Radioimmunoassay of Hepatitis B e-antigen (HBeAg): identification of HBeAg not associated with Immunoglobulins

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(Accepted 30 August 1978)

SUMMARY

A radioimmunoassay for hepatitis B e-antigen (HBeAg) is described. Polystyrene beads coated with IgG prepared from a human serum containing antibodies to HBeAg (anti-HBe) and anti-HBe IgG labelled with \(^{125}\text{I}\)-p-hydroxyphenylpropionic acid \(N\)-hydroxysuccinimide ester were used in the test. The radioimmunoassay was approximately 1000-fold more sensitive than immunodiffusion. At least a transient presence of HBeAg in serum appears to be a common feature of infections by hepatitis B virus. The radioimmunoassay was instrumental in establishing conditions for identification of apparently free monomeric HBeAg. The HBeAg has an approximate mol. wt. of 35000 and was recovered after isoelectric focusing in fractions with a pH between 4.25 and 4.8. Polyacrylamide gel electrophoresis revealed the presence in HBeAg of a polypeptide with an apparent mol. wt. of 17000.

INTRODUCTION

Magnius & Espmark (1972) discovered a new antigen specifically associated with hepatitis B virus (HBV) infections. This antigen, designated as e-antigen (HBeAg), appears to be an indicator of the relative infectivity of hepatitis B surface antigen (HBsAg)-positive sera (Alter et al. 1976; Grady, 1976; Okada et al. 1976; Beasley et al. 1977; Shikata et al. 1977) in agreement with laboratory findings relating the detection of HBeAg in serum with relatively high concentrations of Dane particles, the putative HBV (Nordenfelt & Kjellén, 1975; Takahashi et al. 1976; Tong et al. 1977; Werner et al. 1977).

Recent studies revealed that the antigenic sites of HBeAg are associated with immunoglobulins (Neurath & Strick, 1977). Takahashi et al. (1978) identified another, less abundant form of HBeAg, not associated with immunoglobulins, having a mol. wt. of approx. 125000 (Miyakawa & Mayumi, 1978).

We describe here a sensitive radioimmunoassay (RIA) for HBeAg. This test was instrumental in discerning a population of HBeAg not bound to immunoglobulins; for recognising and overcoming the problems due either to association of HBeAg with serum proteins or to self-aggregation and finally, for the identification of apparently free and monomeric HBeAg.
METHODS

Radioimmunoassay (RIA) for HBeAg and for antibodies to HBeAg (anti-HBe). Polystyrene beads were coated with anti-HBe IgG as described before (Neurath et al. 1978c). Sera containing anti-HBe detectable by rheophoresis at a dilution of at least 1:10 were used as the source for antibodies. IgG was purified by DEAE-cellulose (DE 52, Whatman Ltd, Springfield Mill, Maidstone, Kent, England) chromatography using 0.01 M-phosphate, pH 8.0 as eluant (Fudenberg, 1967). Solid NaCl to a final concentration of 0.5 M was added to the solution of IgG which had passed through the DEAE-cellulose column and was then applied to a column of insolubilized normal human serum linked to Sepharose 4B (2 to 2.5 ml gel per ml of solution) to remove autoantibodies to IgG and albumin possibly present in anti-HBe-positive sera (Matuhasi & Hosokawa, 1972; Kacaki et al. 1977; Tedder & Briggs, 1977; A. R. Neurath, unpublished data). After 1 to 2 h at room temperature and 1 h at 4°C, unbound IgG was eluted from the column with 0.05 M-phosphate-0.5 M-NaCl, pH 7.5.

The purified IgG which did not contain any antibodies to HBsAg as determined by RIA was dialysed against 0.05 M-borate, pH 8.5, for labelling with I25I-Bolton-Hunter reagent (Amersham, Arlington Heights, Illinois). Samples containing 50 μg of IgG were labelled with 500 μCi of the radioactive reagent overnight at 0°C. Two hundred μl of 1.0 M-glycine-0.1 M-borate, pH 8.5, were added to the mixture at 0°C, and after 30 min the labelled IgG was separated from other radioactive products by gel filtration on 0.7 x 20 cm columns of Sephadex G-75 (Pharmacia, Uppsala, Sweden) using 0.05 M-phosphate, pH 7.5, containing 2.5 mg/ml of gelatine as eluant. The iodinated IgG was then dialysed against 0.01 M-tris-0.14 M-NaCl-0.02 % NaN3 (TS) and finally diluted to contain labelled IgG corresponding to 200000 cts/min by adding normal human serum diluted 10-fold with TS.

To determine HBeAg, undiluted serum specimens or samples diluted in normal human serum were incubated overnight at room temperature with anti-HBe-coated beads. The beads were washed with TS and then incubated for 2 h at 37°C with 300 μl of normal human serum (a pool of ~30 sera) diluted 1:10 with foetal calf serum and with 100 μl of the I25I-labelled anti-HBe. Finally, the beads were washed and counted in a γ-counter. Results were expressed in RIA ratio units corresponding to cts/min obtained for a specimen divided by cts/min obtained for normal human serum controls. Samples with an RIA ratio > 2.1 were considered positive for HBeAg.

To determine anti-HBe, equal vol. of specimens (undiluted or diluted in normal human serum) and of an HBeAg-positive serum (from a chronic carrier of HBsAg; see Fig. 1), diluted 1:40 with normal human serum, were mixed and incubated for 30 min at 37°C. Residual HBeAg was determined as described above.

Since the purified IgG contains antibodies to hepatitis B core antigen (anti-HBc) in addition to anti-HBe (Neurath et al. 1978c), the described test would not distinguish between HBeAg and HBcAg. However, the presence of anti-HBc and the absence of free HBcAg in HBeAg-positive sera (Neurath et al. 1978c) renders the test specific for HBeAg if applied to unprocessed serum specimens. To confirm the specificity of the test when applied to preparations of partially purified HBeAg (which would not contain anti-HBe), duplicate samples diluted 1:1 either with normal human IgG or with anti-HBc IgG [isolated from an HBeAg-positive serum; RIA titre 1:1 250 (Neurath et al. 1978c)] were tested. Similar cts/min indicated the absence of HBcAg from the specimen.

Partial purification of HBeAg. Serum from which Dane particles and most of the tubular forms of HBsAg had been removed by high speed centrifugation (Neurath et al. 1976) was
used for purification of HBeAg which was partially purified by affinity chromatography on columns of insolubilized anti-HBe (Neurath & Strick, 1977) either directly from serum; from resuspended pellets obtained after precipitation of serum proteins with polyethylene glycol (final concentration 60 mg/ml); with (NH₄)₂SO₄ (final concentration 1.33 M) or from precipitates obtained by increasing the concentration of (NH₄)₂SO₄ from 1.33 M to 1.9 M.

Two methods were used in attempts to dissociate HBeAg from immunoglobulins: (a) extraction of serum or of partially purified HBeAg with trichloroacetic acid in ethanol (Iwata et al. 1968). The extract was extensively dialysed against TS containing 3 µg/ml of phenylmethyl sulphonfluoride (PMSF; Sigma, St. Louis, Missouri, U.S.A.) and concentrated to the original sample vol. by immersing the dialysis bags containing the extract in Sephadex G-50 (Pharmacia, Uppsala, Sweden). (b) Gel filtration of samples containing partially purified HBeAg and NaSCN (3 M) on 1.5 x 30 cm columns of either Sepharose 6B CL (Pharmacia), Biogel P-300 (BioRad Laboratories, Richmond, California), Sephadex G-150 or G-100. The eluant in all cases was 3 M-NaSCN. Fractions of 1 ml were collected and dialysed against TS containing 3 µg/ml of PMSF. The following proteins, chromatographed in separate experiments on the same columns, served as markers for determination of mol. wt. (Andrews, 1964): cytochrome c, haemoglobin, ovalbumin, human serum albumin and IgG. Ferritin and phenol red were used as markers for the void and included vol. of the columns, respectively.

For reduction and alkylation, samples of HBeAg purified from serum by affinity chromatography were treated with 2-mercaptoethanol (10 µl/ml) in the presence or absence of 8 M-urea for 3 h at room temperature; NaCH₂COO (30 mg/ml) was added, the samples were stored overnight at 4 °C and finally dialysed against TS.

To estimate the size of HBeAg, samples were layered on top of a gradient (4.5 ml in centrifuge tubes for the SW 65 rotor, Beckman Instruments, Palo Alto, California) of 10 to 30 % (w/v) sucrose in 3 M-NaSCN. The tubes were centrifuged for 16 h at 57000 rev/min and fractions of 0.3 ml were collected from the bottom of the tube and dialysed for subsequent determinations of HBeAg. Two marker substances, human serum albumin and IgG, were centrifuged simultaneously in separate tubes.

**Other methods.** Polyacrylamide gel electrophoresis (PAGE); isoelectric focusing; affinity chromatography on insolubilized antibodies to normal human serum proteins; determinations of HBsAg; anti-HBs, HBeAg by rheophoresis, and of serum glutamic-pyruvic transaminase (SGPT) were all performed as described before (Neurath & Strick, 1977; Neurath, et al. 1978a, c).

The following samples were submitted to PAGE: (a) purified HBeAg (Fig. 6) labelled with ¹²⁵I-Bolton-Hunter reagent (Neurath & Strick, 1977) and precipitated by addition of 100 µl of anti-HBe-serum (positive by rheophoresis) after adding Tween 20, to a final concentration of 0.1 %, and 50 µl of HBeAg-positive serum as a carrier. The mixture was incubated for 1 h at 37 °C and overnight at 4 °C. The pellet obtained after centrifugation at 15000 rev/min for 20 min was dissolved in 0.0625 M-tris (hydroxymethyl) aminomethane-8M-urea-1 % (w/v) sodium dodecyl sulphate, pH 7.2, and boiled for 7 min; (b) labelled HBeAg prepared from partially purified preparations by extraction with CCl₄COOH-C₃H₅OH precipitated in the same way; (c) a ‘mock’ preparation of HBeAg prepared as described under (a), except that normal human serum instead of HBeAg-positive serum was used as the starting material; (d) a precipitate obtained as given for (a) except that normal human serum was used as a carrier and that anti-HBe was replaced by a rabbit antiserum to IgG.
RESULTS

Specificity and relative sensitivity of the radioimmunoassay for HBeAg

Table I summarizes the results of RIA tests for HBeAg obtained with specimens from pre-selected groups of individuals. Sera positive for HBeAg (40) by rheophoresis were tested in preliminary experiments. All specimens were positive. HBeAg was detected by RIA in pre-selected groups of sera which were negative for both HBeAg and anti-HBe by rheophoresis: in all of 29 sera from Taiwanese mothers who transmitted hepatitis B to their babies (C. E. Stevens et al. unpublished data); in all of 13 sera from patients with acute hepatitis B and in 37 out of 71 sera from HBsAg-positive blood donors. The RIA for HBeAg is about 1000 times more sensitive than rheophoresis (Fig. 1).

HBeAg was not detected in sera (109) from HBsAg-negative blood donors. However, false positive results were obtained with 29.4% of the sera when foetal calf serum was not added to the anti-HBe coated beads during incubation with 125I-labelled anti-HBe. The non-specific binding of the radioactive antibody was not inhibited by addition of unlabelled anti-HBe to the specimens unlike HBeAg-positive samples (Fig. 2). The inhibition of the attachment of HBeAg to the beads by anti-HBe is the basis for an RIA-inhibition test measuring anti-HBe. This assay is about 200 times more sensitive than rheophoresis (Fig. 2). Anti-HBs or anti-HBc were each negative by the test for anti-HBe.

HBeAg was detected in serum specimens (10) of individuals with acute non-A, non-B hepatitis and in sera containing rheumatoid factor (7; latex agglutination titres between 1:160 and 1:2560). The 20 nm forms of HBsAg, purified as described by Neurath et al. (1978b) did not contain detectable HBeAg.

HBeAg was detected in the serum of all of 31 patients or staff members of haemodialysis units who became HBsAg-positive either transiently (25) or who had not cleared HBsAg at the termination of surveillance (6) [Fig. 3]. In comparison, HBeAg was detected by rheophoresis in the serum of only 18 of these individuals.

In cases of transient HBsAg antigenaemia, HBeAg was first detected by RIA at about the same time as HBsAg and before the appearance of elevated SGPT levels; the level of HBeAg in serum decreased to undetectable levels before clearance of HBsAg and soon thereafter anti-HBe became detectable several months before the appearance of anti-HBs when the titre of anti-HBe had already declined (Fig. 4). Conversion from HBeAg- to anti-HBe-positivity was observed in 10 of 13 individuals who had transient HBsAg antigenaemia.

Isolation and partial characterization of HBeAg not associated with IgG

Takahashi et al. (1978) identified a subpopulation of HBeAg which was not associated with IgG and was precipitated from serum by (NH₄)₂SO₄ when the salt concentration was increased from 1.33 to 2.0 M. Application of this fractionation procedure to 15 distinct sera, positive for HBeAg by rheophoresis, resulted in preparations containing HBeAg detectable by RIA but not by rheophoresis. Isoelectric focusing and molecular exclusion chromatography performed as described before (Neurath & Strick, 1977) indicated that this HBeAg was heterogeneous with respect to both size and isoelectric points, suggesting that the HBeAg was still either aggregated or associated with other serum proteins. Indeed, affinity chromatography on columns of insolubilized antibodies to normal human serum to which HBeAg became attached indicated its association with a serum protein(s).

Therefore, alternative purification procedures were sought in attempts to isolate HBeAg in free form. First, HBeAg was extracted with trichloroacetic acid-ethanol either from
Table 1. Detection of HBeAg by RIA in sera obtained from pre-selected groups of individuals

<table>
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<tr>
<th>Characterization of group</th>
<th>No. of HBeAg positive sera</th>
<th>Total no. of sera screened</th>
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<tbody>
<tr>
<td>(1) Blood donors positive for HBsAg and HBeAg (by rheophoresis)</td>
<td></td>
<td>40/40</td>
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<tr>
<td>(2) Sera negative for both HBeAg and anti-HBe by rheophoresis from</td>
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<tr>
<td>(a) Taiwanese mothers who transmitted hepatitis B to their babies</td>
<td>29/29</td>
<td></td>
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<tr>
<td>(b) patients with acute hepatitis B</td>
<td>13/13</td>
<td></td>
</tr>
<tr>
<td>(c) HBsAg-positive blood donors</td>
<td>37/71</td>
<td></td>
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<tr>
<td>(3) Patients or staff members of haemodialysis units during transient HBsAg antigenaemia</td>
<td></td>
<td>25/25</td>
</tr>
<tr>
<td>(4) HBsAg-negative blood donors</td>
<td>0/109</td>
<td></td>
</tr>
<tr>
<td>(5) Individuals with acute non-A, non-B hepatitis</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>(6) HBsAg-negative individuals positive for rheumatoid factor</td>
<td>0/7</td>
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serum or from partially purified preparations obtained by affinity chromatography. This method yielded an HBeAg with an isoelectric point of 4·8 (Fig. 5). HBeAg extractable by CCl₄COOH-C₂H₅OH appeared in the serum during transient HBsAg antigenaemia at the same time as HBeAg was detected directly in serum specimens (Fig. 4). It was not detected in extracts from normal human serum. Unfortunately, the yield of HBeAg by this method corresponded to about 3 % of the total serum HBeAg and the antigen still appeared heterogeneous with respect to size and was adsorbed to columns of insolubilized antibodies to normal human serum and to anti-albumin, but not to anti-IgG columns.

A more profitable approach resulted from preliminary experiments in which partially purified HBeAg was submitted to rate zonal centrifugation in gradients containing 3 M-NaSCN. Under these conditions, most of the HBeAg detectable by RIA sedimented slower than human serum albumin. In subsequent experiments, HBeAg was submitted to gel filtration on columns of Sepharose 6B CL, Biogel P-300, Sephadex G-100 and G-150 using 3 M-NaSCN as eluant. Results obtained with columns of Sephadex G-100 (Fig. 6) or G-150 calibrated with mol. wt. standards revealed that HBeAg has an apparent mol. wt. of about 35000. HBeAg was also purified 100-fold in this step, corresponding to a total approximate 10⁴-fold purification as compared with the original serum. HBsAg and HBeAg were not detectable by RIA in the purified preparation of HBeAg. After isoelectric focusing, HBeAg was detected in fractions having a pH between 4·25 and 4·8.

Molecular exclusion chromatography of crude HBeAg-positive serum or of HBeAg reduced and alkylated in the absence of urea, a procedure which led to an approximate fivefold increase of HBeAg activity as estimated from calibration curves (Fig. 1), resulted in similar mol. wt. estimates when 3 M-NaSCN was used as eluant. Reduction and alkylation in 8 M-urea destroyed HBeAg which was not inactivated by exposure to 8 M-urea alone or to 8 M-urea + NaCH₂ICOOC. When HBeAg-positive serum was chromatographed on Sephadex G-100 using TS as eluant, the major portion of HBeAg was recovered in fractions corresponding to the void vol. of the column. Re-chromatography of the purified low mol. wt. HBeAg (mixed 1:1 with normal human serum) on Sephadex G-100 using TS as eluant
Fig. 1. Radioimmunoassay of HBeAg. Relationship between ¹²⁵I-labelled anti-HBe bound to polystyrene beads and the dilution of sera from a chronic HBsAg carrier (○—○) and a patient with transient antigenaemia undergoing haemodialysis (●—●). The sera were positive for HBeAg by rheophoresis undiluted and diluted 1:8, respectively.

Fig. 2. Radioimmunoassay inhibition test of anti-HBe. Dose response curves for sera from a chronic HBsAg carrier (●—●) and a patient with transient antigenaemia undergoing haemodialysis after clearance of HBsAg and HBeAg from serum (○—○). The first serum was positive for anti-HBe by rheophoresis at a dilution of 1:10; the second serum was negative. Horizontal broken lines indicate radioactivity corresponding to controls containing normal human serum, and normal human serum + HBeAg, respectively.
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Fig. 3. Results of radioimmunoassays of HBeAg in sera of patients and staff members of haemodialysis units before (○) and after (●) appearance of HBsAg in serum. The results corresponding to each individual were plotted and connected with a line. The time interval between the two specimens from each individual corresponds to the shift between the two points along the abscissa.

Fig. 4. Example of the development of HBeAg and anti-HBe in relation to other serum markers of infection by HBV in a patient undergoing haemodialysis. Short perpendicular lines at the ends of the bars representing different HBV markers indicate positivity at the termination of surveillance. ■, elevated SGPT; □ HBsAg; □, anti-HBs; ●, HBeAg detectable by rheophoresis; □, anti-HBe determined by RIA. Shaded area corresponds to HBeAg detected in trichloroacetic acid-ethanol extracts from serum. The titre of anti-HBe corresponds to 50% inhibition end-points determined from dose-response curves (see Fig. 2). ●●●, HBeAg; ○○○, anti-HBe.
Fig. 5. Isoelectric focusing of HBeAg soluble in trichloroacetic acid-ethanol. [●], HBeAg; [○], pH.

Fig. 6. Gel filtration on a column (1.5 x 30 cm) of Sephadex G-100 of HBeAg partially purified by affinity chromatography. [●], protein; [□], concentration of HBeAg determined from a calibration curve (see Fig. 1). Arrows correspond to the void vol. (1) and the included vol. (2) of the column. 3 M-NaSCN was used as eluant. One ml fractions were collected.
Fig. 7. Polyacrylamide gel electrophoresis of HBeAg isolated by molecular exclusion chromatography (see Fig. 6), labelled with \(^{125}\)I-Bolton-Hunter reagent, and precipitated by anti-HBe-positive serum after adding unlabelled HBeAg as carrier. The peak of radioactivity at the leading edge of the gel (13.8 cm) material corresponds to low mol. wt. material of unknown nature.

(Fig. 6) indicated that the mol. wt. of HBeAg remained the same, i.e. that HBeAg did not become attached to normal human serum proteins under those conditions.

PAGE of labelled HBeAg co-precipitated with non-radioactive unpurified HBeAg by anti-HBe revealed the presence of two major peaks of radioactivity in sections of the gel in which polypeptides with mol. wt. of 23000 to 26000 and 17000, respectively, would be recovered (Fig. 7). When unlabelled HBeAg was replaced by normal human serum as carrier and anti-IgG was used instead of anti-HBe, the polypeptide with an approximate mol. wt. of 17000 (P17) was not detected by PAGE of the radioactive precipitate. This suggests that the two peaks of radioactivity correspond to two distinct proteins and that only P17 is specific for HBeAg. Similar results were obtained with HBeAg isolated by \(\text{CCl}_3\text{COOH-C}_6\text{H}_4\text{OH}\) extraction. P17 was not detected in normal human serum components isolated by affinity chromatography on insolubilized anti-HBe followed by molecular exclusion chromatography under conditions given for Fig. 6.

**DISCUSSION**

Until recently, immunodiffusion was the only technique for detection of HBeAg. The insensitivity of this method and the relatively low levels of HBeAg in HBsAg-positive sera made the attempts to characterize HBeAg frustrating. Takahashi et al. (1977) developed haemagglutination tests for HBeAg and anti-HBe using immunochemically purified reagents obtained by affinity chromatography with relatively low yields. The development of more sensitive RIA using IgG from human sera with a high titre of anti-HBe (Biswas et al. 1978;
Froessner et al. 1978; Mushahwar et al. 1978; Tedder, 1978) or chimpanzee anti-HBe (Fields et al. 1978) was described very recently in brief form. The source for the reagents for the RIA described here was a serum which had an anti-HBe titre of 1:10 as determined by rheophoresis, a titre considered to be low for development of an RIA for HBeAg (G. G. Froessner, personal communication). Screening of hundreds of HBsAg-positive sera in our laboratory failed to detect a serum with an anti-HBe titre > 1:10. Therefore, special precautions were taken to avoid losses of antibody activity during radioactive labelling and to ensure the specificity of anti-HBe. The mild 125I-Bolton-Hunter reagent was chosen for labelling of anti-HBe. The sensitivity of the RIA for HBeAg described here equals that reported by others (Mushahwar et al. 1978) despite the relatively low anti-HBe titre of the serum used as source for our reagents. However, when applied to testing of anti-HBe, the RIA was about 30 times less sensitive as compared with results reported by Mushahwar et al. (1978).

The studies aimed at demonstrating the specificity of the RIA indicate that the appearance of HBeAg in serum for at least a limited time interval is a common consequence of infection by HBV, and therefore has no prognostic significance as has been already suggested (Tong et al. 1977). The conversion of HBeAg positivity to anti-HBe positivity in individuals with transient HBsAg and HBeAg antigenaemia (Fig. 4), observed before (Aikawa et al. 1978; Mushahwar et al. 1978), suggests that at a certain period, HBeAg and anti-HBe co-exist in serum, most likely in the form of complexes. The decline of the level of anti-HBe in serum after clearance of HBsAg seems analogous to the decrease of the titre of anti-HBc reported recently (Neurath et al. 1978c).

The results presented here indicate that HBeAg is probably a single entity, an acidic component with a mol. wt. of about 35,000 and that HBeAg described in previous reports (Neurath & Strick, 1977; Miyakawa, 1978; Mushahwar et al. 1978; Takahashi et al. 1978) was most likely associated with serum proteins or was self-aggregated. The attachment of HBeAg to host proteins may be responsible for the difficulties encountered in its purification by affinity chromatography on columns of insolubilized anti-HBe and for the observation that IgG, IgM and albumin are the major components originating from HBeAg preparations which are detected in HBeAg-anti-HBe precipitin lines (Neurath & Strick, 1977; Neurath, 1978; own unpublished data). The association of HBeAg with other proteins, notably with IgG and albumin, and the presence of rheumatoid factor (Kacaki et al. 1977; Tedder & Briggs, 1977) and anti-albumin antibodies (Matuhasi & Hosokawa, 1972; A. R. Neurath, unpublished data) in some anti-HBe-positive sera might explain the multiple precipitin lines observed in immunodiffusion between HBeAg- and anti-HBe-positive sera (Williams & LeBouvier, 1976). However, other interpretations are possible.

We attempted in earlier experiments to dissociate HBeAg determinants from IgG by 3 M-NaSCN (Neurath & Strick, 1977). However, rheophoresis was not sensitive enough to detect the released HBeAg. Instead, we still observed an antigen reacting with anti-HBe-positive serum in fractions containing IgG obtained after gel filtration of HBeAg preparations in 3 M-NaSCN. It seems likely that the observed precipitin line corresponded to a reaction between idiotypic and anti-idiotypic antibodies since IgG-antigen complexes elicit anti-idiotypic antibodies to self-idiotypes (Klaus, 1978). However, the nature of the binding of HBeAg to IgG remains unknown, since we were not able to demonstrate anti-HBe activity in the void vol. fractions obtained after molecular exclusion chromatography of HBeAg (Fig. 6).

PAGE of the 35,000 mol. wt. HBeAg revealed the presence of Pr17 and of additional components with mol. wt. between 23,000 and 26,000. The latter were repeatedly observed in
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HBeAg-anti-HBe precipitin lines and were identified as immunoglobulin light chains (Neurath, 1978). They probably represent contaminants in the HBeAg preparation. Recently, McAuliffe (1978) obtained indirect evidence for the presence of P17 in HBeAg.

Our results suggest that HBeAg consists of two chains of P17 and that disulphide bonds are essential for the immunological activity of HBeAg. The similarities between HBeAg and HBcAg with respect to isoelectric points and polypeptide composition (Neurath et al. 1978a) raise the question of a possible relationship between these two antigens which might represent conformationally and immunologically distinct structures built from identical subunits in analogy with other complex proteins (Neurath & Rubin, 1971).

This study was supported by grant HL 09011-14 from The National Heart, Lung and Blood Institute and by contract AI-62512 from The National Institute of Allergy and Infectious Diseases. We thank Dr R. S. Tedder, Dr G. G. Froessner and Dr I. K. Mushahwar for helpful and stimulating discussions and Dr R. D. Inman for rheumatoid factor-positive sera.

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(Received 4 July 1978)