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NOTES

Purification of Hepatitis A Virus from Chimpanzee Stools

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Hepatitis A virus antigen was purified from early acute-phase chimpanzee stools by a rapid three-step procedure using 7% polyethylene glycol precipitation, CsCl banding, and Sepharose 2B column chromatography. Electron microscopic examination of the hepatitis A virus antigen preparation revealed highly purified hepatitis A virus particles.

After the discovery of 27-nm-diameter virus-like particles by immune electron microscopy (IEM) in acute-illness-phase stools of adult volunteers experimentally infected with the MS-1 strain of hepatitis A virus (HAV) (6), morphologically and antigenically similar 25- to 30-nm-diameter particles were reported found in a composite stool pool obtained from patients involved in a common-source outbreak of hepatitis A in Phoenix, Ariz. (7). The successful transmission of viral hepatitis A to subhuman primates, such as marmosets and chimpanzees (3, 4, 9, 10), with excretion of similar 27-nm-diameter virus-like particles in their acute-illness-phase stools, has also been well documented. HAV particles have been found in acute-illness-phase chimpanzee liver, bile, and stool specimens (1). Biophysical characterization of HAV particles derived from chimpanzee or human stools has revealed major particle peaks at buoyant densities of 1.32 to 1.34 and/or 1.39 to 1.43 g/cm^3 after isopycnic banding in CsCl gradients (1, 2, 5, 11). HAV particles derived from acute-phase chimpanzee stools appear to band bimodally in CsCl gradients, with a primary peak at 1.33 g/cm^3 and a secondary peak at 1.40 g/cm^3. By contrast, acute-phase human stool-derived HAV particles appear to band in CsCl gradients as a single peak at 1.32 to 1.34 g/cm^3 (1).

Sensitive and specific micro solid-phase immunoradiometric assays have recently been developed for the detection of hepatitis A virus antigen (HA Ag) (8, 12). In contrast to the cumbersome and time-consuming IEM technique, these new radioimmunoassays have made it possible to screen large numbers of samples for the presence of HA Ag. We now report the purification of HA Ag from early acute-phase stools of experimentally infected chimpanzees.

Stools used in this study were obtained from an experimentally infected chimpanzee (no. 0084) on days 14 and 15 postinoculation. The inoculum for animal no. 0084 consisted of an extract prepared from stools (days 18 to 21 post-inoculation) collected from a chimpanzee (no. 722) previously inoculated with the Phoenix antigen strain of HAV (9). HAV particles were visualized by IEM in each of the stool pools described above (9). Subpassage of the animal no. 0084 day-14 to -15 stool pool in another chimpanzee induced enzymatic, histological and serological evidence of hepatitis A, with concomitant excretion of morphologically and antigenically similar HAV particles on day 14 postinoculation. These findings confirmed the infectivity of the no. 0084 crude stool pool.

HAV particles, detected as HA Ag by a modified micro solid-phase immunoradiometric assay test (8), were purified from the early acute-phase chimpanzee feces described above by a combination of polyethylene glycol (PEG) precipitation, CsCl banding, and molecular-sieve chromatography. These three methods take advantage of the fact that HA Ag is a nucleoprotein, possesses a buoyant density that is significantly different from the bulk of the stool material, and has a diameter that excludes it from more than 99% of the material in stools, when chromatographed on Sepharose 2B (C. L. Hornbeck, C. R. Gravelle, F. B. Hollinger, and D. W. Bradley, Intervirology, in press). A 6-ml amount of a fivefold-concentrated, PEG-precipitated stool suspension was isopycnically banded in a linear CsCl gradient (Fig. 1). This initial procedure yielded a partially purified HA Ag. Addition of bovine serum albumin to gradient fraction collecting tubes (0.5% [wt/vol] final concentration) enhanced the recovery of HA Ag. Incorporation of 0.1% (vol/vol) Triton X-100 into preformed CsCl gradients was found to

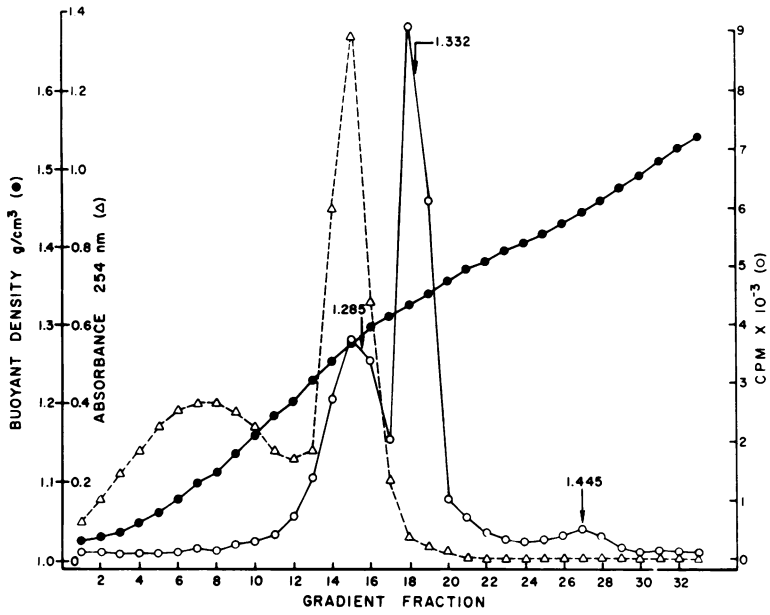

FIG. 1. *Isopycnic banding of HA Ag in a CsCl density gradient. An initial 17% (wt/vol) crude stool suspension was prepared by combining 20 g of stool with 100 ml of water. The suspension was clarified at 8,000 × g for 20 min at 5°C in a fixed-angle rotor. The supernatants were carefully removed from the crude pellets and combined with 7% PEG 6000-7500 in order to precipitate HA Ag and eliminate the bulk of the lower-molecular-weight materials found in the soluble phase. PEG precipitation was found to yield a 20-fold purification of HA Ag from the crude stool suspension. A 6-ml amount of a fivefold-concentration PEG preparation as layered onto a 32.0-ml, linear, preformed CsCl gradient with a buoyant density range of 1.174 to 1.500 g/cm³. The gradient was prepared in 0.05 M phosphate-buffered saline, pH 7.2. The gradient was centrifuged in a Beckman SW27 swinging-bucket rotor for 23 h at 5°C. Thirty-two 1.2-ml fractions were collected and assayed for the presence of HA Ag by micro solid-phase immunoradiometric assay. The buoyant density of each gradient fraction was determined by measurement of refractive index in an Abbe 3L refractometer. Peak HA Ag activity was typically found in gradient fractions with a density of 1.32 to 1.34 g/cm³. IEM examination of gradient fractions 14 to 16 revealed the presence of 27-nm-diameter particles with electron-luscent centers. IEM examination of fractions 18 and 19 revealed 27-nm-diameter particles with electron-dense centers. Some fecal debris was present in each of these gradient fractions. Approximately 97% of the PEG material absorbing at 254 nm was removed from the bulk of the 1.33-g/cm³ HA Ag by CsCl banding.*

substantially reduce the recovery of HA Ag.

Fractions from a CsCl density gradient containing HA Ag activity banding at 1.33 g/cm³ were pooled and chromatographed on Sepharose 2B (Fig. 2). Very little material absorbing at 280 nm was found in fractions containing high-molecular-weight material (fractions 19 to

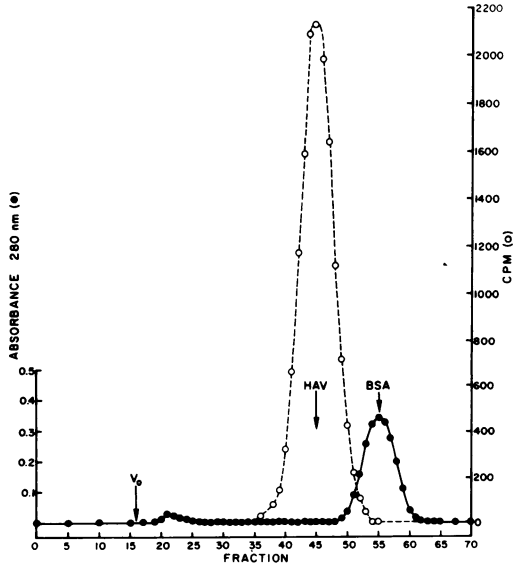

FIG. 2. *Sepharose 2B gel filtration chromatography of CsCl-banded HA Ag. A 2-ml amount of a CsCl gradient fraction pool containing HA Ag activity (and 0.5% bovine serum albumin [BSA]) was loaded onto a glass column (1.6 by 70-mm) packed with 2% agarose (Sepharose 2B). The agarose gel was washed and equilibrated in 2 liters of eluting buffer prior to packing. The sample was eluted through the column by gravity flow with 0.1 M Tris-hydrochloride and 2.0 M NaCl buffer, pH 7.2. Seventy 2.0-ml fractions were collected and assayed for HA Ag activity by micro solid phase immunoradiometric assay. The void volume (V_o) of the column coincided with fraction 16.*

25, Fig. 2). No material absorbing at 280 nm was found in fractions 26 to 48. Fractions 42 to 48 contained highly purified HAV particles, as judged by IEM and direct electron microscopy (7) (Fig. 3). HAV particles and HA Ag eluted coincidentally in fractions 42 to 48, with a peak in fraction 45. HA Ag contained in the latter fractions has been found suitable as a reagent for the radioimmunoassay of specific anti-HA antibody (manuscript in preparation). Bovine

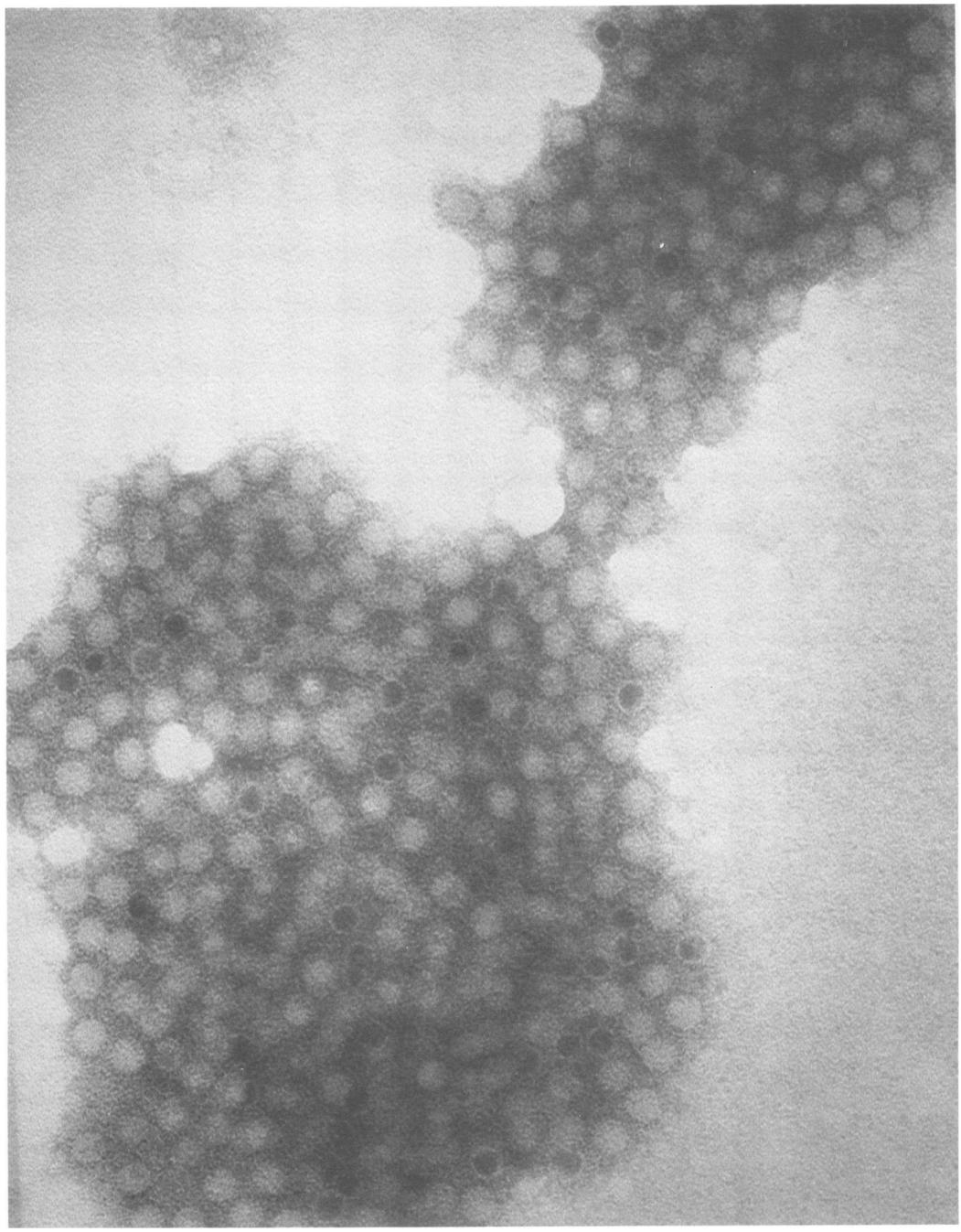

FIG. 3. *Electron micrograph IEM of HAV particles purified by the three-step PEG, CsCl, Sepharose procedure. Particles have been aggregated by a convalescent human serum containing anti-HA activity. ×185,600.*

serum albumin added to the CsCl gradient fraction collecting tube eluted in fractions 49 through 61, with a peak in fraction 55. HA Ag suitable for the production of monospecific antibody in rabbits has been prepared by a modification of the above procedure wherein bovine serum albumin is omitted during fractionation of the CsCl gradient. It should be noted that combinations of PEG precipitation and either CsCl banding or Sepharose 2B chromatography alone were ineffective in yielding a highly purified HA Ag preparation. IEM and electron microscope analysis of HAV particles in these preparations revealed the presence of contaminating fecal detritus. Inoculation of a chimpanzee with 1.0 ml of the highly purified HA Ag preparation induced enzymatic, histopathological, and serological evidence compatible with a diagnosis of viral hepatitis A (S. Plotkin, personal communication). This finding proved the infectivity of the highly purified HA Ag and further established its identity with the agent responsible for hepatitis A.

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