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Infectious Hepatitis A Virus Particles Produced in Cell Culture Consist of Three Distinct Types with Different Buoyant Densities in CsCl

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Although hepatitis A virus (HAV) released by infected BS-C-1 cells banded predominantly at 1.325 g/cm^3 (major component) in CsCl, smaller proportions of infectious virions banded at 1.42 g/cm^3 (dense HAV particles) and at 1.27 g/cm^3 (previously unrecognized light HAV particles). cDNA-RNA hybridization confirmed the banding of viral RNA at each density, and immune electron microscopy demonstrated apparently complete viral particles in each peak fraction. The ratio of the infectivity (radioimmunofocus assay) titer to the antigen (radioimmunoassay) titer of the major component was approximately 15-fold greater than that of dense HAV particles and 4-fold that of light HAV particles. After extraction with chloroform, the buoyant density of light and major component HAV particles remained unchanged, indicating that the lower density of the light particles was not due to association with lipids. Light particles also banded at a lower density (1.21 g/cm^3) in metrizamide than did the major component (1.31 g/cm^3). Dense HAV particles, detected by subsequent centrifugation in CsCl, were indistinguishable from the major component when first banded in metrizamide (1.31 g/cm^3). However, dense HAV particles recovered from CsCl subsequently banded at 1.37 g/cm^3 in metrizamide. Electrophoresis of virion RNA under denaturing conditions demonstrated that dense, major-component, and light HAV particles all contained RNA of similar length. Thus, infectious HAV particles released by BS-C-1 cells in vitro consist of three distinct types which band at substantially different densities in CsCl, suggesting different capsid structures with varied permeability to cesium or different degrees of hydration.

Although the morphology and structure of hepatitis A virus (HAV) closely resemble that of poliovirus, there are marked differences in the biological behavior of these two picornaviruses. Unlike poliovirus, HAV is uniformly noncytopathic in cell culture and replicates relatively slowly (1, 8, 10, 16). Even a virus which is well adapted to cell culture requires a number of days to reach maximum yields. Once established, however, infection has been persistent in all cell types examined and is without apparent effect on cellular macromolecular synthesis (25).

A striking feature of HAV is the wide range of buoyant densities which have been reported for this virus (reviewed by Coulepis et al. [7]). Feinstone et al. (9) initially reported that HAV recovered from human feces banded in CsCl at a density of 1.40 g/cm^3. Similar results were obtained by Locarnini et al. (13). However, a number of subsequent reports described the predominant banding of HAV particles or HAV antigenic activity from a variety of primate specimens at a density of 1.32 to 1.34 g/cm^3 in CsCl gradients (3, 4, 17, 20, 21). Several of these studies, however, also confirmed the existence of a second, usually minor species of HAV virions banding at a density of 1.40 to 1.44 g/cm^3 (3, 4, 20, 21). In addition, HAV particles banding at a density of 1.29 to 1.31 g/cm^3 have been described and appear to represent empty capsids (20, 21). Coulepis et al. (7) have summarized these findings by suggesting the existence of three different HAV particle types: a relatively light, empty capsid structure (1.29 to 1.31 g/cm^3); an intact, normal virion (1.33 to 1.34 g/cm^3); and a minor virion species consisting of particles which are either more permeable to cesium ions or otherwise bind more cesium ions and thus have an increased density (1.40 to 1.45 g/cm^3) when placed in cesium solutions.

Fewer data are available concerning the characteristics of HAV particles produced by infected cell cultures. Siegl et al. (22) found [^{3}H]uridine to be incorporated into HAV particles with densities of 1.33 g/cm^3 (major component) and 1.39 to 1.44 g/cm^3 within infected human hepatoma cells. A minor antigen component banded at 1.30 to 1.32 g/cm^3 but did not incorporate [^{3}H]uridine. In this report, we demonstrate the infectivity of both dense (1.42-g/cm^3) and major-component (1.325-g/cm^3) particle types and describe a third, previously unrecognized, infectious HAV virion which possesses a buoyant density in CsCl of approximately 1.27 g/cm^3 (light HAV particle).

MATERIALS AND METHODS

Cells. Virus was propagated in 490-cm^2 roller-bottle cultures of continuous green monkey kidney (BS-C-1) cells (passage 60 to 67), under conditions described previously (11). After inoculation with virus, cells were maintained in Eagle minimal essential medium supplemented with 2% heat-inactivated fetal bovine serum, glutamine (100 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Medium was replaced weekly.

Virus. HAV strain HM-175 had been adapted to growth in BS-C-1 cells as described previously (1, 11). Virus used in the experiments described in this paper had been passed 10 times in low-passage African green monkey kidney cells and 1 to 4 times in BS-C-1 cells.

Solid-phase radioimmunoassay for HAV. HAV was detected in cell culture supernatant fluids and gradient fractions by a solid-phase microtiter radioimmunoassay de-

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scribed previously (12). Quantitation of viral antigen in gradient fractions was based on the testing of twofold dilutions, the titer being determined by the highest dilution yielding a radioimmunoassay signal greater than or equal to 2.1 times that generated by culture medium alone.

Indirect plaque assay for HAV. Quantitation of infectious HAV was carried out by the radioimmunofocus method (11). This technique is similar to standard viral plaque assays except that foci of viral replication (radioimmunofoci) are detected by autoradiography after fixation of the cell sheet and staining with ^{125}I-labeled anti-HAV. Results are reported in terms of radioimmunofocus-forming units of virus.

CsCl isopycnic centrifugation of HAV. Supernatant fluids were harvested weekly from infected roller-bottle cultures of BS-C-1 cells and clarified by centrifugation at 7,000 × *g* for 30 min. After the addition of 10% polyethylene glycol (PEG) 8000 and 2.3% NaCl, the medium was stirred gently overnight at 4°C. Precipitated virus was collected by centrifugation at 10,000 × *g* for 30 min, suspended in distilled water, and mixed with a solution of CsCl to obtain a final density of 1.34 g/cm^3. In some cases (noted in text), PEG-precipitated virus was initially purified by sedimentation through a 30-ml 10 to 30% (wt/wt) linear, rate-zonal sucrose gradient before isopycnic centrifugation. Rate-zonal gradients were centrifuged at 141,000 × *g* for 130 min at 4°C in a Beckman SW27 rotor (Beckman Instruments, Inc., Palo Alto, Calif.). Isopycnic centrifugation was carried out in 5-ml gradients spun at 200,000 × *g* for 22 h at 4°C in a Beckman SW40 rotor. Fractions (100 μl each) were collected from the bottom of each gradient, and 5 to 10-μl samples of each fraction were tested for HAV antigen by radioimmunoassay. Densities of fractions were estimated from the refractive index determined by an Abbe-type refractometer.

Isopycnic centrifugation of HAV in metrizamide. HAV samples were mixed with 4 ml of a 50% (wt/vol) aqueous solution of metrizamide (Sigma Chemical Co., St. Louis, Mo.), layered onto a 1-ml cushion of 80% (wt/vol) metrizamide, and spun at 200,000 × *g* for 72 h at 4°C in a Beckman SW40 rotor. Fractions were collected from the bottom of the tube and tested for HAV as described above. Densities of metrizamide gradient fractions were estimated from the refractive index.

Immune electron microscopy. Immune electron microscopy was carried out by S. M. Feinstone, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Samples (7 μl) of selected gradient fractions were mixed with 10 μl of a 1:10 dilution of immune chimpanzee serum (CH753) and 50 μl of water. Samples were examined with a JEOL-100B electron microscope at 50,000 magnification.

Extraction of viral RNA. Total cellular RNA was obtained from trypsinized normal and HAV-infected BS-C-1 cells (21 days after infection) by the guanidinium isothiocyanate-cesium chloride technique (14), extracted with chloroform-isoamyl alcohol (24:1), and precipitated with cold ethanol. For extraction of RNA from purified virions, virus-containing gradient fractions were diluted by the addition of 5 ml of extraction buffer (20 mM Tris-hydrochloride [pH 7.4], 50 mM NaCl, 1 mM EDTA, 0.1% β-mercaptoethanol, 0.01% Triton X-100), and virus particles were pelleted by centrifugation at 200,000 × *g* for 6.5 h. Virus was resuspended in 200 μl of extraction buffer, and carrier RNA (10 μg of wheat germ rRNA) was added. Virions were disrupted by incubation for 5 h at 37°C in the presence of 0.5% sodium dodecyl sulfate, 0.5 mg of proteinase K per ml (E. Merck AG, Darmstadt, Federal Republic of Germany), and 25 mM EDTA, and virion RNA was extracted twice with phenol (previously equilibrated with 0.1 M Na acetate)-chloroform (1:1) and precipitated with cold ethanol. Precipitated RNA was washed three times with 70% ethanol and stored at −20°C.

HAV cDNA probes. *Escherichia coli* transformed with the recombinant pBR322 plasmid LB148, which contains an insert of cDNA complementary to a portion of wild-type (marmoset passage 6) HM-175 strain virion RNA, was the gift of J. R. Ticehurst, National Institute of Allergy and Infectious Diseases (24). Plasmid DNA was isolated after cell lysis by repetitive banding in CsCl with ethidium bromide. ^{32}P-labeled cDNA probes were prepared from whole plasmid DNA by alkaline denaturation, followed by transcription with *E. coli* DNA polymerase I (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and random priming with calf thymus oligodeoxynucleotides in the presence of [^{32}P]TTP (New England Nuclear Corp., Boston, Mass.) (23). A control pBR322 probe containing no HAV cDNA sequences was similarly prepared.

HAV cDNA-RNA hybridization. RNA solutions were blotted onto nitrocellulose paper (Schleicher & Schuell, Inc., Keene, N.H.) with the Minifold II slot-blot apparatus (Schleicher & Schuell). For direct blotting of CsCl gradient fractions, 5- to 50-μl samples were diluted into 500 μl of 4.61 M formaldehyde in 7.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and applied directly to the nitrocellulose paper. The paper was dried at 23°C for 30 min and baked at 80°C for 2 h in a vacuum oven. Prehybridization was carried out for 3 h at 42°C with denatured calf thymus DNA (100 μg/ml) in prehybridization buffer (50% formamide, 2.5× Denhardt solution, 5× SSC, 0.1% sodium dodecyl sulfate). Labeled probes (2 × 10^7 to 3 × 10^7 cpm) were boiled for 3 min and added to 20 ml of hybridization buffer (identical to prehybridization buffer except for 1× Denhardt solution) at 45°C, and hybridization was carried out at 42°C for 20 h in sealed plastic bags. Nitrocellulose papers then were washed successively with 2× SSC with 0.1% sodium dodecyl sulfate at room temperature, followed by 0.1× SSC with 0.1% sodium dodecyl sulfate at 52°C, and exposed to XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) overnight in X-Omat cassettes fitted with X-Omatic intensifying screens (Eastman Kodak Co.).

The specificity of the HAV cDNA probe was confirmed in preliminary experiments with blotted whole cell RNA (from normal and infected BS-C-1 cells) and RNA from HAV virions purified from cell culture supernatant fluids by a combination of PEG precipitation, isopycnic centrifugation, and rate-zonal sedimentation. The HAV probe hybridized to purified virion RNA and showed evidence of specificity when tested against infected and uninfected BS-C-1 whole cell RNA (data not shown). The control pBR322 probe did not hybridize to either cellular RNA or purified virion RNA.

Formaldehyde-agarose gel electrophoresis of virion RNA. Extracted RNA samples were electrophoresed in 1% agarose gels containing 2.2 M formaldehyde using a Mini-gel apparatus (Hoefer Scientific Instruments, San Francisco, Calif.). Gels were soaked for 30 min in transfer buffer (20× SSC with 3% formaldehyde) and blotted against nitrocellulose paper overnight at room temperature (14). Papers were then dried, baked at 80°C, and subjected to hybridization with the HAV cDNA probe as described above.

RESULTS

Buoyant density of HAV particles released by BS-C-1 cells. Virus, concentrated from clarified media by precipitation with PEG 8000, was banded isopycnically in CsCl. HAV

antigen was detected in gradient fractions by radioimmunoassay as three distinct peaks of activity banding at different densities (Fig. 1). Fractions comprising each of these three antigen peaks (indicated by arrows in Fig. 1) were separately pooled and subjected to a second round of isopycnic centrifugation under conditions similar to those used previously. Rebanding established the presence of three types of antigenic activities with distinctly different buoyant densities (Fig. 2). Dense, intermediate-density (major-component), and light activities rebanded at approximately 1.42, 1.32, and 1.27, respectively.

Because available tables relating refractive index to density of cesium chloride solutions are valid for solutions at 25°C, virus was rebanded in CsCl at 25°C, and the refractive index was determined at the same temperature to arrive at a more accurate estimate of virion buoyant density. ^{3}H-labeled simian virus 40 (SV40) virions added as a standard density marker banded at a slightly higher density (1.336 g/cm^3) than did HAV under identical conditions (1.325 g/cm^3) (data not shown). All subsequent gradients described in this report were centrifuged at 4°C, with the refractive index of individual fractions determined at 25°C.

Infectivity of HAV antigenic activities with different buoyant densities. Selected peak fractions from each of the three gradients depicted in Fig. 2 were assayed for infectious virus by radioimmunofocus assay (11). Results (also shown in Fig. 2) indicated that each antigenic activity was associated with a peak of infectivity. These data thus indicate the release from infected cell cultures of three distinct, infectious HAV virion types with substantially different buoyant densities in CsCl. Based on the titer of HAV present in each peak fraction, the major component contained approximately 99% of infectious virions released from BS-C-1 cells, whereas dense particles comprised 0.4% and light HAV particles comprised 0.6% of the total.

To obtain an estimate of the ratio of infectivity to antigen content for each particle type, the titer of HAV antigen in peak gradient fractions was determined by radioimmunoassay, and these results were related to infectivity as determined by the radioimmunofocus assay. The highest infectivity-antigen titer ratio was found with the major-component, intermediate-density particle type (Table 1). Based on antigen content, the major component had approximately 15-fold greater specific infectivity than did dense HAV particles and 4-fold that of light HAV particles.

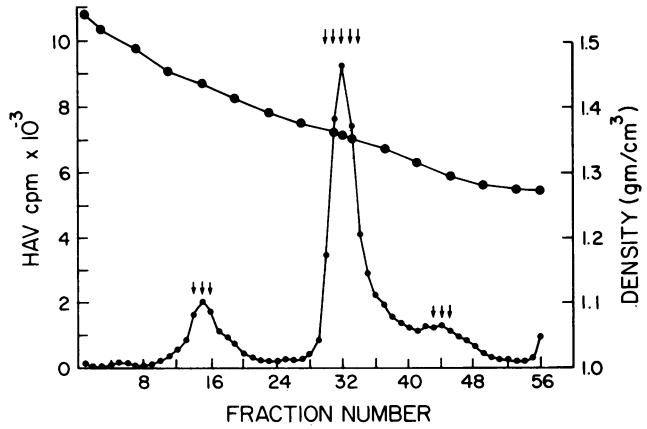

FIG. 1. HAV antigen detected by radioimmunoassay in fractions from a CsCl gradient containing HAV precipitated from cell culture supernatant fluids by the addition of PEG 8000 and NaCl. The density shown is only an approximation because PEG may have interfered with refractive index determinations. Arrows indicate fractions pooled for rebanding in subsequent CsCl gradient (Fig. 2).

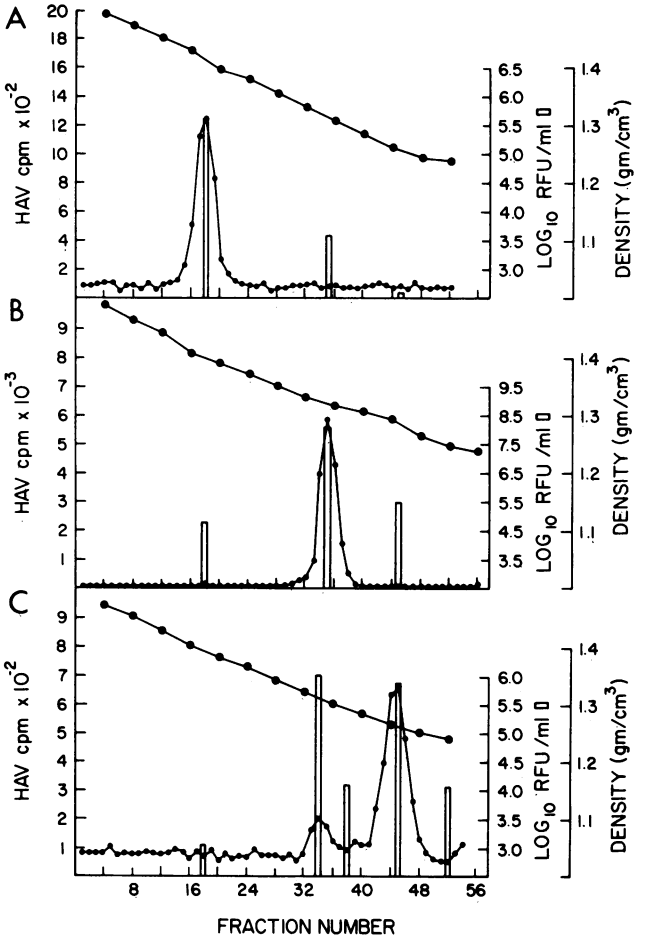

FIG. 2. Rebanding of HAV particle types from the gradient shown in Fig. 1. (A) Dense particles, rebanding of fractions 14 to 16 from the gradient shown in Fig. 1; (B) major component, rebanding of fractions 30 to 34; (C) light particles, rebanding of fractions 43 to 45. Results of radioimmunoassay testing for HAV antigen in each gradient fraction are shown, as are radioimmunofocus assay infectivity titrations (columns) of selected gradient fractions.

Immune electron microscopy of HAV particles. Immune electron microscopy (kindly performed by S. M. Feinstone) of fractions containing major-component and light HAV particles revealed numerous aggregates of particles with a diameter of approximately 27 nm (data not shown). Substantially fewer particles and only occasional aggregates were noted in the fraction containing dense HAV particles (1.42

TABLE 1. Infectivity of HAV virion types separated on the basis of buoyant density in CsCl

Density (g/ml)	Infectivity (RFU[a]/ml)	Antigen titer (reciprocal)	Infectivity/ antigen ratio
1.27	7.2×10^5	4.0×10	1.8×10^4
1.325	1.2×10^8	1.3×10^3	9.1×10^4
1.42	4.8×10^5	8.0×10	6.0×10^3

[a] RFU, Radioimmunofocus-forming units.

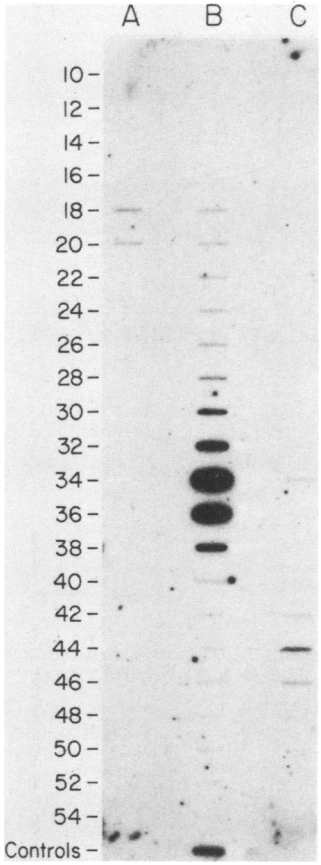

FIG. 3. cDNA-RNA slot-blot hybridizations of fractions from the three CsCl gradients shown in Fig. 2. Numbers to the left represent fraction numbers, collected from the bottom of the gradient. Lane A contains fractions from Fig. 2A (dense particles), lane B contains fractions from Fig. 2B (major component), and lane C contains fractions from Fig. 2C (light particles). Control RNA in lane A is uninfected whole cell RNA, control in lane B is HAV-infected BS-C-1 whole cell RNA, and control in lane C is empty.

g/cm^3). The majority of particles present in each fraction appeared to be complete particles. A small number (<3%) of particles present in the light-particle preparation appeared to be empty, and at least one such particle was noted in the fraction containing intermediate-density virions as well.

HAV RNA content of CsCl gradient fractions. To confirm the radioimmunoassay and infectivity results, HAV RNA was sought in fractions from the CsCl gradients depicted in Fig. 2 by slot-blot hybridization with cloned HAV cDNA as probe (Fig. 3). This probe was prepared from bacterial clones kindly provided by J. R. Ticehurst (24). Preliminary testing indicated that maximum sensitivity was achieved in the slot-blot system by the direct application of gradient fractions diluted in 4.61 M formaldehyde–7.5× SSC to the nitrocellulose paper (see above). In each of the three gradients examined, the presence of HAV RNA correlated well with radioimmunoassay and infectivity results (Fig. 3). Overall, the results of cDNA hybridization confirmed the presence of HAV RNA-containing particles with buoyant densities of approximately 1.42, 1.32, and 1.27 g/cm^3.

Chloroform extraction of HAV particle types. A substantial fraction (up to 30%) of HAV released into cell culture supernatant fluids resists neutralization with specific antibody but can be rendered neutralizable by extraction with chloroform. This, plus the fact that nonneutralizable virus sediments less rapidly in sucrose (S. M. Lemon, L. N. Binn, manuscript in preparation), suggests that some HAV virions released by cell cultures might be associated with lipids. Therefore, to determine whether the light particles (1.27 g/cm^3) might represent lipid-associated virus, fractions comprising dense, major-component-, and light-particle peaks in the three gradients depicted in Fig. 2 were extracted once with an equal volume of chloroform. Extracted virus was rebanded isopycnically in CsCl, and gradient fractions were assayed for antigen by radioimmunoassay (data not shown) and for HAV RNA by slot-blot hybridization (Fig. 4). Chloroform extraction of fractions containing the major component resulted in the rebanding of both HAV antigenic activity and HAV RNA at a density of approximately 1.32 g/cm^3. However, some HAV RNA also was detected at a density of 1.27 g/cm^3. After chloroform extraction of the light particle, viral RNA again was detected at a density of 1.27 g/cm^3, although viral antigen, present at the same density, was nearly undetectable by radioimmunoassay. Neither antigen nor RNA was detected at 1.32 g/cm^3 in this gradient. Thus, the banding of light particles at 1.27 g/cm^3

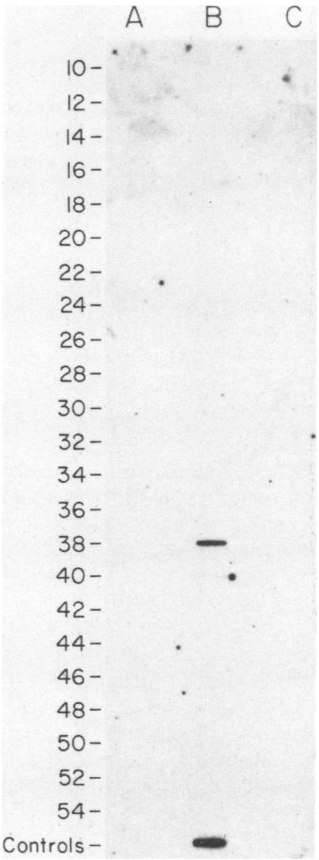

FIG. 4. cDNA-RNA slot-blot hybridizations of fractions from CsCl gradients containing chloroform-extracted peak fractions from the gradients shown in Fig. 2. Lane A contains chloroform-extracted dense particles, lane B contains chloroform-extracted major component particles, and lane C contains chloroform-extracted light particles. In lane B, fraction 38 had a density of 1.31 g/cm^3 and fraction 48 had a density of 1.27 g/cm^3. In lane C, fraction 46 had a density of 1.27 g/cm^3. Controls are as described in the legend to Fig. 3.

could not be related to association of normal virions with lipids. Neither HAV antigen nor HAV RNA could be detected after chloroform extraction of dense HAV particles, suggesting that the dense particle might be relatively unstable (2, 4).

Isopycnic banding of HAV virions in metrizamide. Dense particles of poliovirus (density, 1.40 to 1.44 g/cm^3) have been related to increased virion permeability to cesium ions, with binding of cesium to virion RNA (see below). Therefore, to examine the banding characteristics of HAV in a nonionic density medium, virus was subjected to isopycnic banding in metrizamide after precipitation from culture medium with PEG and partial purification by rate-zonal sedimentation in sucrose. In metrizamide, virus predominantly banded at approximately 1.31 g/cm^3 with a lesser band at about 1.22

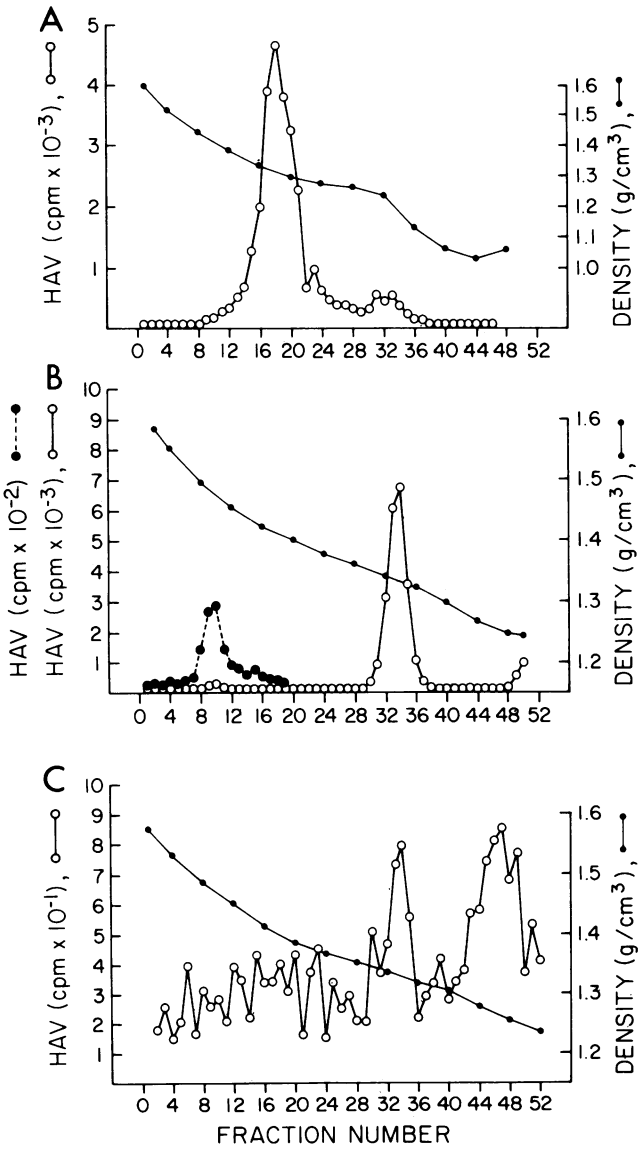

FIG. 5. Isopycnic banding of HAV in self-forming metrizamide gradients. Virus, partially purified by sedimentation through a rate-zonal sucrose gradient, was banded isopycnically in metrizamide (A). Fractions from the major (fractions 17 to 19) and minor (fractions 31 to 33) HAV peaks were separately pooled and rebanded in metrizamide (B and C, respectively).

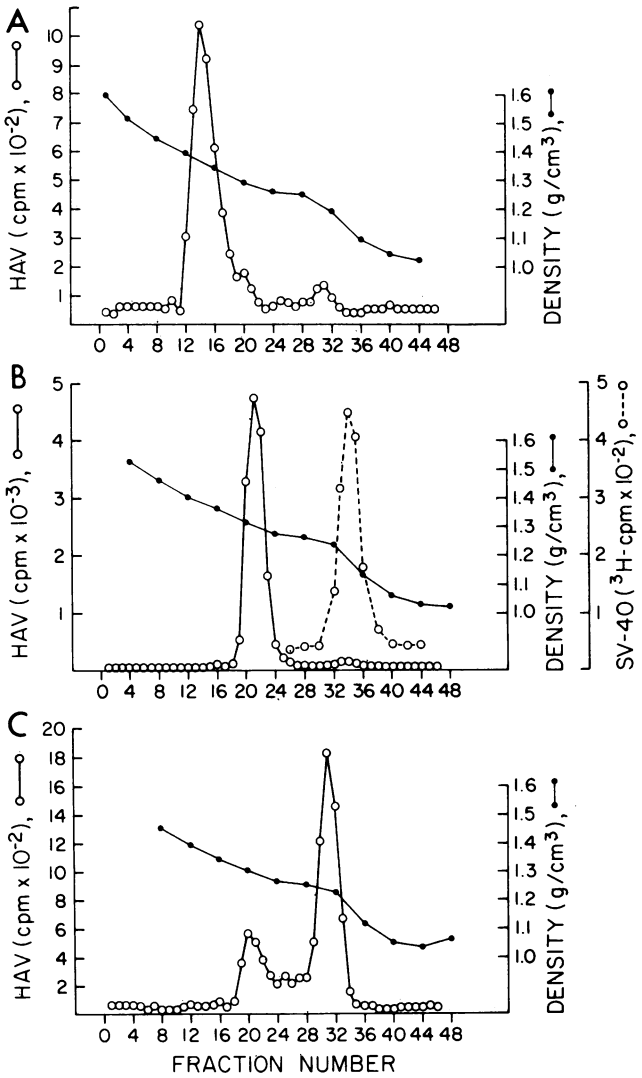

FIG. 6. Isopycnic banding in metrizamide of dense (A), intermediate-density (B), and light (C) HAV particle types recovered from CsCl gradients. The buoyant density of ^{3}H-labeled SV40 in metrizamide also is shown (B).

g/cm^3 (Fig. 5A). Virus in the predominant peak of this gradient was rebanded in CsCl. In CsCl, this virus resolved into two bands representing major-component, intermediate-density particles and a lesser band of dense particles (Fig. 5B). Similar banding profiles were obtained in CsCl whether virus was taken from the central portion or the denser leading edge (fraction number, 14 to 16) of the major peak in metrizamide (Fig. 5A) (data not shown). Fractions comprising the lighter peak in this metrizamide gradient (number, 31 to 33) also were rebanded in CsCl. This virus resolved into two peaks, representing major-component and light HAV particles (Fig. 5C). These virus peaks were at the limit of detection by radioimmunoassay but were clearly demonstrated by cDNA slot-blotting of individual gradient fractions (data not shown). Thus these data demonstrate that dense and intermediate-density HAV particle types have identical buoyant densities in metrizamide but resolve when placed in cesium chloride solution, a finding consistent with the explanation proposed for other picornavirus dense par-

TABLE 2. Buoyant densities of HAV particle types

Particle type	Buoyant density (g/cm³) in:	
	CsCl	Metrizamide
Dense	1.42	1.31 (1.37)[a]
Major component	1.325	1.31
Light	1.27	1.21

[a] After recovery of dense particles from CsCl.

ticles (15, 26). In contrast, light HAV particles were clearly less dense than the major component when banded in metrizamide, indicating that their presence was not an artifact related to the nature of the density gradient medium. In fact, virus examined in this series of gradients seems to have been partially depleted of light particles during the rate-zonal sedimentation in sucrose which preceded the metrizamide gradient.

The banding characteristics of HAV virion types in metrizamide were also examined after initial isopycnic banding in CsCl. In this experiment, PEG-precipitated virus was first banded in CsCl. Fractions containing dense, major-component, and light HAV particles were pooled, extracted with an equal volume of chloroform, and rebanded in CsCl (data not shown). Distinct peaks representing each particle type were obtained, indicating again that light particles were not sensitive to chloroform. Fractions containing dense, major-component, and light HAV particles from this gradient then were separately pooled and rebanded in self-forming metrizamide gradients (Fig. 6). Dense HAV particles isolated from CsCl banded at a higher density (approximately 1.37 g/cm³) than did major-component particles (Fig. 6A and B). Thus, although dense and major-component particles had identical buoyant densities in metrizamide before being placed in cesium chloride solution (Fig. 5), these HAV particle types banded at different densities in metrizamide after removal from CsCl. These observations strongly support the hypothesis that dense HAV particles isolated from cell culture are relatively dense due to binding of cesium cations. Light HAV particles isolated from CsCl banded in metrizamide at a lighter density (about 1.23 g/cm³) than did major-component particles (Fig. 6C). The buoyant density of these particles in metrizamide was not affected by prior banding in CsCl. Buoyant density data are summarized in Table 2.

The relative densities of HAV and SV40 virus were reversed in CsCl and metrizamide, indicating that specific density gradient media may exert a strong but variable influence on the buoyant density of different viruses. Although SV40 was slightly denser than HAV in CsCl (1.336 g/cm³ for SV40 versus 1.325 g/cm³ for HAV), it banded at a density significantly less than that of HAV in metrizamide (1.20 g/cm³ for SV40 versus 1.31 g/cm³ for HAV), possibly reflecting different proportions of nucleic acid and protein in the composition of each virus.

Size of virion RNA in dense, major-component, and light HAV particles. Peak fractions from the gradients shown in Fig. 2 were pooled, and RNA was extracted from each as described above. After electrophoresis in formaldehyde-agarose, RNA was blotted to nitrocellulose paper and hybridized to labeled HAV cDNA. A single band of HAV RNA was detected in peak fractions from the two gradients containing dense and light HAV particles (Fig. 7). In both cases, this RNA appeared to be similar in size to the RNA extracted from the major component particle, and of the same length as the longest HAV RNA species detectable in infected cell cultures. Based on comparison with *Sma* restriction fragments of adenovirus type 2 DNA run concurrently as size markers, the HAV RNA from all three virion types was estimated to be somewhat greater than 7,000 nucleotides in length. A small proportion of the RNA extracted from both the major-component and heavy particles consisted of molecules of less than genomic length and appeared as a faint smear below the prominent RNA band in the Northern blots. Although this subgenomic RNA could represent RNA from defective particles, it also may have resulted from degradation of the RNA after extraction.

DISCUSSION

Based on physical properties, including its polypeptide composition and positive-sense, 3′-polyadenylated RNA genome, HAV has been classified as a picornavirus (7). In general, the buoyant density profile of HAV in CsCl is in keeping with that of other picornaviruses, among which considerable variation is found. Foot-and-mouth disease virus bands in CsCl at a predominant density of 1.42 to 1.44 g/cm³, human rhinoviruses band at about 1.38 to 1.42 g/cm³, and human enteroviruses, including poliovirus, band at about 1.34 g/cm³ (19). However, a small proportion of poliovirus and some other enteroviruses has been shown to band isopycnically at 1.40 to 1.44 g/cm³ in CsCl (15, 18, 26). In addition, defective-interfering particles of poliovirus, which lack sequences near the 5′ end of the RNA genome, band isopycnically at 1.31 to 1.32 g/cm³ (6), and empty poliovirus capsids band at about 1.29 to 1.30 g/cm³ (19). We found the buoyant density of the major component of HAV to be 1.325 g/cm³ in CsCl. Although this is significantly less than the 1.34 g/cm³ previously reported in studies of HAV derived from feces (reviewed in reference 7), Siegl et al. (22) also found cell culture-adapted HAV to be less dense than poliovirus (1.33 g/cm³). The lower values for the buoyant density of HAV may reflect more precise measurement or perhaps a lower density for cell culture-adapted virus relative to wild-type HAV.

Dense (1.40 to 1.44 g/cm³) poliovirus particles have been explained on the basis of a more open capsid structure with increased capsid permeability to cesium ions (15, 26). Cesium presumably displaces cations with less mass which normally neutralize the negatively charged phosphate groups

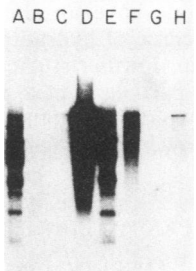

FIG. 7. Northern blot analysis of RNA extracted from isolated HAV virion types. Lanes A through G represent 13-h exposures: A and E, adenovirus DNA size markers; B, is RNA from fractions 15 to 20 of the gradient shown at the top of Fig. 2 (dense particles); C, RNA from fractions 42 to 48 of the gradient shown at the bottom of Fig. 2 (light particles); D, RNA from fractions 34 to 36 of the middle gradient in Fig. 2 (major component); F, whole cell RNA extracted from infected BS-C-1 cells; G, whole cell RNA from uninfected cells. Lane H represents a brief (15-min) exposure of lane D RNA from major-component particles.

on the virion RNA, thus resulting in denser virions. Most poliovirions are impermeable to cesium ions, however, and do not alter their densities when placed in CsCl solutions (15). The dense particle, having a looser and more open capsid structure, also is less stable and more sensitive to RNase digestion when compared with normal poliovirus particles (26). A similar explanation is likely for dense HAV particles (2, 7). We found the buoyant density of dense HAV particles in a nonionic density gradient medium (metrizamide) was significantly altered after banding of the particles in CsCl (Fig. 5 and 6), whereas this was not the case with the major-component or light HAV particles. These observations confirm that dense HAV particles derive their increased density by binding cesium cations and suggest a common origin for HAV and poliovirus dense particles. However, compared with dense poliovirus particles which have been reported to be 5,000-fold less infectious than normal poliovirus (26), our data suggest that dense HAV particles have a specific infectivity much closer to that of the major component (Table 1). In this regard, HAV may be more similar to swine vesicular disease virus in that dense particles of swine vesicular disease virus (1.44 g/cm^3) have a specific activity only fourfold less than that of the predominant swine vesicular disease virus particle type (1.34 g/cm^3) (18).

The light (approximately 1.27 g/cm^3) infectious HAV particle has not been observed previously and is considerably more difficult to explain. To our knowledge, this type of infectious particle has not been found in studies of poliovirus, although a lighter than normal but infectious coxsackie B5 particle (1.30 g/cm^3) has been recently described (5). Unlike the light HAV particle, however, these atypical coxsackie B5 particles were membrane bound and significantly less infectious than normal virions.

Light HAV particles contain encapsidated RNA of approximately unit length (Fig. 7) but are less dense than even the empty capsid is thought to be (20, 21). The light particle appears to be fully infectious and does not have properties of poliovirus defective-interfering particles (6). It does not appear to be lighter than the major virion component because of association with lipids, as its density is not affected by vigorous extraction with chloroform (Fig. 4). On the contrary, the population of light particles appeared enriched after extraction of major-component particles with chloroform. Although light HAV particles are morphologically indistinguishable from the major component, it remains most likely that these virions have an altered capsid structure permitting a higher degree of hydration but not influencing permeability to cesium. Further studies of the capsid structure of these HAV particle types will be necessary to determine the veracity of this hypothesis but will be difficult due to the relatively low yield of light and dense particles in infected cell cultures.

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