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Biophysical and Biochemical Heterogeneity of Purified Hepatitis B Antigen

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Hepatitis B antigen of the D (a+, d+, y−) subtype was purified from plasma of apparently healthy persons and from hepatitis patients. The original samples contained 20- and 42-nm particles and tubular forms (20-nm diameter). Ultracentrifugation during the purification procedure yielded pellets which were then treated at *p*H 2.4. Both the large, 42-nm Dane particles and the tubular forms were lost during the acid treatment of the pelleted particles, yielding a preparation containing a mixture of particles approximately 20 and 25 nm in diameter. This difference in size was substantiated in that two distinct molecular weights were calculated from high-speed equilibrium data, 3.6×10^6 and 4.5×10^6. Further heterogeneity was observed in that hepatitis B antigenic activity was present in purified particles with an isoelectric *p*H of 4.0 and also in those with a *p*H of 4.4. No significant differences were observed in the gross amino acid composition of purified antigen obtained from plasma of three different persons. ^{125}I-labeled, purified antigen was found to contain six distinct polypeptides with molecular weights ranging from 10,000 to 39,000.

The occurrence of virus-like particles associated with the presence of hepatitis B antigen (HB Ag) in the plasma of patients with long-incubation viral hepatitis is now well established. Although the particles were initially thought to be uniform and to contain a single antigenic determinant (5), subsequent reports disclosed considerable heterogeneity among different preparations studied, as evidenced by variations in size, antigenic subtype specificities, and electrophoretic charge. Electron microscopy studies revealed spherical particles 20 nm in diameter, tubular forms measuring approximately 20 nm in diameter and 50 to several hundred nm in length (4), and larger, spherical particles approximately 42 nm in diameter, referred to as Dane particles (2, 7, 14, 16, 18).

Recently, investigators have observed at least two antigenic subtypes of HB Ag, designated D (a+, d+, y−) and Y (a+, y+, d−) (12, 18, 19, 21, 25). Both contain a common antigenic determinant, *a*, in addition to either *y* or *d*. The latter antigens appear to be mutually exclusive so that an individual serum is either subtype Y or D.

In addition to these morphological and antigenic variations, Kim and Tilles have reported a charge heterogeneity in that certain HB Ag particles migrated electrophoretically between $alpha_2$ and beta globulins, whereas others had a beta globulin mobility (18). Electrophoretic mobility of the HB Ag seemed to be related to the specificity of the antisera utilized in the assay procedure.

The present study examines several biophysical and biochemical properties of highly purified HB Ag isolated from plasma of different persons. The data indicate that two particles are present, in each of the purified preparations, with distinct molecular weights and distinct isoelectric *p*H values.

MATERIALS AND METHODS

Purification of HB Ag. Plasma obtained from three anicteric hepatitis patients, with positive HB Ag complement-fixing (CF) titers of 1:2560 or greater, were processed to yield purified HB Ag particles according to procedure I of Dreesman et al. (9). Briefly, 90 to 120 ml of plasma was pelleted by high-speed centrifugation, treated at *p*H 2.4 at room temperature for 1 hr, repelleted, and banded twice on isopycnic CsCl gradients, followed by a rate zonal centrifugation in a preformed linear sucrose gradient. In a few instances, partially purified preparations were used which were prepared by twice pelleting the HB Ag particles at 30,000 rev/min in a Spinco type 30 rotor for 5 hr. All of the preparations included in this study were of the D subtype, as kindly

determined by G. L. LeBouvier (Yale School of Medicine).

Criteria of HB Ag purity. Each preparation of purified HB Ag was examined for the presence of contaminating normal human serum (NHS) proteins by injecting male, 500-g Hartley-strain guinea pigs with 5 μg of HB Ag emulsified in Freunds complete adjuvant on day 0 in the hind footpads and with a similar dose on day 14 in the front footpads. Blood was obtained by cardiac puncture on day 35. Each serum was examined for the presence of antibody to both HB Ag and to NHS by gel diffusion and by two-dimensional CF titrations similar to that described previously (9). None of the preparations elicited an antibody response to NHS proteins.

Electron microscopy examination of negatively stained preparations consistently showed only the presence of particles measuring 17 to 27 nm in diameter. Similar preparations were described in our earlier report (9). Each preparation contained from 10^{13} to 10^{14} particles per ml and 70 to 200 μg of protein per ml.

Serological techniques. The antigenic activity of purified HB Ag in the various fractionation procedures was monitored by a modified microtiter CF test (24) or by a radioimmunoassay procedure employing a second antibody to separate the HB Ag and HB antibody (HB Ab) complexes from free labeled HB Ag (radioimmunoassay-double antibody [RIA-DA] techniques) (15). A line of identity was observed by immunodiffusion in agar gel (9) between preparations obtained after the various biochemical and biophysical methods and a reference, unfractionated HB Ag. The HB Ab sera utilized in the above serological assays were prepared by injecting goats and guinea pigs with highly purified HB Ag as previously described (9).

Buoyant density studies. Buoyant densities of partially purified HB Ag were determined in linear gradients of cesium chloride, sucrose, and potassium tartrate. Preparations consisted of untreated and Tween 80-treated (1%, v/v) HB Ag. HB Ag (0.65 ml) was layered on each preformed linear gradient and centrifuged at 4 C and 35,000 rev/min for 18 hr in a Spinco type SW50L rotor. Fractions were collected by bottom puncture.

Chemical and labeling techniques. Protein determinations were done by the Lowry method by using crystalline bovine albumin (Pentex Inc., Kankakee, Ill.) as standard (22). Labeling of highly purified HB Ag was done by the chloramine-T technique (13) by procedures described in a previous publication (15).

pH Gradient electrophoresis. Electrophoresis of ^{125}I-labeled, purified HB Ag was performed in an LKB electrofocusing apparatus at 4 C and 700 v for 42 hr in a *p*H gradient of 3 to 10. All HB Ag preparations were dialyzed against 1% glycine before electrophoresis. HB Ag-positive plasma diluted 1:50 or 1:100 in 1% glycine was fractionated similarly in an electrofocusing column.

Molecular weight studies. A Spinco model E analytical ultracentrifuge, equipped with both schlieren optics and the Rayleigh interference optical system, was used. Temperature was controlled at 20 C on all runs with the rotor temperature indicator and control unit. Molecular weights were determined according to the high-speed equilibrium method of Yphantis (28) and were carried out in buffer containing 0.01 M phosphate and 0.1 M NaCl at *p*H 7.0. These experiments were performed in a double-sector interference cell in an An-J rotor at speeds of 3,000, 3,600, 4,000, and 5,200 rev/min. Equilibrium was assumed to have been attained when no change was observed in measurements taken 8 hr apart. This usually occurred within 24 hr after speed was changed. Photographs were taken on Kodak spectroscopic plates, type 103a-G, and read on a Nikon model 6C comparator. To compute the molecular weight, the partial specific volume for HB Ag of 0.803 g/cm^3 was used as determined by H. E. Bond (Electro-Nucleonics Laboratories, Inc., Bethesda, Md., *personal communication*).

Amino acid analysis. Lyophilized protein or peptide was hydrolyzed in 5.7 N HCl at 110 C in vacuo for a period of 24 hr. After removal of the acid by lyophilization, the hydrolysates were analyzed with a Spinco model 120C automatic amino acid analyzer equipped for single-column analysis. With range expansion of the recorder, it was possible to assay as little as 10 μg of HB Ag with a precision of ±5%. Routinely, 20 to 40 μg of antigen was analyzed. Cysteine was determined as cysteic acid after performic acid oxidation of the purified preparations. Tryptophan was not quantified, because this amino acid residue is destroyed by acid hydrolysis.

Polyacrylamide gel electrophoresis. Purified HB Ag and ^{125}I-labeled, purified HB Ag were repurified by isopycnic CsCl centrifugation. Material residing at a density of 1.21 g/cm^3 was dialyzed against 0.025 M tris(hydroxymethyl)aminomethane (Tris) and 0.19 M glycine buffer, *p*H 8.8. Subsequently, the sample was solubilized by heating for 10 min at 60 C in 6 M urea, 1% sodium dodecyl sulfate (SDS), and 1% 2-mercaptoethanol (ME) as described by Gerin et al. (10). The solubilized sample was then separated by disc polyacrylamide gel electrophoresis (PAGE) essentially as described by Davis (8). PAGE was performed in a discontinuous system within glass columns (0.6 by 11 cm) containing 0.5 cm of concentrating gel and 10.0 cm of resolving gel. The concentrating gel consisted of the acrylamide monomer (1.25%, w/v), *N*,*N*′-methylenebisacrylamide (Bis) (0.31%, w/v), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) (0.057%, v/v), riboflavine (0.0005%, w/v), SDS (0.1%, w/v), and urea (4 M), which were prepared in 0.06 M Tris-HCl buffer at a final *p*H of 6.6. The resolving gel consisted of the acrylamide monomer (10%, w/v), Bis (0.066%, w/v), TEMED (0.029%, v/v), ammonium persulfate (0.07%, w/v), SDS (0.1%, w/v), and urea (4 M), which were prepared in 0.37 M Tris-HCl buffer at a final *p*H of 8.8 to 9.0. The upper and lower buffer chambers contained 0.025 M Tris, 0.19 M glycine buffer (*p*H 8.6), and 0.1% SDS. The upper chamber buffer also contained 1.0% ME and 0.025 mg of bromophenol blue per 100 ml as the tracking dye. The solubilized sample containing 20% (w/v) of sucrose (0.1 to 0.2 ml) was layered on top of the concentrating gel and electrophoresed for 75 min at 200 v, plus an additional 20 v per gel at a constant

setting of 120 pulses per sec and 0.5 μfarad capacitance by using a pulsed constant-power polarization source (Ortec, Oak Ridge, Tenn.). The gels were sliced into 2-mm fractions and counted in an automatic gamma well counter (Nuclear Chicago, Des Plaines, Ill.). Ribonuclease A, chymotrysinogen A, egg albumin, and bovine serum albumin were run on similar gels, and plots of the logs of the known molecular weights versus relative migration, with regard to the tracking dye, were used to estimate the molecular weight of the HB Ag proteins.

RESULTS

Electron microscopy. Unfractionated plasma containing HB Ag and purified HB Ag were prepared for electron microscopy by the droplet pseudoreplication technique and negatively stained with 2% phosphotungstic acid in KOH, *p*H 6.8, as previously described (23). All photomicrographs were taken with an Hitachi HU11-B electron microscope. Several unfractionated HB Ag-positive plasmas (Fig. 1A) contained a mixture of the various morphological particle forms (17 to 21 nm particles, enveloped 40 to 45 nm particles, and tubular forms) similar to those described by others (2, 4, 7, 14, 16, 17), whereas the smaller particles were predominant in the purified preparations (Fig. 1B). Upon close examination of diluted preparations of purified HB Ag, it was noted that although most of the material in the purified preparations represented the typical small spherical particles with a diameter of 19 ± 2 nm, a population of larger particles could be

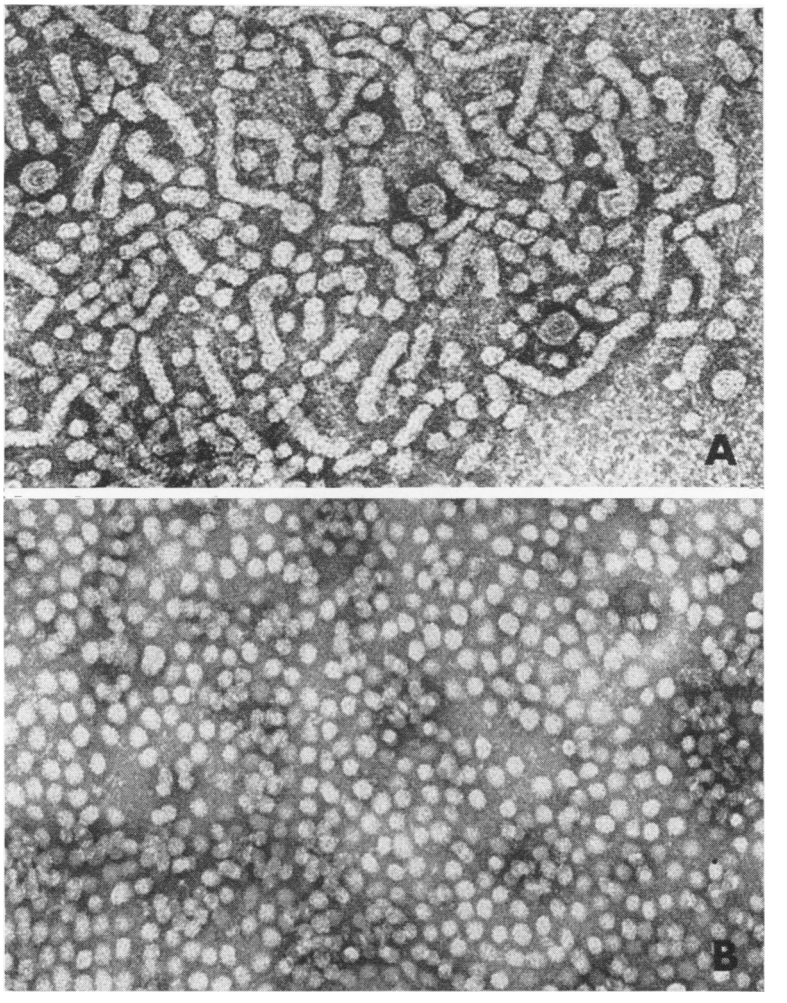

FIG. 1. *Electron micrograph of hepatitis B antigen (HB Ag) stained with potassium phosphotungstate (×110,000). A, Resuspended and sonically treated material obtained by pelleting HB Ag-positive plasma for 5 hr at 30,000 rev/min in a Spinco no. 30 rotor. B, Particles obtained, after pH 2.4 treatment for 1 hr at room temperature, of material described above, followed by a similar centrifugation step and isopycnic density centrifugation in CsCl.*

identified which measured 25 ± 2 nm in diameter. The relative proportions of 19- and 25-nm particles were dependent on the plasma from which the preparations were derived. In one plasma, where relatively few Dane particles were observed in the starting material, 90% of the purified particles had a diameter of 19 nm. However, a plasma rich in Dane particles yielded a purified preparation containing 65% of the 25-nm particles. The loss of the 40-nm particles and tubular forms occurred following treatment of the once-pelleted preparations at *p*H 2.4. The appearance of 25 ± 2 nm particles after this step may represent core material of the larger 40-nm particles from which the outer shell has been removed. This observation prompted the following biochemical and biophysical studies.

Buoyant densities. Buoyant densities, determined on partially purified HB Ag preparations, were 1.21, 1.17, and 1.17 g/cm³ as determined in CsCl, sucrose, and potassium tartrate, respectively (Table 1). Treatment of these preparations with 1% (w/v) Tween-80 decreased the buoyant densities to 1.18, 1.15, and 1.14 in each of the above gradient materials, respectively. An occasional individual HB Ag-positive plasma contained particles with a density of 1.06 to 1.09 g/cm³ in CsCl.

Highly purified HB Ag prepared by procedure I, as outlined in the Materials and Methods section (low *p*H treatment), had a buoyant density of 1.21 g/cm³ both before and after Tween-80 treatment.

Isoelectric pH. Highly purified HB Ag was iodinated with ^{125}I, dialyzed against a 1% glycine buffer, and subjected to electrophoresis to equilibrium in an LKB electrofocusing apparatus. Two radioactive populations were noted with distinctly different *p*H, 4.0 (±0.1) and 4.4 (±0.1) (Fig. 2). Both of these peaks had antigenic activity as monitored by the RIA-DA technique. The relative size of each peak varied, depending on the individual plasma source, in that the HB Ag purified from some individuals yielded rela-

TABLE 1. *Buoyant densities of untreated and Tween-80-treated hepatitis B antigen (HB Ag)*[a]

Gradient material	Buoyant density (g/cm³)	
	Untreated	Tween-80 treated
CsCl	1.21	1.18
Sucrose	1.17	1.15
K Tartrate	1.17	1.14

[a] HB Ag was purified from HB Ag-positive plasma by pelleting and resuspension in 0.01 M Tris-HCl buffer, *p*H 8.0.

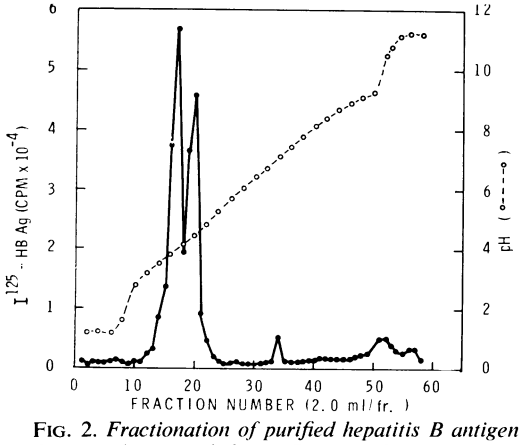

FIG. 2. *Fractionation of purified hepatitis B antigen with ^{125}I with an ampholyte pH range of 3.0 to 10.0 in an LKB isoelectric focusing column.*

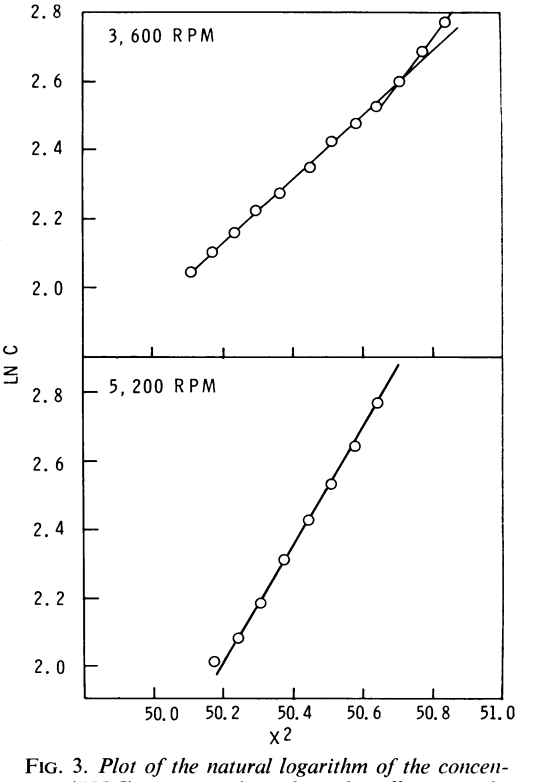

FIG. 3. *Plot of the natural logarithm of the concentration (LN C) at points throughout the cell against the square of the distance from the center of rotation (X^2) for representative high-speed sedimentation equilibrium determination of the molecular weight of purified hepatitis B antigen. Protein concentration was 150 μg/ml and the rotor speeds were as noted in the figure.*

tively higher levels of antigen with an isoelectric *p*H of 4.0, whereas other preparations predominantly contained material with an isoelectric *p*H of 4.4. Conversely, a wider range of antigenic activity and a higher isoelectric *p*H range of 4.3 to 4.7 was observed by using unfractionated HB Ag-positive plasma diluted 1:50 or 1:100 in 1% glycine. This suggests that the purification procedure or iodination has an effect on the overall charge of the HB Ag particles.

Molecular weight studies. The molecular weights of four purified HB Ag preparations were determined by the Yphantis technique (28). In two of these preparations (Table 2 and Fig. 3), two distinct populations of sedimentation particles were observed in the same sample. The average molecular weight of the major population was approximately 3.6×10^6. A second population of more rapidly sedimenting particles was observed, at the slower centrifugation speeds, which had a distinct slope (Fig. 3) and a calculated average molecular weight of 4.5×10^6 (Table 2). An HB Ag preparation (lot 59-26-1) purchased from Electro-Nucleonics Laboratories Inc. (Bethesda, Md.), which had been purified by batch rate-zonal centrifugation in CsCl, only contained particles with a molecular weight of 3.6×10^6.

Amino acid composition. The amino acid composition of the total particle protein was determined on eight preparations of purified HB Ag derived from the sera of three individuals with anicteric hepatitis. All three sera were of antigenic subtype D. The results are summarized in Table 3. No significant differences in the amino acid composition of these individual, purified HB Ag particle preparations were observed. The rather acidic isoelectric *p*H determined correlates well with the higher proportion of acidic amino acid residues to basic amino acid residues which are present in the antigen.

TABLE 2. *Molecular weights of two purified hepatitis B antigen preparations as determined by ultracentrifugal measurements*

Rotor speed (rev/min)	Apparent molecular wt ($\times 10^6$)[a]			
	Prepn 1		Prepn 2	
3,000	4.54	3.30	4.41	3.91
3,600	4.47	3.80		
4,000		3.55		3.38
5,200		3.57		3.42

[a] Each preparation revealed two distinct populations of particles having different molecular weights. Average molecular weights were 3.56 and 4.47.

A comparison of the amino acid composition of HB Ag to that of certain representative viruses and that of KB cells (Table 3) reveals that the HB Ag is distinctive in having significantly higher levels of cysteine (or cystine), proline, leucine, and phenylalanine, and lower levels of both the acidic and basic amino acids (lysine, arginine, aspartic acid, and glutamic acid). Because cysteine was determined as cysteic acid, its role in forming disulfide bonds of structural significance for the HB Ag cannot be directly determined from these data.

The level of protein for each purified HB Ag preparation was quantified by the Lowry method by using bovine serum albumin as a standard. It is noteworthy that protein calculations, based on the total micromoles of amino acid residues recovered from the amino acid analyzer, correlated well with the amount of protein put on the analyzer as quantified by the Lowry method (Table 4). In addition, Lowry protein values correlated well with the protein levels quantified by converting optical density readings read at 280 nm in a Gilford model 240 spectrophotometer to the amount of protein calculated by using an extinction coefficient of 37.26 ($E^{1\%}_{1cm}$ at 280 nm, J. L. Gerin, Rockville Laboratory of the Molecular Anatomy Program, Rockville, Md., *personal communication*).

Polyacrylamide gel electrophoretic profiles.

TABLE 3. *Amino acid composition of purified hepatitis B (HB) antigen and comparison with that of animal viruses and KB cells*[a]

Amino acid	HB antigen	Ad-2[b]	Polio[b]	Herpesvirus[c]	KB cells[b]
Lysine	1.7 ± 0.4	4.4	4.7	6.3	7.6
Histidine	0.6 ± 0.2	1.6	2.4	2.8	2.1
Arginine	3.1 ± 0.5	7.9	4.7	4.7	5.2
Aspartic acid	5.3 ± 0.3	11.8	11.9	8.8	9.4
Threonine	7.8 ± 0.4	6.9	9.1	4.8	5.5
Serine	8.3 ± 0.6	6.7	7.0	6.1	5.9
Glutamic acid	5.6 ± 0.6	9.0	7.7	12.4	12.0
Proline	11.6 ± 1.4	7.2	7.2	6.1	5.2
Glycine	7.7 ± 0.4	7.8	6.8	10.2	7.4
Alanine	4.0 ± 0.4	9.0	7.8	10.4	8.1
Cysteine[d]	4.8 ± 0.6	0.3	0.8	2.0	1.1
Valine	5.9 ± 0.4	6.1	7.2	6.6	7.2
Methionine	1.4 ± 0.6	2.3	1.5	<1.0	2.4
Isoleucine	6.2 ± 0.7	3.4	4.8	5.3	5.2
Leucine	16.7 ± 0.5	7.4	8.5	8.6	8.9
Tyrosine	1.1 ± 0.7	4.4	3.9	1.1	3.0
Phenylalanine	8.0 ± 0.6	3.8	4.4	3.9	3.9

[a] Values are expressed as μmoles of amino acid per 100 μmoles recovered.
[b] From reference 11.
[c] From G. R. Dreesman et al., Virology, *in press*.
[d] Determined as cysteic acid.

TABLE 4. *Protein determination of hepatitis B antigen*

Prepn	Protein (μg/ml)	
	Lowry[a]	Recovered amino acid[b]
A	37.5	43.0
		46.7
B	8.5	13.4
		13.9
C	39.0	32.0
D	27.0	26.1
E	36.0	39.8

[a] Determined by the Lowry method by using crystallized bovine serum albumin as standard.

[b] Calculated from total μmoles of amino acid recovered from amino acid analyzer.

Eight different purified HB Ag preparations were labeled with ^{125}I and repurified by isopycnic CsCl centrifugation. Pooled, iodinated samples were prepared for PAGE in SDS-urea gels (SDS, 6 M urea, and 1% 2-ME) and solubilized by heating at 60 C for 10 min. Six iodinated polypeptides were identified as illustrated in the representative profile shown in Fig. 4. The three large polypeptides (P-1, P-2, and P-3) were similar in size to those described by Gerin et al. (10), with molecular weights of 39,000, 32,000, and 27,000, respectively. These investigators reported that by direct protein staining the larger polypeptide (P-1 in this study) was present in very low concentrations. In the present study, this polypeptide represents the major ^{125}I peak, which may be the result of preferential labeling of this protein. Three smaller polypeptides (P-4, P-5, and P-6) with molecular weights of 22,000, 16,000, and approximately 10,000, respectively, were also detected. The last peak (Fig. 4) was either free iodine or small dialyzable peptides because these counts were not precipitated by 10% trichloroacetic acid.

DISCUSSION

This study demonstrates biophysical and biochemical heterogeneity between populations of particles present in purified preparations of HB Ag. The purification method used in these experiments (9) yielded only small spherical and ovoid particles, although the original material contained varying numbers of the larger 40- to 45-nm particles before fractionation. The loss of these large particles occurred during the early steps of particle purification, namely after the 1-hr acid treatment and 5-hr centrifugation step. These latter particles are approximately 42 nm in diameter and are composed of a 27- to 28-nm diameter core which is surrounded by a 2-nm shell with an outer membrane roughly 7 nm thick (7). Almeida et al. (1) noted that the outer membrane could be preferentially removed with 0.5% Tween-80. Removal of this membrane left the 27-nm cores intact. A closer examination of electron micrographs taken of diluted preparations of purified HB Ag in this study indicated that a mixture of particles was present, namely those with an average diameter of 19 nm and others slightly larger with a diameter of approximately 25 nm. These values are slightly smaller but are most likely analogous to the 20- and 27-nm populations reported by others. When data from high-speed sedimentation equilibrium determinations were plotted, two distinct molecular weights were noted, confirming the presence of two different-size particles in our purified

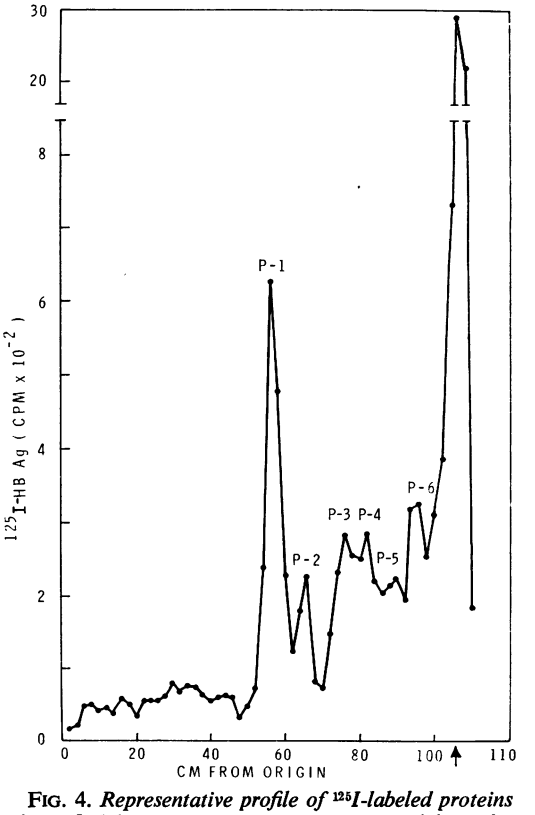

FIG. 4. *Representative profile of ^{125}I-labeled proteins of purified hepatitis B antigen as separated by polyacrylamide gel electrophoresis in 10% sodium dodecyl sulfate (SDS)-urea (pH 8.8) gels. The purified preparations were solubilized by heating for 10 min at 60 C in 6 M urea, 1% SDS and 1% 2-mercaptoethanol. Gels were cut in 2-mm fractions and counted in an automatic gamma counter. Arrow indicates position of bromophenol blue dye marker.*

preparations. The smaller molecular species had a weight of 3.6×10^6 which is similar to that observed by H. E. Bond (Electro-Nucleonic Laboratories, Inc., Bethesda, Md., *personal communication*). It should be noted that the material studied by Bond and Hall was obtained by preparative zonal centrifugation and chiefly contained 22-nm particles (6). The second population had a molecular weight of 4.5×10^6. This may be representative of the 25 to 27 nm particles.

Additional evidence for biophysical heterogeneity of such preparations was observed after isoelectric *p*H determination of iodinated, purified HB Ag. Two distinct isoelectric *p*H values were observed, one at *p*H 4.0 and a second at *p*H 4.4. The relative proportion of these two populations was dependent upon the individual plasma from which the HB Ag was purified, with certain sera containing chiefly one or the other of the particles. Although the particles show size and charge heterogeneity, they have the same density (1.21 g/cm^3) in CsCl. Kim and Tilles (18) had alluded to charge heterogeneity in unfractionated HB-positive plasma. They found that HB Ag migrated electrophoretically either with beta globulin or between alpha$_2$ and beta globulins. Individual sera contained either of these two classes, but a mixture of the two species was not observed (18).

The observed isoelectric *p*H values of the labeled purified particles were more acidic than the values determined for the antigen in unfractionated HB Ag-positive plasmas. The values ranged from *p*H 4.3 to 4.7, which would indicate that possibly a more basic serum protein, perhaps gamma globulin, is dissociated from the particle by the low *p*H treatment.

The heterogeneity observed in several purified preparations by electron microscopy, molecular weight estimations, and isoelectric *p*H determinations was not reflected in the gross amino acid composition among eight preparations obtained from three different donors. The difference seen by the other physical methods will probably become evident once amino acid composition data are available on individual polypeptides isolated from these purified particles. A relatively low concentration of tyrosine and histidine was found, which may account for some of the difficulty encountered in preparing iodinated HB Ag with a high specific activity. It is also noteworthy that HB Ag has a relatively high concentration of cysteine (or cystine) when compared to the levels reported for certain animal viruses (Table 3). In view of the marked morphological alterations which occur upon reduction of HB Ag (26), it is likely that the cysteine is involved in disulfide linkages which are important to the structural integrity of the antigen.

The polypeptide analyses of ^{125}I-labeled purified preparations were remarkably similar to those reported by Gerin et al. (10). However, three additional smaller peptides were noted with molecular weights of 22,000, 16,000, and roughly 10,000, respectively. The occurrence of at least six distinct polypeptides is not entirely unexpected for a virus particle of this size. Poliovirus, a picornavirus which has a diameter of 27 nm (similar to that of HB Ag-containing particles), has four distinct proteins (27) with molecular weights of 35,000, 28,000, 24,000, and about 8,000 (Table 5) (3). Such a comparison indicates that HB Ag may be more complex than the poliovirion, which would not be unexpected because, unlike poliovirus, HB Ag contains lipid as well as protein. A second possibility for the increased number of peptides may be that more than one distinct particle is present in the purified population, as suggested by the above data. It should be emphasized that the PAGE in this study was done with solubilized ^{125}I-labeled HB antigens. Consequently, polypeptides which failed to iodinate would not be detected. However, preferential labeling of selected polypeptides might also result in detection of protein not detectable by conventional protein staining procedures.

The heterogeneity observed in this study indicates that at least two distinct populations are present in purified preparations in which no tubular forms or Dane particles are observed. The identity and function of each of these different populations can only be speculated upon at this time. The larger 25-nm particle with a supposedly higher molecular weight may represent: (i) the cores of the Dane particles or (ii) dissociated subunits of the tubular forms. Others have suggested that the HB virus may reside in the inner core of the Dane particle (1, 17). The smaller 19-nm population may represent a second virus or a new or modified cellular component whose synthesis is specified by, or specifically derepressed by, the HB virus—similar to the

TABLE 5. *Molecular weights of proteins of hepatitis B antigen (HB Ag) and poliovirus*[a]

HB Ag		Poliovirus	
Protein	Mol wt	Protein	Mol wt
P-1	39,000	VP-1	35,000
P-2	32,000		
P-3	27,000	VP-2	28,000
P-4	22,000	VP-3	24,000
P-5	16,000		
P-6	10,000	VP-4	8,000

[a] Taken from reference 3.

hypothesis of LeBouvier and McCollum (20). Development of a tissue culture technique so that the virus could be propagated in vitro would be of great value in resolving these as well as other problems. Two reports indicate replication of HB Ag-containing virus in organ cultures (16, 29). One of these studies (16) found a preponderance of Dane particles in the tissue culture fluid, whereas the other group of workers observed only smaller 20-nm particles (29).

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