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# Characterization of the Snow Mountain Agent of Viral Gastroenteritis

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**Snow Mountain agent (SMA) is a 27- to 32-nm virus which is the etiologic agent of outbreaks of acute gastroenteritis in Colorado and Vermont. SMA is morphologically similar to but antigenically distinct from the Norwalk and Hawaii agents of viral gastroenteritis but, like those agents, has not been cultivated in vitro. We purified and characterized SMA directly from human stool specimens containing the virus. The density of the SMA virion was 1.29 g/cm$^3$ and 1.21 to 1.22 g/cm$^3$ on potassium tartrate-glycerol gradients and 1.33 to 1.34 g/cm$^3$ on cesium chloride gradients. SMA had an S value of 170 to 183S on a sucrose velocity gradient. The purified virion was iodinated, immunoprecipitated with acute and convalescent sera from volunteers challenged with SMA, and analyzed on polyacrylamide gels. The virion contains one major structural protein of 62,000 molecular weight, which is similar in size to the 59,000-molecular-weight protein found in the Norwalk virion. The biophysical properties and single structural protein of SMA most closely resemble those of the calicivirus group.**

Norwalk-like viruses are a group of small, round viruses of similar size (26 to 32 nm), morphology, and density in cesium chloride (1.36 to 1.41 g/cm$^3$; 1, 2, 6–8, 10, 14, 15, 22). They are etiologically associated with outbreaks and sporadic cases of acute, infectious, nonbacterial gastroenteritis, often occurring in explosive outbreaks of gastroenteritis in families or communities, with frequent secondary infections (4, 8, 9). Multiple antigenic types exist, as illustrated by the Norwalk, Hawaii, and W-Ditchling agents (1, 15, 22, 23), which are antigenically distinct from each other. In addition, the Marin County agent and the Snow Mountain agent (SMA) appear to be distinct from one another (18) and from the Norwalk and Hawaii agents (11, 18) and thus may represent additional serotypes. The viruses are shed in stools of infected individuals in limited amounts and have not yet been cultivated in vitro (4, 6, 8, 9). For these reasons, they have not been extensively characterized.

Greenberg et al. (12, 13), however, developed a radioimmunoassay (RIA) for Norwalk virus and used this tool to purify and characterize the Norwalk virus proteins. A major structural protein of 59,000 molecular weight is associated with the virion, and a virus-specific soluble protein of 30,000 molecular weight is found in fecal samples (12). The nature of the viral genome has not been established. The only group of animal viruses with one major virion structural protein (60,000 to 71,000 molecular weight) is the calicivirus group (19). These viruses are of similar size (35 nm) and density in cesium chloride (1.36 to 1.39 g/cm$^3$) as the Norwalk virus (19), although the characteristic morphology of caliciviruses has not been observed in electron microscopy of Norwalk-like viruses.

SMA, another Norwalk-like virus, has been etiologically associated with outbreaks of acute, infectious, nonbacterial gastroenteritis in Granby, Colo. (17), and Chittenden County, Vt. (5). This agent is antigenically distinct from the Norwalk, Hawaii (11), and Marin County (18) agents by immune electron microscopy. The virus is morphologically similar to Norwalk virus (11), but the density in cesium chloride is unknown. This laboratory recently developed an RIA for SMA (R. Dolin, K. D. Roessner, J. J. Treanor, R. C. Reichman, M. Phillips, and H. P. Madore, J. Med. Virol., in press) using sera and stool specimens from a volunteer challenge study with SMA (11). This RIA has enabled us to further characterize SMA. In this report, we describe the purification of SMA virion from feces, determination of its density and sedimentation velocity, and characterization of the virion-associated proteins.

## MATERIALS AND METHODS

**Viruses and cells.** The source of SMA was stool from a volunteer infected with the agent, who subsequently had a seroresponse by RIA. Feline calicivirus (Bolin strain; VR652) and fetal cat tongue cells (Fc3Tg; CCL-176) were obtained from the American Type Culture Collection, Rockville, Md. Cells were grown in Eagle minimum essential medium supplemented with 10% fetal calf serum. Feline calicivirus was propagated in Fc3Tg cells. The pooled cells and supernatant fluid were harvested upon development of maximum cytopathic effect, sonicated, and stored at −70°C. The titers of feline calicivirus were determined on Fc3Tg monolayers in 6-well plates (Costar, Cambridge, Mass.) with a 0.6% agarose–10% fetal calf serum–minimum essential medium overlay. After 2 to 3 days at 37°C in a 5% $CO_2$ incubator, the cells were fixed and stained with a 10% Formalin–0.5% crystal violet solution, and the plaques were read.

**Feline calicivirus purification.** The procedure for feline calicivirus purification was adapted from that described by Ashley and Caul (3). Briefly, confluent roller bottles (Costar) of Fc3Tg monolayers were infected with feline calicivirus (Bolin strain) and incubated at 37°C until development of a 4+ cytopathic effect. The cells and medium were sonicated (Ultrasonic Cleaner; Branson Sonic Power Co., Danbury, Conn.) and clarified by centrifugation at 1,600 × *g* for 10 min, and then ammonium sulfate (final concentration, 60% [vol/vol]) was added to the supernatant fluid. The precipitate was centrifuged at 10,000 × *g* for 10 min, suspended in a small volume of TNE buffer (0.05 M Tris, 0.15 M NaCl,

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0.001 M EDTA [pH 7.4]), and then run on a preformed 50% (wt/wt) potassium tartrate–30% (wt/wt) glycerol–TNE buffer gradient in an SW27 rotor at 100,000 × *g* for 16 h at 4°C. The virus band (1.29 g/cm$^3$) was collected, diluted in TNE buffer, and concentrated by centrifugation in an SW40 rotor at 150,000 × *g* for 3 h at 4°C. The virus pellet was suspended in TNE buffer and run on a 10 to 40% (wt/vol) sucrose–TNE buffer gradient at 120,000 × *g* for 1 h at 4°C. The virus band was collected, reconcentrated by centrifugation as described above, suspended in 0.01 M phosphate buffer (pH 7.3), and stored at −70°C.

**SMA purification.** The procedure for SMA purification was adapted from that described by Ashley and Caul (3). Samples from each step were monitored for the presence of SMA-specific antigen by RIA. A 10-g portion of stool was mixed with 15 ml of TNE-Triton buffer (0.05 M Tris, 0.15 M NaCl, 0.001 M EDTA, 0.1% Triton X-100 [pH 7.4]) and sonicated for 1 to 2 min in a Branson Ultrasonic Cleaner. Trichlorotrifluoroethane (25 ml) (Genetron 113) was then added, and an emulsion was formed with a Vortex-GENIE (Scientific Industries, Springfield, Mass.). The emulsion was centrifuged for 15 min at 400 × *g*, and the supernatant fluid (no. 1) was decanted and saved. The Genetron was removed from the interface, and equal volumes (10 ml) of TNE-Triton buffer and Genetron were added. The interface was re-extracted and centrifuged, and the supernatant fluid (no. 2) was pooled with supernatant no. 1. Saturated ammonium sulfate was mixed with the pooled supernatant fluid to a final concentration of 60% (vol/vol), the precipitate was allowed to form for 1 h at room temperature, and then the preparation was centrifuged at 10,000 × *g* for 10 min at room temperature. The pellet was suspended in 4 to 8 ml of TNE-Triton buffer, and 2 to 4 ml was layered per gradient of 50% (wt/wt) potassium tartrate–30% (wt/wt) glycerol in TNE buffer. The gradient was centrifuged to equilibrium in an SW27 rotor at 100,000 × *g* for 16 h at 4°C. The gradient fractions were assayed for SMA antigen by RIA, the densities (in grams per cubic centimeter) of the fractions were calculated from their refractive indices, and a standard curve was calibrated by weighing potassium tartrate-glycerol gradient fractions. The virus peak was pooled (6 ml), diluted with TNE-Triton buffer (6 ml), and then centrifuged in an SW40 rotor at 150,000 × *g* for 3 h at 4°C. The pellet was suspended in 0.25 to 0.5 ml of TNE-Triton buffer, layered on a 10 to 40% (wt/vol) sucrose gradient in TNE-Triton buffer, and centrifuged in an SW40 rotor at 120,000 × *g* for 1 h at 4°C. The fractions (0.5 ml) were assayed for SMA antigen by RIA. The virus peak was pooled (2 ml), diluted with 3 ml of 0.01 M Tris buffer (pH 7.4), and centrifuged in an SW65 rotor at 150,000 × *g* for 3 h at 4°C. The pellet was suspended in 100 μl of 0.01 M Tris buffer (pH 7.3) and stored at 4°C for further analysis. The fractions of the potassium tartrate-glycerol and sucrose gradients containing nonvirion-associated (soluble) SMA antigen as measured by RIA were pooled and stored at −70°C.

**CsCl equilibrium gradient.** A sample of the purified SMA virion preparation was layered onto a discontinuous 1.2- to 1.6-g/cm$^3$ CsCl gradient in 0.01 M Tris buffer (pH 7.4) and centrifuged to equilibrium in an SW40 rotor at 150,000 × *g* for 22 h at 4°C. Fractions (0.8 ml) were assayed for SMA antigen by RIA, and their densities were calculated from the refractive indices.

**RIA.** The RIA for SMA antigen (Dolin et al., in press) was similar to that developed for Norwalk antigen (13). Pre- and postchallenge sera taken 2 weeks apart from a volunteer challenged with SMA were diluted to 1/10,000 in phosphate-buffered saline (PBS; pH 7.3) plus 0.02% sodium azide (PBS-NaAz), and 100 μl of each serum was added to duplicate V-bottom wells of a 96-well plate (no. 1-220-25; Dynatech Laboratories, Inc., Alexandria, Va.) and then incubated overnight at 4°C. Excess serum was removed, the wells were washed three times with PBS-NaAz, and then the wells were filled with PBS containing 1% bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, Mo.; 1% bovine serum albumin–PBS), incubated overnight at 4°C, and washed three times with PBS-NaAz. Fetal calf serum (25 μl) was added to the duplicate pre- and postchallenge serum wells, followed by the sample(s) to be tested (25 μl). A positive control (stool with SMA antigen) and a negative control (PBS-NaAz) were included in each assay. The plates were incubated for 24 h at 4°C and washed six times with PBS-NaAz. Immunoglobulin G from the serum of another volunteer positive for SMA antigen was purified and iodinated as previously described (13). A 50-μl sample (200,000 cpm) was added to each well, and the plates were incubated for 4 h at 37°C, washed extensively with PBS-NaAz, and then air dried. The wells were cut from the plates and then counted in a gamma counter (Beckman Instruments, Inc., Palo Alto, Calif.). After background subtraction, the ratio of counts per minute in the duplicate postchallenge serum wells to those in the duplicate prechallenge serum wells was determined. A positive/negative ratio of ≥2 was considered positive.

**Antisera.** Paired pre- and postchallenge sera from volunteers (11) with little or no antibody (prechallenge) and significant antibody (postchallenge) against SMA antigen by RIA were chosen for immunoprecipitation analyses.

**Iodination of virion preparations.** Virion was purified from stool as described above, the pellet was suspended in 100 μl of 0.01 M $PO_4$ buffer (pH 7.4), and then the preparation was lyophilized. The preparation was dissolved in 20 μl of 0.25 M $PO_4$ buffer (pH 7.3) followed by 7.5 μl of $^{125}$I (100 mCi/ml), carrier-free NaI (Amersham Corp., Arlington Heights, Ill.), and 15 μl of chloramine-T (3.5 μg/μl). After 15 to 20 s, 20 μl of sodium metabisulfite (4.8 μg/μl) was added, followed by 30 μl of a 22.5% (wt/vol) sucrose–potassium iodide (2 μg/μl)–0.025% phenol red solution. Labeled protein was separated from $^{125}$I by being passed through a G-25 Sephadex column equilibrated with 1% bovine serum albumin–PBS. $^{125}$I-labeled virion fractions (1 to 2 ml) were concentrated by ethanol precipitation in 0.01% bovine serum albumin, suspended in 200 μl of 1% Triton X-100–1% deoxycholate–PBS-NaAz buffer (RIP buffer), and stored at 4°C.

**Immunoprecipitation procedure.** $^{125}$I-virion preparations (5 × 10$^6$ cpm) were mixed with an equal volume (10 μl) of pre- or postchallenge serum diluted in RIP buffer and incubated for 1 h at 37°C and then overnight at 4°C. Sepharose-protein A (25 μl; Sigma) was added, and this was followed by incubation for 1 h at 4°C and washing of the precipitate (six times) in 500 μl of RIP buffer (10,000 rpm for 2 min; Eppendorf Microfuge, Brinkmann Instruments, Inc., Westbury, N.Y.).

**Polyacrylamide gel electrophoresis.** Samples were prepared by boiling for 2 min in the appropriate volume of sample buffer (0.067 M Tris [pH 6.8], 2% sodium dodecyl sulfate, $10^{-2}$ M dithiothreitol, 10% glycerol, 0.002% bromphenol blue) and then run on discontinuous 3% spacer–10% resolving gels as described by Laemmli (16). Unlabeled marker proteins (92,500 to 14,000 molecular weight; Bio-Rad Laboratories, Richmond, Calif.) and feline calicivirus, $^{125}$I labeled as described above, were added to the gels as markers. Gels

were dried and then autoradiographed with XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) and two Cronex Lightning-Plus screens (Du Pont Co., Wilmington, Del.) (20).

## RESULTS

**Purification and biophysical characterization of SMA.** The crude 50% (wt/vol) stool homogenate from a volunteer infected with SMA was chosen to purify and characterize SMA. The volunteer manifested clinical symptoms of gastroenteritis, which included diarrhea and vomiting, and had a significant rise in antibody titer to SMA by RIA. The crude stool homogenates had a positive/negative ratio of 3.5 to SMA by RIA. A 10-g sample of the stool was suspended in TNE-Triton buffer and extracted with Genetron, and the proteins in the sample were precipitated with ammonium sulfate as described in Materials and Methods. The precipitate was resuspended in TNE-Triton buffer and then centrifuged to equilibrium on a potassium tartrate-glycerol gradient (Fig. 1). A distinct peak of the SMA virion was at a density of 1.22 g/cm$^3$, and a minor peak, representing virus-specific soluble proteins, was present at the top of the gradient. The virion peak was within one of the four bands of protein in the gradient that were visible to the naked eye. The virion band (fractions 9 to 11) was pooled, concentrated by centrifugation, and then run on a 10 to 40% (wt/vol) sucrose velocity gradient as described in Materials and Methods (Fig. 2). Feline calicivirus ($10^7$ PFU) was run on a 10 to 40% (wt/vol) sucrose velocity gradient in parallel with the SMA virion (Fig. 2). The SMA and the calicivirus virions sedimented at the same velocity. SMA-specific soluble proteins were not detected at the top of the sucrose gradient. The SMA virion band detected by RIA was not visible to the naked eye. The purified SMA virion (fractions 13 to 16) was pooled, concentrated by centrifugation, and stored at 4°C as described in Materials and Methods.

The density of the sucrose gradient-purified SMA virion then was compared directly with that of the feline calicivirus virion by cosedimentation with feline calicivirus to equilibrium on another potassium tartrate-glycerol gradient (Fig. 3a). The broad SMA virion peak was at 1.21 g/cm$^3$, with a minor peak of virus-specific soluble protein at the top of the gradient. This pattern suggested that some degradation of the SMA virion had occurred. The feline calicivirus virion had a major peak at 1.29 g/cm$^3$ and a minor peak at 1.21 g/cm$^3$ as measured by PFU. In another purification of the SMA virion from a different homogenate of the same stool specimen, however, the SMA virion equilibrated at 1.29 g/cm$^3$ on the potassium tartrate-glycerol gradient (Fig. 3b). A subsequent purification from this stool specimen yielded SMA peaks at 1.21 and 1.29 g/cm$^3$ on the same gradient (data not shown).

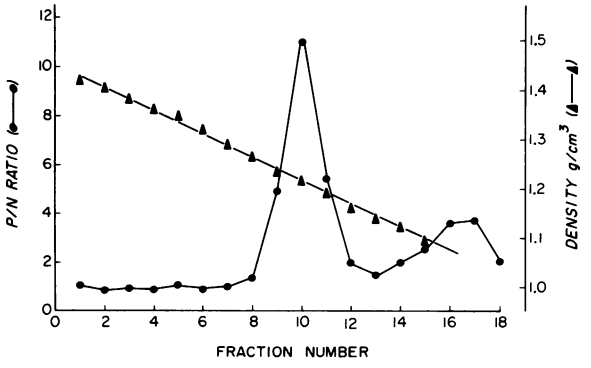


FIG. 1. Potassium tartrate-glycerol equilibrium gradient of SMA. A crude 50% (wt/vol) stool homogenate containing SMA was Genetron extracted, ammonium sulfate precipitated, and run on a potassium tartrate-glycerol gradient as described in Materials and Methods. The virus-specific proteins were detected by RIA. A positive/negative (P/N) ratio of ≥2 is indicative of the presence of virus-specific antigen. The density of the SMA virion peak was 1.22 g/cm$^3$. The virion peak (fractions 9 to 11) was pooled for further purification.

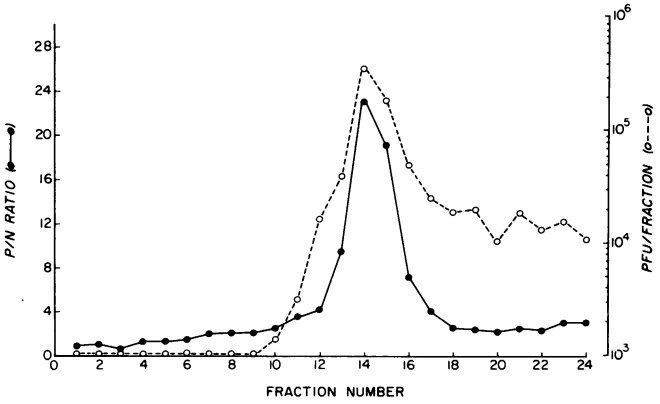


FIG. 2. Rate zonal sedimentation of SMA virion in a 10 to 40% (wt/vol) sucrose gradient. The partially purified SMA virion was run on a sucrose velocity gradient as described in Materials and Methods. Feline calicivirus was run on a parallel sucrose velocity gradient, and PFU were assayed as described in Materials and Methods. SMA was measured by RIA. The virion peak (fractions 13 to 16) was pooled and concentrated by centrifugation. P/N, Positive/negative.

The SMA virion from the initial purification by potassium tartrate-glycerol and sucrose gradients was also cosedimented with feline calicivirus to equilibrium on a cesium chloride gradient (Fig. 4). The SMA virion peak was at 1.34 g/cm$^3$, with a shoulder at 1.37 g/cm$^3$. The feline calicivirus peak was at 1.37 g/cm$^3$, with an indistinct peak at 1.33 g/cm$^3$. SMA virion purified from the stools of other volunteers also had a density of 1.33 to 1.34 g/cm$^3$ in CsCl (data not shown), including a preparation that was concentrated by high-speed centrifugation and not ammonium sulfate precipitation prior to sedimentation on the CsCl gradient.

**Analysis of SMA virion-associated protein.** The relationship of SMA to other Norwalk-like viruses and caliciviruses was examined characterization of the virion-associated proteins. The SMA virion, purified as described in Materials and Methods, was iodinated with chloramine-T, immunoprecipitated with pre- and post-SMA-challenge sera from volunteers, and then analyzed on a discontinuous sodium dodecyl sulfate-polyacrylamide electrophoresis gel, followed by autoradiography as described in Materials and Methods (Fig. 5). The iodinated virion preparation had four polypeptide bands of 62,000, 51,000, 36,000, and 30,000 molecular weights (lane E). The 62,000-molecular-weight polypeptide was the most abundant. Purified, iodinated feline calicivirus virion contained a major structural polypeptide of 65,000 molecular weight (lane F). Only the 62,000-molecular-weight polypeptide of the iodinated SMA virion preparation was immunoprecipitated by post-SMA-challenge serum from volunteer no. 1 (RIA titer, 1/1,600; lane B). Pre-SMA-challenge sera from volunteers no. 1 (RIA

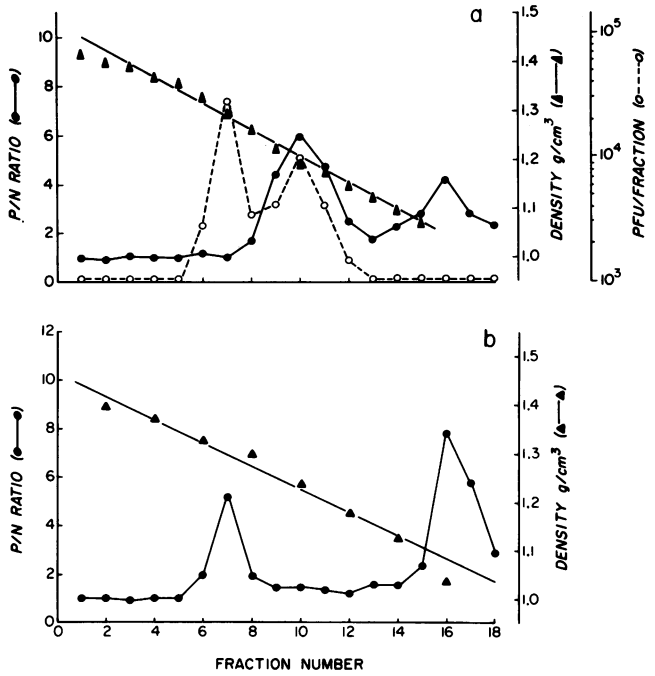


FIG. 3. Density comparison of SMA and feline calicivirus virions on a potassium tartrate-glycerol gradient. (Panel a) Purified SMA virion and feline calicivirus ($10^6$ PFU) were cosedimented to equilibrium on a potassium tartrate-glycerol gradient. SMA was measured by RIA, and feline calicivirus was measured by plaque assay as previously described. The density of the SMA virion peak was 1.21 g/cm$^3$. (Panel b) Purified SMA virion from a different stool homogenate was run to equilibrium on a potassium tartrate-glycerol gradient. No feline calicivirus was present, and SMA virion was measured by RIA. The density of the SMA virion peak was 1.29 g/cm$^3$. P/N, Positive/negative.

titer, 1/100; lane A) and 2 (RIA titer, 1/100; lane D) did not immunoprecipitate any polypeptides. The residual polypeptide bands present in lanes A to D represent nonspecific attachment of the iodinated polypeptides to Sepharose-protein A alone. This residual radioactivity could not be removed even after extensive washing. Even further dilution of prechallenge serum from volunteer no. 1 from 1/5 (lane A) to 1/25 (lane C) gave the same amount of residual bound radioactivity.

## DISCUSSION

This report describes the purification of the SMA virion, determination of its biophysical characteristics, and characterization of the major virion-associated structural protein. The purification procedure was adapted from the work of Ashley and Caul (3), who recommended potassium tartrate-glycerol gradients as an alternative to cesium chloride gradients for the purification of astroviruses, caliciviruses, and Norwalk-like agents. Cesium chloride appeared to render these virions more susceptible to degradation during subsequent concentration by centrifugation. When we ran a stool preparation on a potassium tartrate-glycerol gradient, the majority of the SMA antigen was virion associated, with little virus-specific soluble protein (Fig. 1). The virion obtained from the potassium tartrate-glycerol gradient appeared to remain intact after concentration to a pellet by centrifugation, yielding a homogeneous band on a sucrose velocity gradient, with no soluble virus-specific proteins in evidence (Fig. 2). The entire purification protocol yielded a small amount of virion containing four polypeptides as determined by iodination.

The SMA virion equilibrated on potassium tartrate-glycerol gradients at densities of 1.21 to 1.22 g/cm$^3$ and 1.29 g/cm$^3$ (Fig. 1 and 3). Information on the density of other Norwalk-like agents on potassium tartrate-glycerol gradients was not available for comparison. Ashley and Caul (3), however, found that small, round viruses in human feces, morphologically indistinguishable from the Norwalk agent, had the same density as feline calicivirus (1.28 g/cm$^3$) on a potassium tartrate-glycerol gradient. We observed feline calicivirus at a density of 1.29 g/cm$^3$ on potassium tartrate-glycerol gradients, but we also detected another, smaller peak of infectious feline calicivirus at 1.21 g/cm$^3$. These values coincide with those observed for the SMA virion. Thus, the values 1.28 to 1.29 g/cm$^3$ for SMA and calicivirus agree with previous data on these viruses (3, 19) and most likely represent the intact virions. Whether the 1.21- to 1.22-g/cm$^3$ particles represent an alternate form of the virion or changes in density resulting from the purification process is presently unknown. In this regard, an SMA virion preparation with a density of 1.29 g/cm$^3$ that was frozen at −70°C, thawed, and rerun on a potassium tartrate-glycerol gradient remained at a density of 1.29 g/cm$^3$ (data not shown). In addition, SMA virions of both densities were isolated from different stool preparations. Because of the limited number of specimens, we did not analyze these differences in density in more detail.

The density of the SMA virion in cesium chloride gradients was 1.33 to 1.34 g/cm$^3$, less than the 1.36 to 1.41 g/cm$^3$ reported for the other Norwalk-like viruses (1, 2, 14, 22). A shoulder in the broad peak of the SMA virion in the CsCl gradient, however, suggests that a portion of the SMA virion may be at the same density as the feline calicivirus and other Norwalk-like agents (1.37 g/cm$^3$). The 1.34-g/cm$^3$ peak was present in preparations concentrated by ammonium sulfate precipitation or high-speed centrifugation. The heterogeneity in density of SMA was observed in both potassium

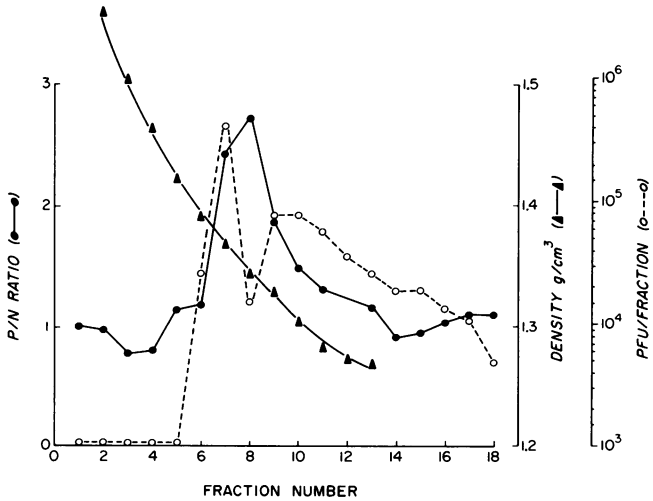


FIG. 4. Density comparison of SMA and feline calicivirus virions on a cesium chloride gradient. The purified SMA virion and feline calicivirus ($10^7$ PFU) were cosedimented to equilibrium on a cesium chloride gradient. SMA was measured by RIA, and feline calicivirus was measured by plaque assay as described in the text. The density of the SMA virion peak was 1.34 g/cm$^3$. P/N, Positive/negative.

tartrate-glycerol and CsCl gradients. In contrast, on a sucrose velocity gradient, the SMA virion formed a homogeneous, symmetrical peak, with the same S value as that of feline calicivirus. This value has been estimated to be 170 to 183S (19). The density differences, therefore, do not appear to represent substantial differences in the size or mass of the virus particle.

The SMA virion contained one major structural protein with a molecular weight of 62,000. The purified virion also contained other proteins of 51,000, 36,000, and 30,000 molecular weights that appeared to be contaminating rather than virus-specific proteins on the basis of immunoprecipitation analysis. The limited amount of virion available precluded attempts at further purification. In the same gels, the major feline calicivirus virion structural protein had a molecular weight of 65,000, a value consistent with previous work (19). In comparison, Greenberg et al. (12) reported that Norwalk virus has one major structural protein of 59,000 molecular weight. They at times also observed faintly visible bands of 40,000 and 34,000 molecular weights after 3 to 4 weeks of exposure of the autoradiographs containing the immunoprecipitated Norwalk virion. In our gels, the 36,000- and 30,000-molecular-weight polypeptides were not immunoprecipitated and may not be analogous to these polypeptides. Unfortunately, purified, iodinated Norwalk virus that was not immunoprecipitated was not included in their gel analyses, so the presence of other polypeptides in the Norwalk virus could not be determined. Our results suggest that SMA, like Norwalk virus, has properties which resemble those of the calicivirus group.

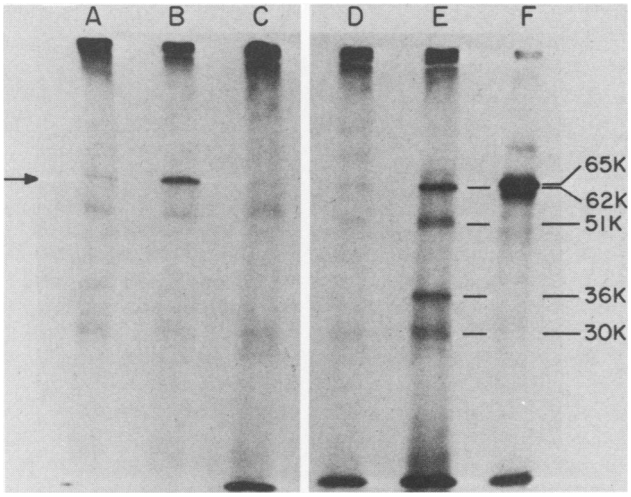


FIG. 5. Immunoprecipitation and polyacrylamide gel analysis of purified SMA virion. Purified SMA virion was iodinated, immunoprecipitated with pre- and post-SMA-challenge sera from volunteers, run on a polyacrylamide gel, and autoradiographed as described in Materials and Methods. Lanes: A, volunteer 1, prechallenge serum, 1/5 dilution (110,000 cpm added to gel); B, volunteer 1, postchallenge serum (2 weeks), 1/5 dilution (75,000 cpm added to gel); C, volunteer 1, prechallenge serum, 1/25 dilution (140,000 cpm added to gel); D, volunteer 2, prechallenge serum, 1/5 dilution (83,000 cpm added to gel); E, iodinated SMA virion preparation added directly to gel (250,000 cpm added to gel); F, iodinated, purified feline calicivirus virion added directly to gel (100,000 cpm added to gel). Unlabeled molecular weight marker polypeptides from Bio-Rad (92,500, 66,200, 45,000, 31,000, 21,500, 14,400) were run on a gel, stained with Coomassie blue, and used to calibrate the molecular weights of the feline calicivirus and SMA polypeptides. The arrow indicates the 62,000-molecular-weight SMA polypeptide; 65K = 65,000 molecular weight.

The fecal extracts also contained SMA-specific antigen not associated with the virions. The amount of soluble protein in the preparations varied from a small proportion relative to virion-associated antigen to all of the SMA-specific antigen in several preparations. In one instance, soluble protein appeared after purified virion was run on a potassium tartrate-glycerol gradient, suggesting degradation of intact virion to produce soluble protein. Greenberg et al. (12) found that soluble protein represented 50% of the Norwalk virus-specific antigens in fecal extracts and determined its molecular weight to be 30,000. When sufficient amounts have been obtained, we will characterize the SMA-specific soluble proteins in stool preparations.

The biophysical properties and polypeptide composition of the SMA virion suggest relationships to the calicivirus group and the Norwalk virus. The surface morphologies of the SMA and the Norwalk agent are not distinct (11, 15) when compared with the cuplike depressions of the caliciviruses (19). The nature of these morphological differences remains unresolved. Terashima et al. (21) have recently characterized the virion of a human calicivirus isolated from the stool of a patient with acute gastroenteritis. The virion has the morphological characteristics of a calicivirus, a buoyant density in cesium chloride of 1.37 to 1.38 g/cm$^3$, and a major structural protein of 62,000 molecular weight. However, the virus is not serologically related to Norwalk virus. Elucidation of the precise relationships among the Norwalk-like viruses and the human caliciviruses awaits further antigenic, biochemical, and genomic characterization of the viruses.

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