery or extensive investigation is required, there is some risk to the staff involved. Renal dialysis units provide an extreme example of this situation and numerous outbreaks of hepatitis involving staff, patients, and home contacts of the patients have now been recorded (Jones, Goldsmith, Wright, Roberts, and Watson, 967; *Lancet*, 1969; London, DiFiglia, Sutnick,

and Blumberg, 1969b; Ringertz and Nyström, 1969).

Other groups requiring many blood transfusions, but not immunologically deficient, are subject to a considerable risk of hepatitis, but since they usually carry the antigen for fairly short periods there is less opportunity for others to become secondarily infected.

Mongols are a group whose ability to deal with infection is impaired and they are likely to carry Australia antigen for very long periods (Sutnick, London, Gerstley, Cronlund, and Blumberg, 1968). However, in mental institutions, although close contact must facilitate natural transmission of the agent, there is no significant opportunity for parenteral spread. In one institution (Giles *et al*, 1969) half the cases of hepatitis occurring amongst a very large number of mentally subnormal patients was of the antigen-positive type. It seems from these rather scattered observations that hepatitis should be considered as a widespread cross-infection problem affecting hospitals of many different types.

The discovery of Australia antigen and its association with a specific type of hepatitis has already proved to be a valuable tool in the investigation of a disease whose infectious agent has resisted all attempts to cultivate it *in vitro*. It has also produced many theoretical puzzles which as yet await elucidation.

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