THE LANCET

More about Australia Antigen and Hepatitis

THE discovery of Australia antigen and its association with hepatitis by BLUMBERG et al.[1] ushered in a new and exciting era of research on viral hepatitis. PRINCE called the antigen S.H. antigen because of its presumed association with serum rather than infectious hepatitis,[2] and, more recently, the designation of hepatitis-associated antigen (H.A.A.) has found favour because the antigen has been detected in both types of hepatitis. The spate of papers on Australia antigen created some confusion because the distinction between serum and infectious hepatitis seemed to be breaking down. The newer work makes this distinction clearer, though it suggests that the names "serum" and "infectious" may be inappropriate.

The two types of hepatitis were originally delineated by G. M. FINDLAY and F. O. MACCALLUM in the U.K., who worked with volunteers; their line of investigation was continued in the U.S.A.[3] Serum hepatitis came into prominence as an infection spread by inadequately sterilised syringes, by blood, or by blood products; but the work of KRUGMAN et al.[4] at Willowbrook State School showed that serum hepatitis, which they called MS2, can infect by mouth as well as parenterally and may also be spread by contact, though less readily than infectious hepatitis. There is some evidence, never confirmed, that serum-hepatitis virus may be excreted in urine.[5] Moreover, infectious hepatitis can be transmitted parenterally as well as by mouth.

Because viræmia is more prolonged in serum hepatitis—it may persist for many years—parenteral transmission is much commoner than with infectious hepatitis; but this is not a reliable diagnostic criterion for individual cases. A second distinguishing feature is the incubation period. Work with volunteers has shown that infectious hepatitis has an incubation period of around 30 days, whether virus is administered by mouth or parenterally; and this figure has been confirmed by epidemiological methods—for example, the classic work of PICKLES in Wensleydale.[6] Serum hepatitis, on the other hand, has a longer incubation period, almost always over 40 days and usually in the range 60–100 days. The Willowbrook investigations showed a shorter incubation period when the disease was transmitted parenterally than when it was transmitted orally. Unfortunately the incubation period cannot always be determined accurately, so that this diagnostic criterion is also suspect in individual cases. A third difference is that serum hepatitis is often a more prolonged and severe illness than infectious hepatitis. Moreover, in general, infectious hepatitis is commoner in children than is serum hepatitis, although this depends upon exposure to the agent of serum hepatitis, which may be high, for example, in institutions for retarded children. In summary, experimental work points to two forms of hepatitis distinguishable by differences in immunity, usual mode of transmission, incubation period, severity and duration, and age-incidence.

The work on Au/S.H. antigen strongly suggests that this is associated with serum hepatitis rather than infectious hepatitis. Firstly, re-examination of materials used in experimental induction of jaundice shows that icterogenic sera and blood products contain Au/S.H. antigen.[7–10] Reappraisal of the Willowbrook findings [8] shows clearly that MS2 (serum-hepatitis source-materials) contained Au/S.H. antigen and that recipients became antigenæmic, whereas MS1 (infectious-hepatitis materials) did not contain the antigen or render recipients antigenæmic. It is also clear that some people who receive Au/S.H. antigen become jaundiced, some get subclinical liver damage, and others have completely silent infections. Au/S.H. antigenæmia and liver damage may persist for many months or years. Point-source epidemics of infectious hepatitis, like the one in the Holy Cross football team,[11] have been found to be unassociated with Au/S.H. antigen—additional confirmation of the distinction. Also some work lately reported from Scotland [12,13] shows that, in the general population, Au/S.H. antigen is extremely rare under the age of 18.

It is not clear why the severity of hepatitis associated with Au/S.H. is so variable. BLUMBERG et al.[14] think that genetic factors may be of prime importance in the increased susceptibility of individuals with leukæmia and Down's syndrome to infection; but

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the explanation could lie also in their greater exposure to virus, whether in transfused blood or in institutional environments. These workers also believe that genetic factors may be responsible for the persistent antigenæmia without severe liver disease in individuals with leukæmia, Down's syndrome, and leprosy. Their studies of families in the Philippines and New Guinea suggest that susceptibility may be transmitted as a simple autosomal-recessive trait. Another factor may be the immune response of the host, as suggested by ALMEIDA and WATERSON,[15] who described three cases: one patient had chronic antigenæmia without disease, and had made no antibody detectable by electron microscopy; the second, who had chronic active hepatitis, showed immune complexes with excess free antigen; and the third, who died of acute hepatitis, had massive immune complexes with excess antibody. In outbreaks in renal-dialysis units [16] it is usual for the patients with impaired immunological responsiveness to have much milder disease than staff members. Although there is no evidence that γ-globulin prevents infection, it might possibly reduce the severity of the disease by impairing the immune response, rather as anti-D immunoglobulin prevents Rh hæmolytic disease. Another factor may well be differences in virulence of various strains of hepatitis virus.

Another question is the relationship between Au/S.H. and the virus of infectious hepatitis. ALMEIDA et al.[17] have drawn attention to electron microscopical differences between Au/S.H. antigen and viruses, and DANE et al.[18] and others have observed particles of about 40 nm. in Au/S.H.-positive sera which may be the virus. These particles are like some arboviruses, but this cannot be confirmed until the virus can be grown. COSSART and FIELD [19] have lately called attention to the similarity between Au/S.H. antigen and the appearances when proteins from some plant picornaviruses [20] are allowed to aggregate on a suitable polymer. It may be that S.H. virus is similar to an arbovirus—the frequency of antigenæmia in the tropics is in accord with this—or, like cowpea chlorotic virus, it may be a member of the picornavirus family; or it may be some other morphological type of virus. ALMEIDA and her colleagues [21] have observed picornavirus-like particles in the livers in two patients with Au/S.H.-positive hepatitis. These particles were similar to the kind seen in duck hepatitis, which suggested that they might be the virus of serum hepatitis. ALMEIDA et al. did not observe the particles in aggregation with Au/S.H. antigen, though this was known to be present, and they advanced the view that Au/S.H. antigen may be an aggregation of protein subunits derived from the virus. This material, which could be produced as a result of breakdown of unstable virions or excess production in infected cells, might be antigenically distinct from the virus just as the H antigen of poliovirus is antigenically distinct from the antigen on native virus.[22]

What practical steps can be taken to control the incidence of serum hepatitis? ALTER et al.[23] have made a plea for screening all blood-donors, and there is much sense in this. The most usual test is the gel-diffusion technique, whose sensitivity can be increased by previous concentration of specimens; electrophoresis also increases the sensitivity, as used, for example, by LOUS et al.,[24] and so does the use of radioactive indicator serum, as described by ROWE.[25] Electron microscopy is a valuable technique, either directly or with added antiserum to bring down antigen aggregates. (The structures described by ALMEIDA et al.[17] and DANE et al.[18] is characteristic.) This method also illustrates that free antigen may not always be present, so that complement fixation might be more sensitive; this technique has been widely used and found to detect more positive sera.[9,26,27] The complement-fixation test is, however, very insensitive for detecting antigen. BARKER et al.[9] found that an icterogenic serum produced disease in volunteers at a thousandfold lower dilution than it reacted in the complement-fixation test. A more sensitive test—perhaps a radioimmune assay—is badly needed.

In the U.K., the incidence of Au/S.H. antigenæmia is very low, especially in people under 18, and so too is the risk of hepatitis following blood-transfusion. However, wherever the risk of hepatitis is high, screening is important. The most serious problem relates to renal-dialysis units. The reason for the high incidence in these units is unknown, but the infections probably start with the introduction of transfusion hepatitis into one patient, followed by the spread of virus through the unit. Certainly, the aseptic techniques in many units would not preclude the spread of a blood-borne agent. Further evidence of the unsatisfactory nature of the precautions in these units has just been published.[28] Also, the infection may well be spread by contact in these units. Finally, some cases of kidney failure may be the result of Au/S.H.-immune-complex disease, for there are striking similarities with the late renal disease in mice infected with lymphocytic-choriomeningitis virus.[29] All patients admitted for renal dialysis should

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be regularly checked for Au/S.H. antigenæmia, and special aseptic precautions should be taken with carriers. Unfortunately γ-globulin seems ineffective, but specific antibody may be effective in that it prevents experimental reinfection..

The discovery of Au/S.H. has greatly increased our understanding of viral hepatitis, but there is still a long way to go before the disease can be controlled. This new approach to the aetiology of serum hepatitis may well be applicable also to infectious hepatitis. Indeed, the first reports are already appearing. Two weeks ago, FERRIS and his colleagues described in this journal a rabbit antiserum which reacted with extracts of faeces from some patients with infectious hepatitis; and last week, DEL PRETE and his colleagues reported work which may, they believe, lead to the serological differentiation of infectious hepatitis. These reports require confirmation, but they may open the way to as big an advance in infectious hepatitis as the discovery of Au/S.H. was in serum hepatitis.

L.A.T.S.

THE long-acting thyroid stimulator (L.A.T.S.) was first described in 1956 by ADAMS and PURVES,[1] who found that injection of serum from a patient with thyrotoxicosis into suitably prepared animals caused thyroid stimulation with a later onset and longer duration that that obtained with thyroid-stimulating hormone (T.S.H.). Since then many studies have indicated that L.A.T.S. may be the substance causing thyrotoxicosis in those cases not due to an autonomous thyroid adenoma.[2,3] L.A.T.S. has been shown to be an immunoglobulin G (IgG) with the properties of an antibody against thyroid: it combines highly specifically with thyroid extracts and can be recovered from such combination in conditions which dissociate antigen-antibody complexes. Lymphocytes from patients with thyrotoxicosis can produce L.A.T.S. in vitro.[4] T.S.H. differs from L.A.T.S. in a number of respects [5] and cannot be extracted from the IgG fraction of serum which contains L.A.T.S. Conversely, L.A.T.S. cannot be isolated from the pituitary gland. T.S.H. levels are normal or low in thyrotoxicosis, and no case of hyperthyroidism due to a T.S.H.-secreting pituitary tumour has been described in man.[6]

At present, the only methods available for measuring L.A.T.S. are based on the mouse bioassay technique of MCKENZIE [7] which has definite limitations. With prior concentration of serum IgG, L.A.T.S. can be detected in up to 80% of patients with thyrotoxicosis,[8] but with untreated serum it can be found in only a quarter to a half of such patients. Thus Professor SELLERS and his colleagues, whose article we publish this week, detected L.A.T.S. in 37% of 248 patients with thyrotoxicosis and concluded that L.A.T.S. did not cause the thyroid overactivity. However, there are other reasons for believing that it may do so. Congenital thyrotoxicosis is a transient thyroid overactivity in children born to mothers with high serum-L.A.T.S. levels. L.A.T.S. is initially found in the child's blood,[9,10] and as it disappears the child becomes euthyroid. Similarly, injection of L.A.T.S.-containing serum into normal volunteers causes transient thyroid stimulation.[11]

Because L.A.T.S. has the properties of an antibody against thyroid tissue, other evidence of autoimmune disease has been sought in patients with thyrotoxicosis. Thyroid autoantibodies have been demonstrated in 85% of such patients [12] and there is an increased incidence of pernicious anæmia and other autoimmune conditions in them and in their relatives. Thyrotoxicosis itself tends to run in families, and L.A.T.S. has been detected in 9 of 43 apparently euthyroid relatives of affected patients.[13] This interesting finding suggests that circulating L.A.T.S. on its own does not stimulate the thyroid. A similar situation exists in patients with endocrine exophthalmos.[14] A coexistent thyroiditis could explain the findings in some of these subjects, but would be unlikely to be present in all the symptomless relatives found to have L.A.T.S. In addition, L.A.T.S. has been detected in some normal subjects [15]; and lymphocytes, apparently sensitised both to thyroglobulin and to the thyroid component with which L.A.T.S. combines, have been found not only in each of 19 patients with thyrotoxicosis, but also in 19 of 20 normal subjects.[16] These findings are puzzling and difficult to reconcile with the idea that L.A.T.S. alone causes thyrotoxicosis. Possibly a thyroid-gland abnormality or some conditioning mechanism is required for L.A.T.S. to exert its stimulating action.

The exact mechanism by which L.A.T.S. acts on the thyroid gland remains unknown. Both L.A.T.S. and T.S.H. stimulate the adenyl-cyclase system in thyroid cells with the formation of 3'5'-cyclic adenosine monophosphate (cyclic A.M.P.), which is the final common path of many stimulating hormones.[17,18] T.S.H. is

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