Progress report

Long-incubation (virus B, HAA-associated) hepatitis

Since the war of 1939-45, the distinction between short-incubation ('infectious' virus A) and long-incubation ('serum', type B) hepatitis has been recognized. The discovery of Australia (hepatitis-associated HAA) antigen has allowed the distinction to be more clearly defined (Table I).

<table>
<thead>
<tr>
<th></th>
<th>Virus A</th>
<th>Virus B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synonym</td>
<td>Infectious</td>
<td>Serum</td>
</tr>
<tr>
<td>Incubation (days)</td>
<td>15-50</td>
<td>50-160</td>
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<tr>
<td>Willowbrook</td>
<td>MS₁</td>
<td>MS₂</td>
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<tr>
<td>Australia antigen</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Spread of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parenteral</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Oral</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Faecal</td>
<td>++</td>
<td>?+</td>
</tr>
<tr>
<td>Urinary</td>
<td>?+</td>
<td>?+</td>
</tr>
<tr>
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<td>Marmoset</td>
<td>Chimpanzee</td>
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<td>Seasonal incidence</td>
<td>Autumn—winter</td>
<td>Year round</td>
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<td>Age preference</td>
<td>Children—young adults</td>
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<td>Associations</td>
<td>Epidemic—rural</td>
<td>Sporadic—urban</td>
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<tr>
<td>Severity</td>
<td>Usually mild</td>
<td>Often severe</td>
</tr>
<tr>
<td>Value of gamma globulin</td>
<td>Good</td>
<td>Uncertain</td>
</tr>
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</table>

Table  Short-(A) and long-(B) incubation hepatitis contrasted

Virus B hepatitis is differentiated from virus A hepatitis by the presence of Australia antigen in the serum. This most interesting antigen was discovered incidentally. In 1965, Blumberg, a geneticist, and his colleagues were working in Philadelphia on the development of antibodies against human serum lipoproteins in multiply transfused subjects. They found that in two haemophilic patients there was an antibody which reacted with an antigen in a single serum in their panel and this came from an Australian Aborigine. The antibody, an immunoglobulin G, was found in many patients who had been multiply transfused. Later it was detected in some 20% of patients with viral hepatitis. Because of its discovery in an Aboriginal serum, the antigen was called Australia antigen. The more general term, hepatitis-associated antigen (HAA), is also employed. The antigen is present in about 82% of sera from patients suffering from long-incubation hepatitis if tested within 12 days of the onset of symptoms. It is usually cleared from the serum by three to four weeks. Patients transfused with blood containing the antigen will either develop icteric or anicteric hepatitis or will remain asymptomatic.

Virus B hepatitis has an incubation period of 50 to 160 days compared with virus A infection which has an incubation period of 15 to 50 days.
The onset of virus B hepatitis is more insidious than for the virus A type, often extending over a period of two to three weeks. During this prodromal period and in the early stages of the illness, a serum-sickness-like syndrome may be seen. This is marked by arthritis involving distal joints, usually the peripheral interpharangeal ones, fever, and an erythematous or urticarial rash. This polyarthralgia was indeed well described by Sir Robert Graves in 1843. In an epidemic of hepatitis involving 300 patients, Mirick found 55% showing allergic manifestations such as urticaria, arthralgia, and angio-neurotic oedema. This serum-sickness-like syndrome has been attributed to circulating immune complexes, for the C₃ component of complement, which would be included in such complexes, is very low in these patients.

Otherwise, the clinical course is very similar for the two types, except that the mortality is higher for the virus B infection. For the short-incubation disease a figure of 2 per 1 000 is quoted whereas for the long-incubation disease the mortality can be as high as 1 in 8 for the post-transfusion patients and 1 in 200 for the naturally acquired disease.

Cross-immunity studies of the two types have been greatly restricted by the lack of suitable volunteers. Limited investigations in the 1940s suggested that there was no cross immunity between the two. Since then, much has depended on observations by Krugman and his group at the Willowbrook State School. This group has described two strains of virus hepatitis, MS₁, which is short-incubation and without the presence of Australia antigen in the serum, and MS₂, which is long incubation and which is associated with Australia antigen. Oral challenge of those recovered from one type with material containing the same strain results in no disease, whereas challenge with the other leads to virus hepatitis having a different incubation period.

Spread of Long-incubation (Virus B) Hepatitis

It is generally agreed that the short-incubation disease affects predominantly children and young adults often in an epidemic, rural setting and most frequently in autumn and spring. The usual mode of infection is ano-oral although blood is also infectious. In contrast, the long-incubation disease affects all ages, at all times of the year. The classical infection by virus B is parenteral through infected blood, transfusions for instance, or the use of contaminated syringes and needles. In urban areas, the increasing incidence of drug abuse exposes larger numbers to syringe-borne infection, and close contacts of addicts, who are not themselves drug users, can contract the disease. Similarly, hospital-acquired disease, for instance, in renal units, can spread to the general community.

Mass screening of blood donors and epidemiological surveys have made it clear that the prevalence apparent in blood donors varies greatly from country to country and in various areas within the same country. This is a reflection both of the general prevalence of antigenaemia in the community and of the type of person donating blood. Positive donors are more frequently found in those paid for the blood, and so include alcoholics, narcotic addicts, and convicts, rather than the Red Cross-type volunteer.

Other ways in which the blood can be infective by parenteral or oral routes include kissing, menstrual discharge, contaminated tooth and shaving brushes, dental instruments, infected scratches, and accidental pricks during attendance on a patient with a positive Australia antigen test. Non-parenteral
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spread of infection is also seen in Swedish track finders who develop hepatitis as a result of scratches and wounds acquired during the cross-country races and washing afterwards under suboptimal hygienic conditions.

There are probably sources of infection other than blood. Faecal spread is uncertain. If it does occur this must be rare. However, faecal antigens have been described. In Melbourne, antigen was detected in the faeces of 14 patients with Australia-antigen-positive viral hepatitis. Swiss workers have also found the antigen in the faeces on several occasions. Urinary spread is also possible from patients with renal transplants who have become chronic carriers of the antigen and may excrete it in the urine. The Royal Free Hospital, London, and the Wellcome group found antigen particles in the urine not only in the presence of Australia-antigen-positive liver disease but in apparently healthy persons whose serum antigen testing was negative. It is, however, still uncertain whether such urine is infectious to others.

Akdomar and co-workers failed to demonstrate Australia antigen in bile and gastric juice of individuals with antigenaemia. Recently, however, Alp and Wright have reported the finding of Australia antigen by electron microscopy and immunoelectrophoresis in the bile of one patient who was a chronic antigen carrier in his blood.

Nature of Australia Antigen

The exact relationship of the Australia antigen to the causative agent of virus B hepatitis remains uncertain. Unfortunately, the agent has not been propagated in tissue culture. Chimpanzees in captivity have been found to be antigen positive and this suggests that at last a susceptible animal has been found. For obvious reasons, however, this preparation is unlikely to be useful for routine testing. The relation of the antigen to virus B hepatitis rests on the close association between its presence in the blood and acute hepatitis and the infectious nature of blood containing it when transfused into a susceptible human being.

Under the electron microscope three types of particles can be seen in the serum of patients with the long incubation disease who are antigen positive—small, 20 nm (200A) spheres, tubules 20 nm in diameter and 100 nm long, and thirdly the more complex 42 nm Dane particles. It is possible that these large particles represent the actual virion of virus B hepatitis. The rest of the Australia antigen may be virus-coat material not incorporated into intact virus (see Fig.). The inner spherical core remaining after detergent treatment of Dane particles may be formed by the liver cell nucleus whereas the smaller particles may be the actual Australia antigen and be produced by multiplication in the cytoplasm of the liver cell. The inner core bears some resemblance to a rhinovirus. Both inner and outer components must be present to form the complete virion which is presumably the actual infectious agent. The particles may have antigenic differences, for components are agglutinated by convalescent sera of patients who have had HAA-positive hepatitis and who are now HAA negative, but not by HAA itself. There are now sensitive methods of detecting antibody. Persistence in the inner portion when the antigen has disappeared from the serum may be useful in the late diagnosis of those who have recovered from type B hepatitis.

Biochemically, the antigen has been shown to consist largely of protein with a minor lipid moiety. It is unaltered in its reaction with specific antibody
Sheila Sherlock

VIRION
(Rane Particle)

Fig. The infectious agent or virion of long-incubation hepatitis may be identical with the large Dane particle seen under the electron microscope. Australia antigen may be only a part of the virion, probably the outer coats.

after exposure to heat, freezing and thawing, putrefaction, acid and alkaline, and most catalytic agents. Extraction of the lipids does not affect the antigenic reactivity. These results suggest that the antigenic determinant(s) of HAA is protein. The presence or absence of nucleic acid is still in doubt. Recently, Polish workers have shown the presence of 2 to 5% RNA. The particles used for analysis by these workers were coreless. If complete particles had been used, the quantitative constitution might have been different.

A weak, RNA-dependent DNA polymerase has been found in concentrated preparations containing Australia antigen from three patients with evidence of hepatitis. This type of reverse transcriptase has been associated with larger tumour cell viruses. It may be the link between HAA and primary hepatocellular carcinoma.

ANTIGENIC SUBTYPES

The relationship of Australia antigen particles to the infective agent itself is still unresolved, but present evidence suggests that they are fragments of a viral lipoprotein envelope. It now seems that Australia antigen is heterogeneous. There is one antigenic specificity common to all, designated a, and there are three additional determinants, b, x, and y. ‘x’ is apparently common to all Australia antigen positive sera and may be a host factor; ‘b’ and ‘y’ are markedly exclusive. This discovery has considerable practical implications both in epidemiology and in the relation to various clinical types both acute and chronic, of long-incubation hepatitis. The MS2(Willowbrook) hepatitis is of ‘y’ + ‘b’ — type as are many instances of sporadic long-incubation hepatitis. The ‘b’ + ‘y’ — subtype seems to have a greater association with chronic carriage of antigen, for instance ‘in healthy’ blood donors, and in those with chronic liver disease. Much more needs to be done in subtyping the particles associated with the various clinical entities.

Mosley and his group, from Los Angeles, have applied subtyping to an epidemiological survey of Australia antigen-positive patients in a renal dialysis unit. All antigen-positive patients in the unit, whether the household
contacts of patients or the staff were ‘d’—. In the same hospital, all non-dialysis blood-transfusion-associated cases were ‘d’+. This implies that patients on the renal unit were contracting their hepatitis from one another and not from the blood transfused as part of their treatment. It strongly suggests that the patients' own blood was the source of the infection of their relatives and the staff rather than blood being used for transfusion purposes. Finally, the fact that ‘d’-patients after recovery experienced no ‘d’+ infection despite numerous subsequent blood transfusions provides some evidence for cross protection among subtypes.

**Mechanisms of Disease Production and of Chronicity**

Contact with material containing Australia antigen has variable consequences. No obvious liver injury may occur, the antigen either never being detectable or appearing and persisting as a carrier state. An acute hepatitis of varying severity, from subclinical to fulminant, may also develop, following which the antigen is usually cleared from the serum and the liver returns to normal or the patient is left with slight postnecrotic scarring. In some cases, however, the antigen persists in the serum and the patient is a symptomless carrier or has evidence of continuing liver damage. Liver biopsies in such patients can show a normal liver, postnecrotic scarring, chronic persistent hepatitis, or chronic aggressive hepatitis with or without cirrhosis. Primary liver cell carcinoma may be another long-term complication. Various factors influence the outcome in the individual patient. These include the dose, virulence, and strain of the agent, the mode of transmission, and the immunological response of the patient.

Differences in the virulence in different epidemics are illustrated by the experience of recent outbreaks of hepatitis in renal haemodialysis units in the United Kingdom. There were 11 outbreaks involving 161 patients of whom 10 died (mortality 6%). One hundred and forty-five staff were affected and six died (4%). Nineteen relatives also suffered from hepatitis. Of the 16 deaths, 10 were encountered in one outbreak in Edinburgh involving 42 persons. This emphasizes the severity of the infection in the Scottish unit compared with the other 10 units among which there were only six deaths.

The infection is probably a virus-cell interaction of non-cytopathic type. It would therefore be unrelated to the amount of antigen in the serum. It is indeed found that titres of Australia antigen are highest in the chronic carriers and lowest with the severest liver injury, namely, fulminating hepatitis and chronic aggressive hepatitis. Assuming that the serum HAA titre accurately reflects the amount of infective agent in the body, this indicates that the virus might not be solely responsible for the liver damage in these patients. If the virus was directly responsible for the liver injury, the highest HAA titres should be associated with the greatest hepatic injury—which is the opposite to what was found. Nevertheless, it is likely that the more liver cells infected with virus, the more severe the attack. The patient's immunological response to the virus is probably more important.

The liver injury could be related to the humoral immune response. Antibody is produced to HAA and this with the antigen forms complexes which in the presence of complement can cause lysis and cell death. Several reports support this concept of immune-complex liver injury. Almeida and Waterson in three patients suggested that antibody production with the formation of
immune complexes paralleled the severity of the liver injury. Immunofluorescent studies show immune complexes involving immunoglobulin, HAA, and complement in the tissues of patients with chronic HAA disease. There are, however, points against this hypothesis. If immune complexes are important in the pathogenesis of liver cell injury, complement will be utilized, and hence a low serum complement will be expected in patients in whom the liver damage is greatest. The serum levels of the third component of complement, which closely reflects the total complement activity, is within the normal range in the majority of patients, except in those with massive necrosis of the liver in whom it is always low. In fact, this marked depression in fulminant hepatic necrosis occurs whatever the aetiology of the liver injury and is likely to be due to reduced synthesis of complement.

Improved techniques have allowed HAA antibody to be measured at all stages of long-incubation virus hepatitis. The simultaneous detection of HAA and anti-HAA in these patients at a time when liver damage is beginning or at its peak suggests that the immune reaction is important in the pathogenesis of the hepatitis. However, no striking differences in the clinical course of the disease are found among the patients who had transient HAA at the same time as anti-HAA early in the illness, a chronic carrier of HAA who continued to circulate HAA and anti-HAA simultaneously for at least 18 months after the first exposure, and two chronic carriers of HAA in whom anti-HAA was not detected. Both antigen and antibody can therefore be present in the serum without either a severe acute attack of viral hepatitis or the development of chronicity.

There is some evidence of depression of delayed type hypersensitivity reactions in those who develop chronic antigenaemia and chronic liver disease. The acute condition may progress through to cirrhosis in those with agammaglobulinaemia. This suggests that chronicity is independent of humoral antibody response. Diseases such as chronic renal failure, where the cellular immune response is particularly impaired, are associated with antigenaemia and chronic hepatocellular disease. The lymphocytes from patients who have recovered from virus B hepatitis and have Australia-antigen-negative serum respond differently from normal when exposed to serum rich in the antigen. This suggests a specific immunological state of delayed hypersensitivity to Australia antigen in the post-hepatitic patient. Finally, prednisolone, which would be expected particularly to reduce cellular immune responses, does lead to clinical improvement and a fall in serum transaminase levels in antigen-positive liver disease. This suggests that changes in cell-based immunity are concerned in the pathogenesis of the liver injury.

Types of Chronic Australia Antigen-positive Liver Disease

Pathology
The relation between acute viral hepatitis and chronic hepatitis with cirrhosis has always been in doubt. In certain cases, serial liver biopsies have shown progression from acute viral hepatitis to cirrhosis. Massive necrosis and scar formation are intermediate stages leading to cirrhosis. Since the discovery of Australia antigen it is now known that the following pathological sequelae may be associated with persistence of a positive antigen test in blood, post-necrotic scarring, chronic hepatitis, and cirrhosis.

Post-necrotic scarring is characterized by inactive slender scars, intersecting
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the parenchyma. The lobular architecture is distorted but not destroyed, inflammation is slight and there is no histological evidence of continuing liver cell necrosis.

Chronic hepatitis may be defined as continuing liver inflammation lasting for more than one year. It is essentially a reversible condition marked by portal cellular infiltration and varying degrees of fibrosis, focal hepatocellular necrosis, and minimal piecemeal necrosis at the periphery of the lobule. Two types are distinguished, chronic persistent and chronic aggressive hepatitis.

Chronic persistent hepatitis is characterized by portal inflammatory infiltration, preserved lobular architecture, and slight to absent fibrosis. Piecemeal hepatocellular necrosis is not conspicuous.

Chronic aggressive hepatitis is characterized by chronic inflammatory infiltrate involving not only portal veins but extending into the parenchyma with piecemeal necrosis and formation of intralobular septa. The architecture is distorted but there is no nodular regeneration. This progressive hepatitis undoubtedly proceeds in some instances to cirrhosis, a condition which is progressive and irreversible. Hepatocellular necrosis is a key factor initiating the aggressive hepatitis. This transcends zonal boundaries running from one lobule to another and from portal zone to central zone. This feature when seen in connexion with viral hepatitis has been called bridging. Recently, the importance of active fibrous septa engulfing liver cells in the pathogenesis of cirrhosis has been stressed. The active fibrosis is contrasted with benign, inactive septa. The hepatic histological picture may be identical to other forms of aggressive hepatitis such as that associated with the 'classic' lupoid variety. However, histological evidences of a previous acute hepatitis, for instance, centrilobular hepato-cellular drop-out and irregularity, do suggest that the active chronic hepatitis in that particular instance is associated with a positive antigen test.

Hepatic cirrhosis is marked by complete disorganization of the lobular architecture of the liver with hepatocellular nodule formation and widespread fibrosis.

Clinical

In general, it seems that the more florid and acute the original attack, the less likely are chronic sequelae to develop. In these circumstances immunological responses are presumably adequate to free the patient of antigen. Persistence of antigen in the serum after the acute attack usually implies the development of one or other form of chronic hepatitis. Nielson and coworkers from Copenhagen have studied 112 patients with Australia-antigen-positive hepatitis. In 88, the antigenaemia lasted less than 13 weeks but of 11 in whom antigenaemia persisted eight developed chronic aggressive and two persistent hepatitis.

There is a suggestion that the administration of corticosteroids during the acute attack may favour a later chronic hepatitis, but this is not proven. Chronic sequelae seem much more common in men than in women.

Patients with chronic persistent hepatitis often present when low transaminase levels fail to return to normal after an acute attack of hepatitis or are subsequently found to be elevated at a routine medical examination. Symptoms are vague, usually tiredness and indigestion. They are those of the post-hepatitis syndrome. Clinical stigmata of chronic liver disease are absent.
The serum transaminase levels may fluctuate, but the level of gamma globulin is virtually normal—a helpful prognostic sign. The prognosis of chronic persistent hepatitis is excellent and cirrhosis does not develop\textsuperscript{44}. The patient must be strongly reassured.

\textit{Chronic aggressive hepatitis} is somewhat similar clinically to the well known classical lupoid type which predominantly affects young women\textsuperscript{43}. The patient may be anicteric or mildly jaundiced, hepatosplenomegaly and vascular spiders may be found. In contrast to the 'classical' lupoid type of hepatitis, the patients tend to be male and older. Associated lesions of lupoid hepatitis such as acne, striae, diabetes, ulcerative colitis, renal tubular acidosis, and thyroiditis are not found. The onset is more abrupt, and antinuclear antibodies and LE cells are usually absent\textsuperscript{45}. Serum globulin levels are only moderately raised. Smooth muscle antibodies which are present in two-thirds of patients with the classical lupoid disease, if present, are only in low titre. The prognosis is probably better than for the lupoid type although this has not hitherto been accurately evaluated. The place of corticosteroid therapy also remains uncertain. Although of proven benefit in the classical, lupoid type\textsuperscript{44} its position in the Australia antigen group is uncertain. If the disease is clinically and biochemically active, prednisolone therapy should probably be given in the same way as it is for lupoid hepatitis. The end stages of chronic aggressive hepatitis are the same as for chronic hepatocellular failure with the problems of coma, ascites, jaundice, and portal hypertension to be faced\textsuperscript{48}.

\textbf{Primary Liver Cell Cancer}

Earlier reports failed to mention any association between Australia antigen and primary liver cell cancer\textsuperscript{47}. These negative results could have been related to relatively insensitive methods of detection. More recently, there have been many accounts of patients with hepatocellular cancer having positive HAA tests\textsuperscript{47}. Recent results from the Royal Free Hospital, London, showed that 10 of 46 patients with primary liver cell cancer in whom Australia antigen was sought gave positive results. There are considerable geographic differences in the percentage of positives. In Greece, the figure 38\% has been quoted\textsuperscript{48} and in Taiwan 80\%\textsuperscript{49}. Surprisingly, in a large series of 114 Singapore Chinese patients with liver cell cancer, only three (2.6\%) were positive whereas antigen was detected in 69 (4.2\%) of 1,632 blood donors\textsuperscript{51}.

In general, however, and particularly in Africa, the association with Australia antigen is frequent. This suggests that viral hepatitis could be an important antecedent of hepatocellular carcinoma, although of course, not the only one. The stages by which this arises remain uncertain. Hepatocellular carcinoma is usually found in association with cirrhosis. This would support the progression of the acute viral hepatitis through chronic aggressive hepatitis to macronodular cirrhosis whence one regeneration nodule ultimately becomes malignant. Another possibility is that the Australia antigen has oncogenic properties.

\textbf{The Place of Gamma Globulin in Prophylaxis}

It is now generally agreed that gamma globulin is effective in preventing or modifying the short-incubation virus A infection\textsuperscript{51,52}. Its place in the pre-
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vention of the long-incubation disease is still uncertain. In a very large cooperative study involving 14 hospitals and 5189 transfused patients undergoing cardiovascular surgery, hepatitis was not prevented by 10 ml of gamma globulin given during the first, fourth, and seventh postoperative weeks. In Germany, however, Creutzfeldt and his associates, in a controlled trial, showed that a 5% gamma globulin solution, treated enzymatically for intravenous use, and added to the blood before transfusion, had a prophylactic effect. More recently, R. Katz and colleagues from Chile have shown a significant reduction in the incidence of posttransfusion hepatitis with jaundice in patients receiving modified gamma globulin added to blood before transfusion as compared with controls receiving blood alone. Gamma globulin was modified by the Swiss method (hydrolysis), 10 ml of a 6% solution being added to each unit of blood before transfusion. The incidence of anicteric hepatitis in the two groups was not significantly different.

If these results are confirmed and such preparations of gamma globulin are indeed of value in preventing posttransfusion hepatitis, a considerable strain will be placed on world supplies of gamma globulin. Restraint will have to be exercised. The total current production of 16% gamma globulin in the United States is said to be between 6000 and 10000 litres per annum. It is also estimated that the total amount of gamma globulin required to modify posttransfusion hepatitis might be equivalent to 22200 litres of 16% gamma globulin. Although present facilities are insufficient to prepare this amount, an output of 18000 litres might be achieved. This is 81% of that required. Priority for receipt of gamma globulin should be given to those likely to require multiple blood transfusions or those likely to become chronic carriers of Australia antigen, for instance, haemophiliacs or patients in renal haemodialysis units.

Alternative methods of prevention will have to be found. One possibility would be to use serum with a high titre of specific antibody. One such high titre-specific gamma globulin gave detectable antibodies in recipients which lasted for at least two months. Their preventative value remains to be established.

It is possible that exogenous antibody may depress the immune response and so increase the chances of the development of a chronic carrier state. It might also impair the ability of the patient to rid himself of the infection.

A heat-denatured preparation of Australia antigen is also a possible means of prevention. Krugman and his colleagues have such a preparation. This would be more useful than antibody as it would stimulate natural immunity.

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References

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