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FURTHER CHARACTERIZATION OF HBsAg BINDING WITH POLYMERISED HUMAN SERUM ALBUMIN BY ELISA TECHNIQUE

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The basic mechanism of hepatitis B virus (HBV) entry into hepatocyte during infection is still an unresolved problem. Circulating polymerised human serum albumin (pHSA), which is usually formed during HBV infection, binds to HBsAg and thus plays an important role in the attachment of HBV to hepatocyte by acting as a linker between HBV and hepatocyte membrane. Recently, binding between HBsAg and pHSA has been studied in more details. However, the binding activity in relation to HBe-markers still remains controversial. The high binding activity in presence of HBeAg, as reported earlier was presumed to be due to high HBsAg concentration, however, Hopf et al (1984, Liver 4: 372-378), ruled out the concentration effect and attributed it to the nature of HBsAg particles varying in their binding efficiency. In an attempt to further characterise this binding process, we developed a highly sensitive ELISA technique using pHSA coated microtitre plate as the solid phase and HRPO bound horse anti-HBs as the conjugate.

This technique was found to be highly specific with a sensitivity of 0.2 ug HBsAg/ml. An analysis of HBsAg negative (25) and HBsAg positive sera from healthy HBV carriers (31) and patients group of acute viral hepatitis (44), fulminant hepatitis (14), cirrhosis of liver (10) and chronic active hepatitis (5), indicated the binding activity only in HBsAg positive sera and not in HBsAg negative sera. When the binding activity was analysed in relation to the presence of HBe-markers in each patient groups, it was detectable both with and without HBe-markers (50-100 %). However, the mean binding activity was comparatively high in presence with HBeAg. In order to see the role of human serum immunoglobulins (IgG, IgM and IgA) in the binding process, an inhibition assay, using rabbit anti-human IgG, IgM and IgA, was performed and only anti-IgM was found to be reducing the binding activity (>50 %). This stresses upon the possible involvement of serum IgM in the binding process. This is further supported by the presence of binding activity in 70-100 % sera samples positive for HBsAg/IgM complex as compared to merely 0-14 % sera samples in absence for this complex. The results of the present study indicate that binding depends on the nature of HBsAg particles and is least affected by the presence of HBe-markers in serum. The serum IgM that circulates with HBsAg as HBsAg/IgM complex, represents anti-pHSA and helps in binding process. HBsAg bound pHSA gets attached to the hepatocyte membrane thereby concentrating HBV on the site where they get entry into the cell by a mechanism to be still resolved.