Electrofocusing of Hepatitis B Antigen

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SUMMARY

Hepatitis B (Australia) antigen was isolated from normal serum proteins by gel filtration and electrofocusing. The latter technique revealed a heterogeneity of the small spherical particles associated with hepatitis B antigen activity, there being a variance in determined isoelectric points according to the serological subtype studied. Hepatitis B antigen isolated in this manner was aggregated by concanavalin A.

Early studies by staining and flotation experiments disclosed the lipoprotein nature of the hepatitis B (Australia) antigen which is closely associated with the human hepatitis type B virus (serum hepatitis virus). Several techniques for the isolation of hepatitis B antigen from normal serum proteins by equilibrium and rate zonal sedimentation have been described (Gerin et al. 1969; Vyas et al. 1972), but these methods are time-consuming and there is evidence to suggest the isolated hepatitis B antigen is still closely associated with normal human serum proteins (Millman et al. 1970). A procedure is described which offers the possibility of isolating hepatitis B antigen by a simple two-step procedure which negates the need for equilibrium and rate-zonal sedimentation and reveals a heterogeneity of the predominant morphological form as viewed in the electron microscope.

Isoelectric focusing may be used for analytical or preparative separation from heterogenous mixtures of individual ampholytes, particularly proteins, and the technique has recently been extended to the study of the protein components of viruses (Shortridge & Biddle, 1970; Hung, Robinson & Robinson, 1971). The technique offers almost complete recovery of total protein after separation. Prior concentration of the sample is rarely necessary.

The plasma samples used for electrofocusing were obtained from a number of apparently healthy carriers of hepatitis B antigen, one of whom had recently been implicated in two deaths from post-transfusion hepatitis in transfused recipients. The identity of the antigen in each sample was confirmed by several tests including immunodiffusion, complement fixation and radioimmunoassay. The predominant morphological type was the small spherical particle 20 to 25 nm diam., as revealed by immune electron microscopy (Memorandum, 1970). Plasma was clarified by preliminary centrifuging at 15000g for 30 min at 4°C and the supernatant fluid was subjected to gel filtration in order to remove the major portion of the normal plasma proteins. Samples of 75 ml were applied to a 100 x 5 cm diam. Sephadex G 200 column and the hepatitis B antigen eluted in the void vol. with 0.05 M-tris-hydrochloric acid buffer at pH 7.4. Total protein content of the void vol. was found to be 5% of that in the applied sample as estimated by the method of Lowry et al. (1951). There was little or no loss of complement fixation titre using the above conditions.

Samples of the partially purified hepatitis B antigen containing not more than 10 mg of protein were used in the formation of a 0 to 40% (w/v) sucrose gradient. A 40% (w/v) solution of a mixture of carrier ampholytes (Ampholine) was added to the gradient at a final concentration of 1% (w/v). The anode was protected with a 1.4% solution of orthophosphoric acid in 60% (w/v) sucrose and the cathode with a 2% solution of ethanolamine in water. In some experiments, the final concentration of carrier ampholytes was increased...
to 2% (w/v) in order to enhance the solubility of focused proteins, or the nonionic detergent Brij 35 added to a final concentration of 0.1%. The pH gradient was established in an electric field maintained at an output of 3 W during the initial 24 h and the sample was fractionated in the gradient after a further period of 10 h.

The elution profile of focused protein from a typical experiment is shown in Fig. 1. The areas under peaks I and II were found to be positive for hepatitis B antigen by immunodiffusion, counter-immunoelectrophoresis (Memorandum, 1970), latex particle agglutination and solid-phase radioimmunoassay. In addition, the hepatitis B antigen was detected by complement fixation after exhaustive dialysis of separated proteins against phosphate-buffered saline, pH 7.2, and when examined by immune electron microscopy. Normal serum proteins were not detected in either peak of hepatitis B antigen by immunodiffusion against horse whole human protein antiserum. The protein in both peaks was aggregated upon addition of concanavalin A (330 µg/mg separated protein) at room temperature in phosphate-buffered saline. The effect was reversed upon addition of α-methyl-D-mannoside (0.1 mol).

The appearance of the separated hepatitis B antigen particles by immune electron microscopy is shown in the plate. There is thus at least one antigenic determinant in common with both peaks of antigenic activity. There is no significant difference in diam., although the particles isolated in peak I (Fig. 2b) appear to be not as clearly defined when compared to peak II (Fig. 2a). There has recently been some suggestion that the small spherical particles may be either 20 nm or 25 nm in diam. with a corresponding difference in determined mol. wt. (Dreesman et al. 1972). The isoelectric points of isolated hepatitis B antigen were determined as 3.65 and 4.33 for peaks I and II, respectively, for the material initially characterized as being of the ‘ad’ subtype. One sample of the ‘ay’ subtype was available for study, the values being determined as 3.95 and 4.90. This difference almost certainly reflects the antigenic composition of the particle surface. The low values of determined isoelectric points could be due also to the presence of phosphatidyl serine in the lipid component of the antigen, but lipid analyses of hepatitis B antigen purified by sedimentation precludes this possibility (Kim & Bissell, 1971; T. Takahashi, personal communication). Interestingly
enough, similar values of determined isoelectric points have recently been obtained for Qβ phage and several plant viruses (Rice & Horst, 1972) as well as for Rous sarcoma virus (Hung et al. 1971).

The extinction in peak I increased at the expense of peak II after prolonged storage of partially purified material. This may be due to an ageing effect on the apoprotein. Also of interest is the observation that purified hepatitis B antigen in both peaks is aggregated by concanavalin A. Carbohydrate has been reported to be present (Bond, 1972) and this may contribute to the antigenic composition of the particle surface (Burrell et al. 1973), as well as to the overall ionic properties of the antigen.

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**Hepatitis Research Unit**
Department of Microbiology
London School of Hygiene & Tropical Medicine
London, WC1E 7HT

C. R. HOWARD
A. J. ZUCKERMAN

**REFERENCES**

New York: Greive and Stratton.


**Fig. 2.** Electron microscopic appearance of hepatitis B antigen isolated in (a) peak II and (b) peak I.
Short communications


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