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Protein Composition of Coronavirus OC 43

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A human coronavirus, strain OC 43, was propagated in suckling mouse brain and purified 5000-fold with a 90% yield. Purity of the virus was confirmed by electrophoretic, ultracentrifugal, and electron microscopic procedures. Immunodiffusion and immunoelectrophoresis tests revealed one precipitin line with normal mouse brain, three with purified virus, and four with crude virus when tested against anti-pure virus or anti-crude virus animal serums. The association of a host cell antigen with the virion was confirmed by standard HI and CF tests. Polyacrylamide gel electrophoresis of solubilized purified virus revealed a minimum of six polypeptides with apparent molecular weights of 191,000 (No. 1), 104,000 (No. 2), 60,000 (No. 3), 47,000 (No. 4), 30,000 (No. 5), and 15,000 daltons (No. 6). A seventh band was occasionally found in the 165,000-dalton region of the gels. Four polypeptides contained carbohydrate and one contained lipid. Polypeptide No. 5 comprised 26% of the total viral protein and glycopolypeptide No. 3 comprised 23%. Three other components accounted for most of the remaining protein: polypeptide No. 4 (16%), glycopolypeptide No. 6 (14%), and glycolipopolypeptide No. 1 (13%). Glycopolypeptide No. 2 was 8% of the total protein. Bromelin digestion of the viral projections (spikes) removed glycopolypeptides No. 2 and No. 6. Association of the remaining polypeptides with structural components of the virion is only tentatively postulated. The buoyant density in potassium tartrate of the bromelin-treated virus was 1.15 g/cm^3 and of the intact OC 43 virion was 1.18 g/cm^3. By analytical ultracentrifugation the corrected sedimentation coefficient ($s^0_{20,w}$) of the OC 43 virion was determined to be 390 ± 16 S, and the apparent molecular weight (MW_a) was calculated to be 112 ± 5 × 10^6 daltons.

INTRODUCTION

Coronaviruses, a recently described group of human and animal viruses, resemble myxoviruses in size and structure, although various physical and host-sensitivity characteristics set them apart. These factors have been thoroughly outlined in reviews by Estola (1970) and Bradburne and Tyrrell (1971).

Antigenic characterization of the avian infectious bronchitis virus, the first of the coronaviruses to be described, has revealed three soluble viral antigens in allantoic fluids of infected embryonated eggs (Tevethia and Cunningham, 1968). Similar studies on human coronaviruses have only recently been conducted (McIntosh *et al.*, 1969; Bradburne, 1970; Kaye *et al.*, 1970). In this report we describe the structural protein and antigenic composition of OC 43 virus harvested and purified from suckling mouse brain (SMB).

MATERIALS AND METHODS

Source and purification of virus. OC 43 strain of human coronavirus was grown to high infectivity titers (10^7LD$_{50}$/.02 ml) in SMB and harvested as a 20% suspension in 0.01 *M* phosphate-buffered saline (PBS). The virus was extensively purified by temperature-dependent adsorption to and elution from fresh human "O" erythrocytes and by batch $CaHPO_4$ chromatography (Kaye *et al.*, 1970). The final purified virus suspen-

sion was concentrated either by ultrafiltration with XM100A or XM300 Diaflo membranes (Amicon Corporation[1], Lexington, MA), by pelleting at 20,000 rpm (26,400*g*) for 2 hr in a Beckman No. 40 rotor, or by hydrogel extraction (Lyphogel, Gelman Instrument Company, Ann Arbor, MI), depending upon the tests to be performed on the product.

Preparation of immune sera. Antiserums to crude virus (20% suspension in PBS), normal SMB (20% in PBS), and purified virus were prepared in adult Swiss white mice, both as serum and ascitic fluid, in adult guinea pigs (Hartley strain), and in adult White Leghorn chickens. For the production of antiserum in mice, five weekly ip injections of 0.5 ml antigen were given, and the mice were exsanguinated 2 weeks after the last injection. For production of ascitic fluid in mice, four weekly ip injections of 0.5 ml antigen were given (Days 0, 7, 14, 21), followed by 0.2 ml of a 10% suspension of sarcoma 180/TG ip on Day 25; 0.5 ml antigen ip on Day 28; paracentesis on Days 36, 39, and 42; and exsanguination on Day 42.

The schedule for producing antiserum in guinea pigs consisted of five weekly sc injections of 2.6 ml of a 50–50 mixture of antigen emulsified in Freund's incomplete adjuvant; guinea pigs were exsanguinated 2 weeks after the final injection. In chickens a brief schedule was used to enhance the titer of precipitating antibodies. Two milliliters of antigen were given ip and 2 ml of a 50–50 mixture of antigen in Freund's complete adjuvant were given im in four sites (0.5 ml/site) on Days 0 and 7; the chickens were exsanguinated on Day 13.

Preimmunization serum samples were obtained from all experimental animals, and serum was obtained from control animals to monitor adventitious infections.

Serologic tests. Hemagglutinin (HA) titrations were performed in the microtiter system with 0.01 *M* PBS diluent, pH 7.2, and 0.5% adult chicken red blood cells (RBC) (Hierholzer *et al.*, 1969). Indirect HA (IHA) tests for incomplete hemagglutinins were performed in the following manner. Serial dilutions of test antigens were mixed in 0.025-ml vol with 0.025 ml of 2 hemagglutinin-inhibition (HI) antibody units of guinea pig anti-pure virus serum and incubated for 2 hr at room temperature. Two HA units of crude virus in 0.025 ml were added and the test reincubated for 1 hr at room temperature. After addition of 0.05 ml of 0.5% chicken RBC suspension and incubation for 1 hr at room temperature, tests were observed for absence of inhibition in wells containing dilutions of test materials.

Complement fixation (CF) tests. CF tests were performed by the standardized microtiter method with overnight fixation of 5 units of complement (Casey, 1965).

Protein measurements. Protein determinations were made by the method of Lowry *et al.* (1951) and read in a Beckman DB spectrophotometer at 750 nm against a BSA standard curve.

Immunodiffusion (ID). Ouchterlony double-diffusion tests were carried out on 25 × 75-mm glass slides containing 4 ml 0.7% agarose (Kallestad Laboratories, Minneapolis, MN) in 0.01 *M* PBS, with 0.01% merthiolate as preservative. Wells 3 mm in diameter and 9 mm apart, center-to-center, were filled three times. The plates were incubated at room temperature for 2 days, photographed, stained with amido black (see IE procedure), and rephotographed.

Immunoelectrophoresis (IE). IE was performed on 25 × 75-mm slides containing 4 ml 1% agarose in 0.025 *M* Tris–barbital–sodium barbital buffer, pH 8.8. Antigen wells 1.5 mm in diameter were filled with 0.02–0.03 ml sample. The samples were electrophoresed for 75 min at 24° and 250 V at 5–8mA/slide in a 0.50 *M* Tris–barbital–sodium barbital buffer, pH 8.8. Arcs of precipitate developed after application of appropriate antisera to 1-mm troughs and incubation for 1 day at room temperature.

After immunoelectrophoresis the agar slides were stained for protein and protein-bound lipid (Uriel, 1964). The slides were washed for 2 days in 2% NaCl, rinsed for 2 days in distilled water, and dried overnight under filter paper at room temperature. For

[1] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

protein staining, the slides were immersed in amido black 10B (0.1% stain in 10% acetic acid and 45% methanol) for 20 min and destained through several rinses in the acetic acid–methanol solvent. For lipids, slides were stained with oil red 0 (saturated solution in 60% ethanol) and destained in 50% ethanol.

Polyacrylamide gel electrophoresis (PAGE). Polyacrylamide gel electrophoresis was performed based on the method of Holowczak and Joklik (1967). Gels, 6 × 60 mm, were composed of 7.5% acrylamide, 0.20% *N*,*N*′-methylenebisacrylamide, 0.1% sodium lauryl sulfate (SLS), 0.0575% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, and 0.07% ammonium persulfate, in 0.01 *M* sodium phosphate buffer, pH 7.2. Samples to be electrophoresed were solubilized by incubation for 1 hr at 37° with equal volumes of the 0.01 *M* phosphate buffer containing 4% SLS, 2% 2-mercaptoethanol (ME), and 1.0 *M* urea. Samples were then heated at 100° for 1 min. The materials were dialyzed overnight at room temperature against the phosphate buffer containing 0.1% SLS, 0.1% ME, and 0.05 *M* urea. Samples thus treated and containing 75–135 μg of protein were stabilized to 10–20% sucrose and applied to the gels. The gels were electrophoresed at 78–86 V and at 3.5 mA per tube at room temperature until a tracking dye, 0.05% bromphenol blue, migrated to 1 mm from the distal end of the gel (about 60 min). The reservoir buffer in both chambers during electrophoresis was 0.01 *M* sodium phosphate, pH 7.2, with 0.1% SLS.

The gels were removed from the support tubes and stained for protein, protein-bound lipid, and protein-bound carbohydrate. For protein staining, the gels were fixed with 10% trichloroacetic acid (TCA) for 4 hr, stained with 0.05% Coomassie blue in 10% TCA for 8–12 hr, and destained for 3–5 days in 10% TCA (Chrambach *et al.*, 1967). Alternately, gels were stained with 0.1% Coomassie blue in 50% methanol/7.5% acetic acid and power-destained as described by Maizel *et al.* (1970). For lipid staining, gels were immersed in saturated oil red 0 in 60% ethanol for 2 hr, destained with 50% ethanol for 2 hr, and rehydrated in distilled water (Crowle, 1961). For carbohydrate staining, gels were oxidized with 1% periodic acid in 3% acetic acid for 1 hr, rinsed in distilled water for 1 hr, stained with 0.5% reduced acidified basic fuchsin (Schiff's reagent) for 45 min, destained in distilled water, and stored in 1% sodium metabisulfite (Clarke, 1964).

All gels were scanned for accurate detection and quantitation of bands with a Gilford model 2410 linear transport coupled to a model 2000 recording spectrophotometer (Gilford Laboratories, Oberlin, OH). Gels stained with Coomassie blue were scanned at 640 nm, those with oil red 0 at the absorption maximum of 492 nm, and those with Schiff's reagent at the absorption maximum of 534 nm.

Molecular weight determinations of the separated polypeptides were performed by PAGE as described by Shapiro *et al.* (1967). Solubilized cyt. *c*, RNAse, hemoglobin, carboxypeptidase A, pepsin (all from Nutritional Biochemicals Company, Cleveland, OH), trypsin, ovalbumin, bovine serum albumin (Schwarz/Mann, Orangeburg, NY), and fetuin (GIBCO, Grand Island, NY), and nonsolubilized purified human IgG (courtesy of Dr. H. Daugharty, Center for Disease Control) were used as molecular weight standards in coelectrophoresis and companion electrophoresis runs. Fetuin was also used as a carbohydrate standard.

Enzyme treatment. Purified virus was treated with bromelin (NBC, Cleveland, OH) in a reducing buffer with dithiothreitol as described by Compans *et al.* (1970) and was subsequently repurified on a 5–40% potassium tartrate gradient by equilibrium centrifugation at 75,000*g*.

Electron microscopy (EM). Negative contrast electron microscopy was carried out with concentrated samples sprayed onto carbon-coated grids, stained with 2.0% sodium phosphotungstate (PTA), and examined at 80 kV with a Philips EM-300 electron microscope.

Ultracentrifugal analysis. Sedimentation coefficients and estimates of molecular weight were determined at 15, 16, and 18,000 rpm and at 20.0° with a An-H titanium rotor and 12-mm Kel-F cells in a Beckman Model E ultracentrifuge equipped with schlieren,

interference, and uv optical systems. The virus samples were dialyzed against 0.01 *M* sodium borate buffer, pH 8.0, with 0.16 *M* NaCl, and contained 1–2 mg protein per 0.4 ml. Apparent molecular weight (MW_a) was calculated from equations derived by Svedberg and Pedersen (1940) and as applied by Burness and Clothier (1970).

RESULTS

Virus purity. The virus used throughout these studies was purified to a 5000-fold decrease in total protein, with a yield of 90%. The purity of the virus (at an HA titer of 1.3×10^5) was confirmed by immunologic procedures, density gradient centrifugation, electron microscopy, and analytical procedures. Electron microscopy at low and high magnifications showed very clean fields of particles exhibiting the pleomorphism typical of this virus (Almeida and Tyrrell, 1967; Almeida and Waterson, 1970; Apostolov *et al.*, 1970; McIntosh *et al.*, 1970; Bradburne and Tyrrell, 1971). Analytical ultracentrifugation revealed a sharp peak at 15,000 rpm with as little as 1.0 mg protein in the sample; this peak had the same sedimentation rate as one of several broad peaks observed when crude virus (at 2–4 mg protein/.4 ml) was sedimented under identical conditions. Acrylamide gels loaded with untreated concentrated supernatant fluids obtained from centrifugation (23,800*g*, 2 hr) of purified virus suspensions revealed no protein bands.

Immunization and serology. Mice, guinea pigs, and chickens were immunized with normal SMB (NSMB), crude SMB-grown OC 43, and purified virus. All sera obtained before and during the course of immunization were tested for HI, CF, and precipitating antibody. Preimmunization serum samples and sera from normal animals were consistently negative. Among the postimmunization serum samples, mouse ascitic fluid and chicken antisera had the lowest HI and CF antibody titers to OC 43 virus and guinea pig antisera had the highest titers (Table 1). Sera from mice and guinea pigs immunized with NSMB showed low-level HI and CF activity against the purified OC 43 antigen.

Immunodiffusion with unsolubilized virus. Mouse ascitic fluids and mose antiserums prepared against crude virus and purified virus gave predominantly single bands (of identity) with pure or crude virus in standard ID tests. However, tests with guinea pig and chicken anti-crude and anti-pure virus sera showed two precipitin bands of complete identity between pure and crude virus, and one additional band of identity between pure virus, crude virus, and NSMB (Fig. 1A). These results suggest that the OC 43 virion contains at least one mouse brain antigen. Additional evidence of host antigen in the intact virus is seen by the identical line between anti-NSMB serum and the three concentrated antigens (Fig. 1B). Immunoelectrophoresis of these same antigen–antiserum combinations demonstrated three or four precipitin arcs with crude virus and two–three arcs with purified virus, and con-

TABLE 1

SERUM ANTIBODY TITERS OF ANIMALS IMMUNIZED WITH NORMAL SMB AND WITH CRUDE AND PURIFIED OC 43 VIRUS[a]

Immunizing antigen	Mouse		Mouse ascitic fluid		Guinea pig		Chicken	
	HI[b]	CF[c]	HI	CF	HI	CF	HI	CF
Normal SMB	20	8	<10	<8	20	8	<10	—
Crude virus	1280	128	320	32	5120	512	160	—
Purified virus	1280	256	160	32	2560	1024	640	—

[a] All titers listed represent the average antibody titer of five animals per immunizing antigen. All tests performed with purified virus antigen.

[b] Hemagglutination-inhibition titer expressed as the reciprocal of the end point serum dilution.

[c] Complement-fixation titer expressed as the optimal dilution of serum as determined by the optimal dilution of antigen in a block titration.

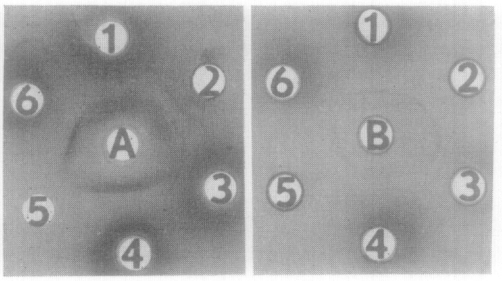

FIG. 1. Immunodiffusion tests with 80× concentrated crude virus (1), purified virus (2), and normal SMB (3), and unconcentrated crude virus (4), normal SMB (5), and purified virus (6). Center well A contains guinea pig anti-crude virus serum, and B contains guinea pig anti-NSMB serum.

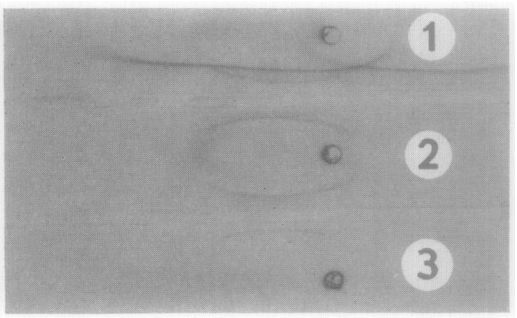

FIG. 2. Immunoelectrophoresis of 80× concentrated crude virus (1), purified virus (2), and normal SMB (3) antigens reacted against guinea pig anti-crude virus serum (both troughs).

firmed the presence of a mouse brain protein associated with the purified virus (Fig. 2).

PAGE with solubilized virus. The purified virus was solubilized with SLS–ME–urea, and the resulting proteins were separated by PAGE. The staining patterns with Coomassie blue revealed a minimum of six bands, five of which might be considered major (Fig. 3). One additional very faint band was occasionally observed between bands one and two.

The molecular weights of the viral proteins, as determined by co- and companion electrophoresis with proteins of known molecular weights, were estimated to be 191,000; 104,000; 60,000; 47,000; 30,000; and 15,000 in decreasing order (Fig. 4). The very faint band was located in the 165,000-dalton region of several gels. Gels were also stained for lipid and carbohydrate (Fig. 5).

Polypeptide No. 5 (26%) and glycopolypeptide No. 3 (23%) constituted nearly one-half of the total virus protein. Polypeptide No. 4 (16%), glycopolypeptide No. 6 (14%), and glycolipopolypeptide No. 1 (13%) were similar in concentration and comprised most of the remaining viral protein. The lipid content of glycolipopolypeptide No. 1 was more evident in gels in which the sample was not heated, than in those in which it was heated at 100°C for 1 min. Glycopolypeptide No. 2 (8%) was found in the smallest quantity (Table 2).

Composition of the projection antigen. The spikes on the virion surface have previously been associated with HA activity and are thought to comprise the major antigens measured by both the HI and CF tests. Hence these surface spikes or "projection antigens," unlike influenza virus, appear to consist of a single species (Kaye *et al.*, 1970). Absence of neuraminidase was confirmed using a highly sensitive assay system (Laver and Kilbourne, 1966; Laver and Valentine, 1969): overnight incubation of 10^5 HA units of virus with fetuin failed to yield any detectable sialic acid.

Attempts to determine directly which polypeptides comprise the external antigen were unsuccessful. Bands from acrylamide gels were eluted in PBS, dialyzed, and titrated for direct and indirect hemagglutination. In both tests, the apparent residual SLS adversely affected the erythrocytes.

Repeated attempts to determine the composition of the projection antigen by selectively removing the entire projection from the virus envelope also were of limited value. The agents used represented the major chemical groups often employed in similar studies: reducing agents (6.0 *M* guanidine + 5.0 m*M* dithiothreitol (DTT); 0.003 and 0.03 *M* DTT; 0.005, 0.05, and 0.5 *M* 2-mercaptoethylamine); oxidizing agents (67.0 and 85.7% dimethylsulfoxide; 0.1 and 0.2% β-propiolactone); ionic detergents (0.1, 0.5, 1.0, and 2.0% desoxycholate; 0.5% Tween 20); and nonionic detergents (1.0% Nonidet P-40; 1.0% P-40 + 0.5 *M* urea). These chemical treatments, all used in selected buffer, salt, pH, and temperature environments, were uniformly nonselective and were overly destructive to the entire virion.

The bromelin procedure described by

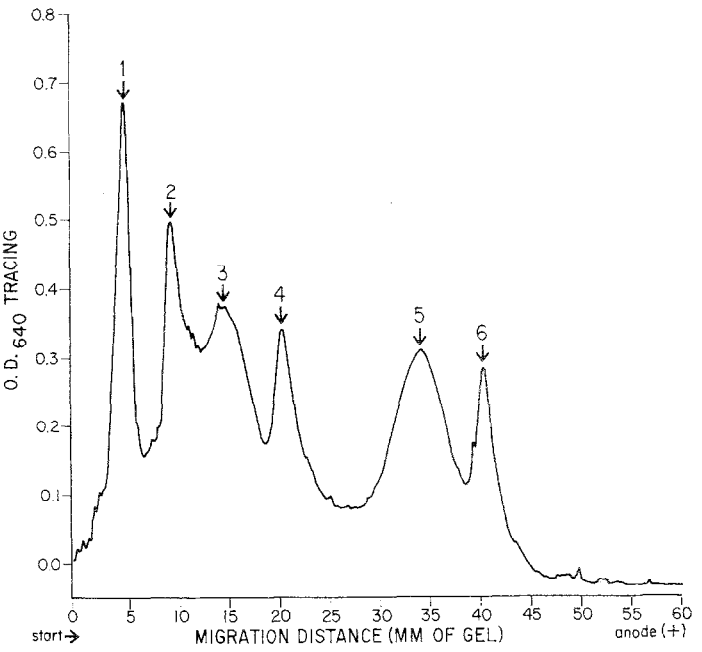

FIG. 3. Protein components of OC 43 virus separated by polyacrylamide gel electrophoresis.

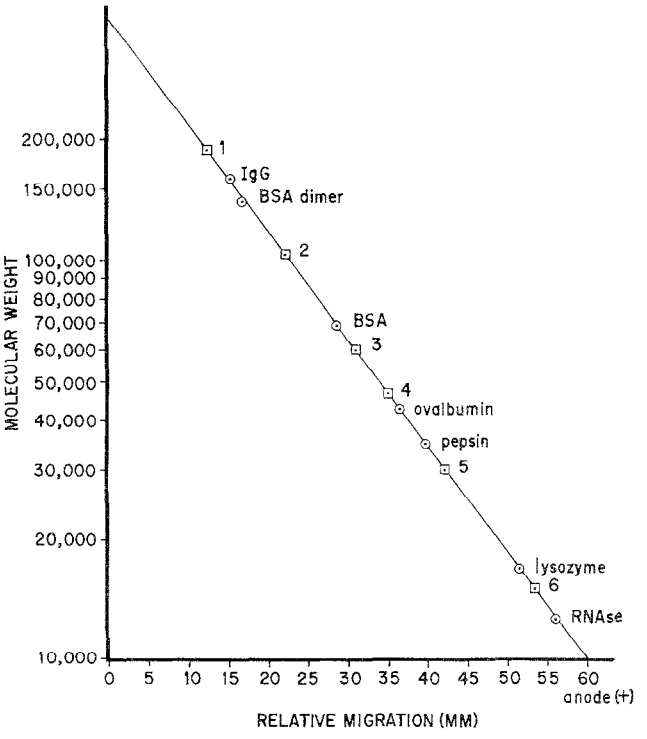

FIG. 4. Molecular weight of OC 43 virus proteins as determined by acrylamide gel electrophoresis.

Compans *et al.* (1970) for influenzaviruses was employed as an indirect means of locating the proteins comprising the projection antigen. The effect of bromelin treatment on purified virus was monitored every 30 min under the electron microscope. The projections were completely degraded after 2 hr of incubation at 37°C with 0.13% bromelin.

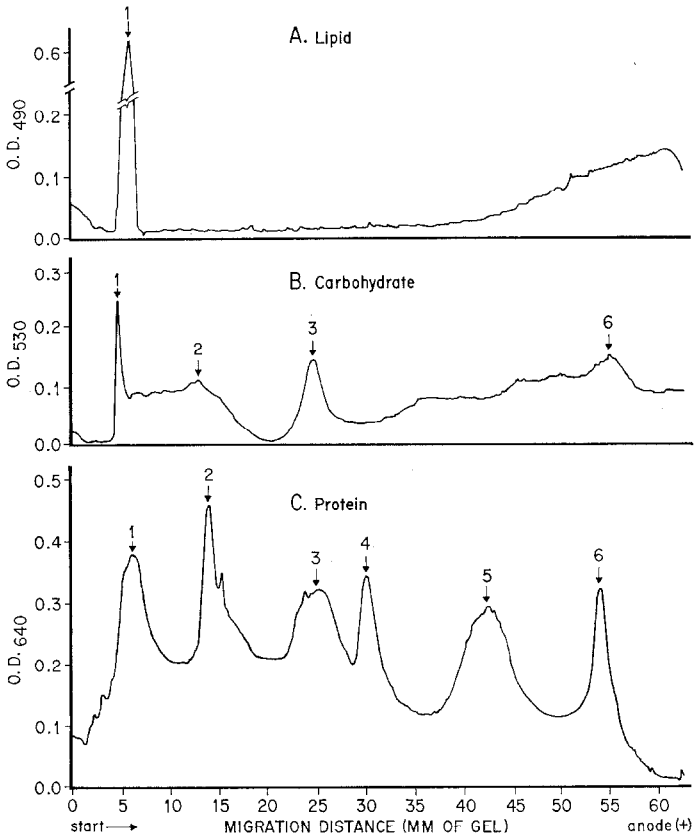

FIG. 5. Chemical staining reactions of polypeptides of OC 43 virus separated by polyacrylamide gel electrophoresis. A, gels stained with oil red O and scanned at 490 nm; B, companion gels from the same run stained with Schiff's reagent and scanned at 530 nm; and C, companion gels stained with Coomassie blue and scanned at 640 nm.

The 2-hr test samples, along with the appropriate virus and enzyme controls, were repurified on 5–40% aqueous neutral potassium tartrate gradients in an SW39L rotor at 75,000*g* for 8 hr. The untreated control virus banded in the 1.18 g/cm^3 region of the gradient and was associated with a sharp HA peak. By EM this band was found to consist of typical virions (Fig. 6A, B). The bromelin-treated virus banded in the 1.15 g/cm^3 region of the gradient; by EM this band contained completely "despiked" but fully enveloped virus particles (Fig. 6C–E). All biological activity was lost by the enzyme treatment (Table 3). The repurified virus control, the purified "despiked" particles, and the comparable area from the gradient purification of the bromelin control sample were examined by PAGE. Polypeptides 2 and 6—both glycoproteins—were absent in the bromelin-treated sample (Fig. 7).

Sedimentation coefficient and molecular weight of virion. Analytical ultracentrifugation of intact, purified virus (HA titer 2.6 × 10^5) at 15, 16, and 18,000 rpm revealed a single distinct peak with a sedimentation coefficient ($s_{20,b}$) of 368 ± 14 × 10^{-13} sec. Corrections for solvent density and viscosity and for partial specific volume of the virus resulted in an $S^c_{20,w}$ value of 380 ± 15 S. The virus concentration was extrapolated to infinite dilution from the data of Burness and Clothier (1970) to give a fully corrected sedimentation coefficient ($S^c_{20,w}$) of approximately 390 ± 16 S. The apparent molecular weight (MW_a) was calculated to be 112 ± 5 × 10^6 daltons, so that the particle

TABLE 2

POLYPEPTIDES OF OC 43 VIRUS

Polypeptide band No.	Approximate MW ($\times 10^3$)[a]		Percent of total protein	Staining reactions
	Mean	Range		
1	191	(171–210)	13	Lipid, carbohydrate, protein
2	104	(93–115)	8	Carbohydrate, protein
3	60	(53–66)	23	Carbohydrate, protein
4	47	(40–52)	16	Protein
5	30	(27–34)	26	Protein
6	15	(13–17)	14	Carbohydrate, protein

[a] Mean of a minimum of 38 gels from 14 electrophoresis runs on seven different lots of purified virus.

weight of one OC 43 virion is approximately $18 \pm 1 \times 10^{-17}$ g.

DISCUSSION

The coronavirus virion has been the subject of a number of studies during the past few years and much is now known about its morphology. The coronaviruses contain RNA (Hamre *et al.*, 1967; Becker *et al.*, 1967; Bradburne and Tyrrell, 1971) which may be present as a form of ribonucleoprotein (RNP) or internal nucleocapsid. Tevethia and Cunningham (1968) thought that "antigen 2" of avian infectious bronchitis (AIB) virus was RNP since it was sensitive to both ribonuclease and proteolytic enzymes. Becker *et al.*, (1967) described an "inner shell" which may have been an internal nucleocapsid. More positive proof is offered by Apostolov *et al.* (1970) who found in AIB virus a threadlike internal component 7–8 nm in diameter.

Most reports agree that coronaviruses possess an outer double membrane and an inner layer (Becker *et al.*, 1967; Apostolov *et al.*, 1970; Uppal and Chu, 1970; Oshiro *et al.*, 1971). For AIB and 229E viruses, Becker *et al.* (1967) described a 7–8-nm thick outer shell, a 4–8 nm electronlucent zone, and a 9–17-nm thick inner shell. Oshiro *et al.* (1971) found a similar pattern in the Linder strain of 229E. Apostolov *et al.* (1970) describe this envelope as a "3-layered unit membrane structure." The viral envelope of AIB and 229E appears to contain host lipoprotein (Becker *et al.*, 1967; Berry and Almeida, 1968, Apostolov *et al.*, 1970). This finding is consistent with the mode of virus reproduction—budding from intracytoplasmic cisternae (Becker *et al.*, 1967; Hamre *et al.*, 1967; Apostolov *et al.*, 1970; Oshiro *et al.*, 1971).

The projections of coronaviruses are approximately 10 nm across the club or petal-shaped terminus and are 15- to 20-nm long (Almeida and Tyrrell, 1967; Bradburne and Tyrrell, 1971). They may be composed of two antigens (Tevethia and Cunningham, 1968).

The number of antigens associated with the coronaviruses is still not clear. Part of the problem may relate to the inability to effectively disrupt the virus. Two–three bands were seen in ID tests and three–four precipitin arcs were seen in IE tests with crude OC 43 virus and anticrude or anti-pure virus sera. These were only observed with highly concentrated antigen preparations. The addition of SLS or of Na *N*-lauroylsarcosinate in various concentrations failed to increase the number of precipitin lines. Our findings support the multiplicity of bands observed in other studies (Tevethia and Cunningham, 1968; McIntosh *et al.*, 1969, Bradburne, 1970).

The one arc found at the antigen well in ID and IE tests was probably whole virus. Another precipitin line was always continuous with the NSMB material and was, therefore, host related. The host-related antigen may correspond to the anti-host activity measured in HI and CF tests. This is consistent with the observation of Berry and Almeida (1968) that AIB virus contains host cell lipoprotein in the envelope. The additional one or two bands seen, especially

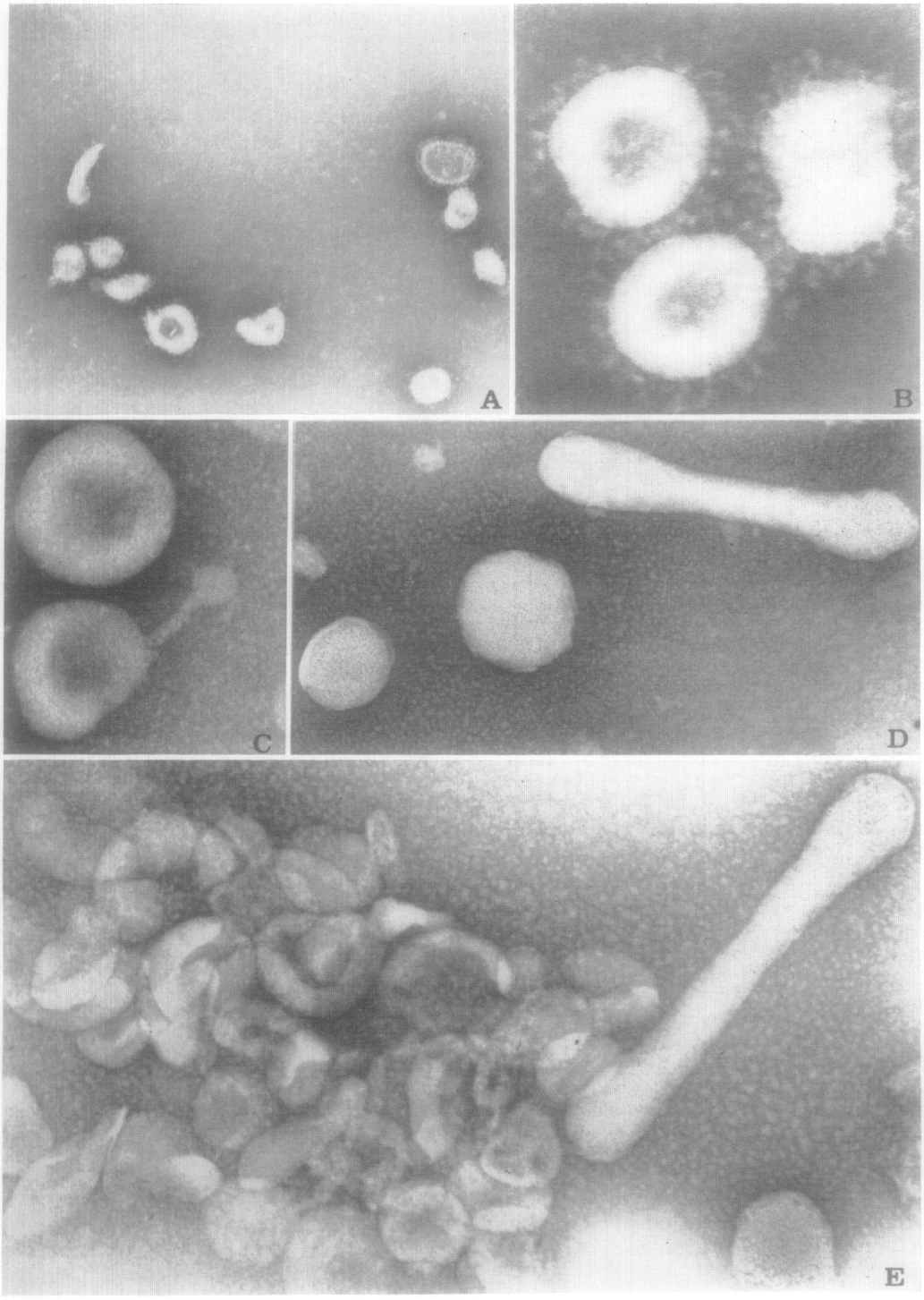

FIG. 6. Electron micrographs of OC 43 virus from control preparations showing typical pleomorphism and corona of surface projections (A, ×50,000; B, ×225,600), and from bromelin-treated preparations showing the complete absence of spikes (C, ×192,600; D, ×112,000) and the intact but weakened envelope (E, ×139,400).

TABLE 3

PROPERTIES OF BROMELIN-TREATED OC 43 VIRUS

Sample[a]	Buoyant density (g/cm³)	Infectivity in SMB (log_{10} LD_{50}/0.02 ml)	Antigen titer[b]		No. of bands in PAGE	No. of precipitin arcs in IE
			HA	CF		
1 Control virus	1.18	9.8	32,768	128	6	3
2 Bromelin-treated virus	1.15	0.7	<1	<2	4	1
3 Bromelin control	—	0.0	<1	<2	0	0

[a] Harvests from potassium tartrate gradients after equilibrium centrifugation at 75,000*g* for 8 hr: No. 1 was purified virus incubated for 2 hr at 37° with 0.1 *M* Tris-HCl buffer, pH 7.2, containing 0.001 *M* EDTA and 0.005 *M* DTT (final concentrations), and collected from the 1.18–1.19 g/cm³ region of the gradient; No. 2 was purified virus treated with 0.13% bromelin in the above buffered medium at the same conditions, and collected from 1.15 to 1.16 g/cm³ region of the gradient; and No. 3 was the equivalent amount of bromelin and buffered medium incubated with PBS, and collected from a gradient in a manner identical to sample No. 2.

[b] Titer expressed as reciprocal of end point dilution.

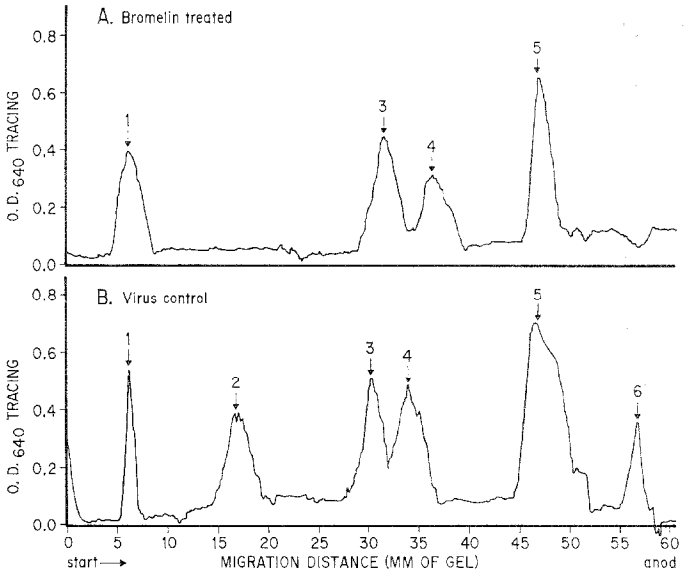

FIG. 7. Polyacrylamide gel electrophoresis patterns of purified virus treated with 0.13% bromelin for 2 hr and repurified on a tartrate gradient (A) and of the control preparation from the same experiment (B).

in IE tests, probably were the results of electrophoretic breakdown of the virus spikes; indeed, IE tests performed with bromelin-treated (despiked) particles did not exhibit these lines. In these tests only a single arc developed, and this represented a structure with considerably greater electrophoretic mobility than was exhibited by whole virus. Such observations suggest the antigens reactive in the ID or IE tests are probably associated with the virion surface. At least one or two additional precipitin lines might be expected under conditions of complete virus disruption and release of internal components. Indeed this would be suspected by our studies with PAGE.

A minimum of six polypeptide components were demonstrated under the described conditions of solubilizing and examining purified virus by PAGE. These components ranged in molecular weight from 15,000 to 191,000 daltons. However, these molecular weight

figures can only be considered as approximations since the reliability of the PAGE procedure when used for molecular weight determinations is greatest for proteins in the range of 15,000–165,000 *MW* and since several sources of error are inherent in the PAGE technique. The six or possibly seven bands found in acrylamide gels most likely represented distinct polypeptides and not oligomers because the inclusion of 1.0 *M* urea as a hydrogen bond-breaking agent in the solubilizing mixture and the use of heat at 100° would minimize protein aggregation.

The classification of the polypeptides as glyco- or glycolipoproteins must be considered as tentative at this point, since the chemical nature of these complexes cannot be precisely defined by differential staining techniques. The use of more sensitive procedures to confirm the nature of these polypeptides awaits the successful adaptation of OC 43 coronavirus to growth in tissue culture systems.

By indirect methods several of these polypeptides can be related to virus structural components. Glycopolypeptides No. 2 (*MW* 104,000) and No. 6 (*MW* 15,000) which together constituted 22% of virus protein were associated with the surface projections. Because of its lipid content, one might speculate that the high molecular weight glycolipopolypeptide (No. 1) representing 13% of the total protein may be the outermost layer of the three-layered envelope.

The remaining polypeptides are not readily localized. Glycopolypeptide No. 3, representing 23% of the total virus protein, polypeptide No. 5, representing 26%, and polypeptide No. 4, a relatively low *MW* (47,000 daltons) protein making up 16% of the protein are still unaccounted for. These proteins may comprise the remaining two layers of the virus membrane and the protein moiety of the RNP, respectively. The faint band (approximately 165,000 daltons) occasionally observed in acrylamide gels could not represent more than 2% of total virus, and may not be structural protein. In any event, the presence of a minimum of six or seven polypeptides in a virus with a molecular weight of 112 million suggests a highly complex structural organization.

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