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Structure of Infectious Bursal Disease Virus

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Infectious bursal disease virus of chickens was purified, and its structure was examined by the negative-staining technique in the electron microscope. The buoyant density of infectious bursal disease virus in CsCl was found to be 1.34 g/cm^3. The morphological details suggest that the capsid of the virion consists of a single layer of 32 capsomeres arranged in 5:3:2 symmetry. The virion measured about 55 nm in diameter and had no envelope.

Infectious bursal disease (IBD), formerly termed Gumboro disease, is a viral disease of young chickens that causes necrosis of lymphocytes. The disease specifically affects the bursa of Fabricius (BF), initially causing severe edema followed by atrophy. IBD virus was tentatively classified as a member of the reovirus group (3). The classification was based upon the morphology under ultrathin-section electron microscopy of the BF from infected chickens (4, 8, 17, 18) and upon limited biochemical characterization (5, 9). Almeida and Morris (1) recently showed by means of immuno-electron microscope techniques that the virus has a hexagonal outline and a diameter of 55 to 60 nm. In a previous study, we suggested particles with a diameter of 55 nm, probably consisting of 92 capsomeres arranged in an icosahedral form (K. Hirai, S. Shimakura, and E. Kawamoto, manuscript in preparation). Little information is available on the ultrastructure of the IBD virus.

In this paper, electron micrographs of purified IBD virus, negatively stained with phosphotungstate, are presented.

MATERIALS AND METHODS

Virus and cell. The two strains of IBD virus were used. The strain GBF-1 was described previously (11); it was prepared from the 12th passage of the virus in susceptible chickens. An egg-adapted strain 1/PV was kindly supplied by G. Cervio of Instituto Zooprofilattico Sperimentale della Lombardia E dell' Emlia, Italy. It replicates in embryo fibroblasts producing cytopathic effect (22). Primary chicken embryo fibroblast cell monolayers were prepared from 11-day-old chicken embryos. The cells were grown in Eagle medium supplemented with 5% bovine fetal serum and were maintained in Hanks balanced salt solution containing 0.5% lactalbumin hydrolysate and 0.5% bovine fetal serum. Virus infectivity titrations were done in tube cultures of chicken embryo fibroblast cells at 37 C, and the 50% end point was calculated according to the method of Reed and Muench (23). For the preparation of radioactively labeled virus, the IBD virus was grown in chicken embryo fibroblast cells maintained in lactalbumin hydrolysate and 0.5% bovine fetal serum containing [^{3}H]uridine (3 μCi/ml).

Virus purification. The purification materials were typically affected BF (strain GBF-1) and infected cultural fluid (strain 1/PV).

The typically affected BF were harvested 4 days after inoculation, weighed, suspended 1:1 (wt/vol) with Tris-hydrochloride-buffered saline, consisting of 0.01 M Tris-hydrochloride (pH 7.0) and 0.1 M NaCl, and then homogenized in a Waring blender and Teflon homogenizer. The resulting homogenate was frozen and thawed 5 times and was sonically treated (30 s at 20 kc). After slow centrifugation (5,000 rpm for 60 min) to remove cellular debris, the supernatants were treated twice more with fluorocarbon. The virus was concentrated by centrifugation at 30,000 rpm for 3 h. The pellet was resuspended in Tris-hydrochloride-buffered saline. The virus was recentrifuged at 30,000 rpm for 3 h, and the pellet, after brief sonic treatment to disperse aggregates, was isopycnically banded three times in CsCl at 30,000 rpm for 24 h (RPS 40 rotor). Following centrifugation, fractions were collected from the bottom of the centrifuge tube, and either the infectivity or radioactivity of each fraction was determined. The buoyant densities of representative fractions were calculated from their refractive index measurement.

The infected culture fluids, exhibiting 4+ cytopathic effects after 2 to 3 days at 37 C, were harvested, and cellular debris was then removed from the culture fluids by low speed sedimentation; solid, dry-heat-sterilized NaCl (24 g/liter) was added to the supernatant fluids and polyethyleneglycol 6,000 was added as a 10% (wt/vol) solution. The polyethyleneglycol mixtures were left for at least 1 h at 4 C before the sedimentable phase was collected. The virus was suspended in Tris-hydrochloride-buffered saline and treated twice more with fluorocarbon. Thereafter, purification procedures were performed as described above.

Electron microscopy. All fractionated samples were prepared by the conventional negative-staining technique for electron microscope examination. Sam-

ples to be examined were dialyzed in the cold against 0.01 M Tris buffer to remove CsCl and were mixed 1:1 with stain (4% phosphotungstic acid in double-distilled water adjusted to pH 6.0 with KOH) containing 0.1% sucrose. After 2 to 3 min, a drop of the mixture was transferred to carbon-coated copper grids, and a further 2 to 3 min were allowed for particles to adsorb to the grid surface. After removal of the excess fluid, the grids were air-dried at room temperature and examined in a Hitachi HU-12 model electron microscope operating at 100 kV. Magnifications were calibrated against a diffraction grating replica.

RESULTS

Buoyant density. Isopycnic sedimentation in CsCl of IBD virus derived from the infected BF (virulent strain GBF-1) revealed the presence of a single band with a density of 1.34 g/cm^3 (Fig. 1A). Sedimentation in CsCl of [^{3}H]uridine-

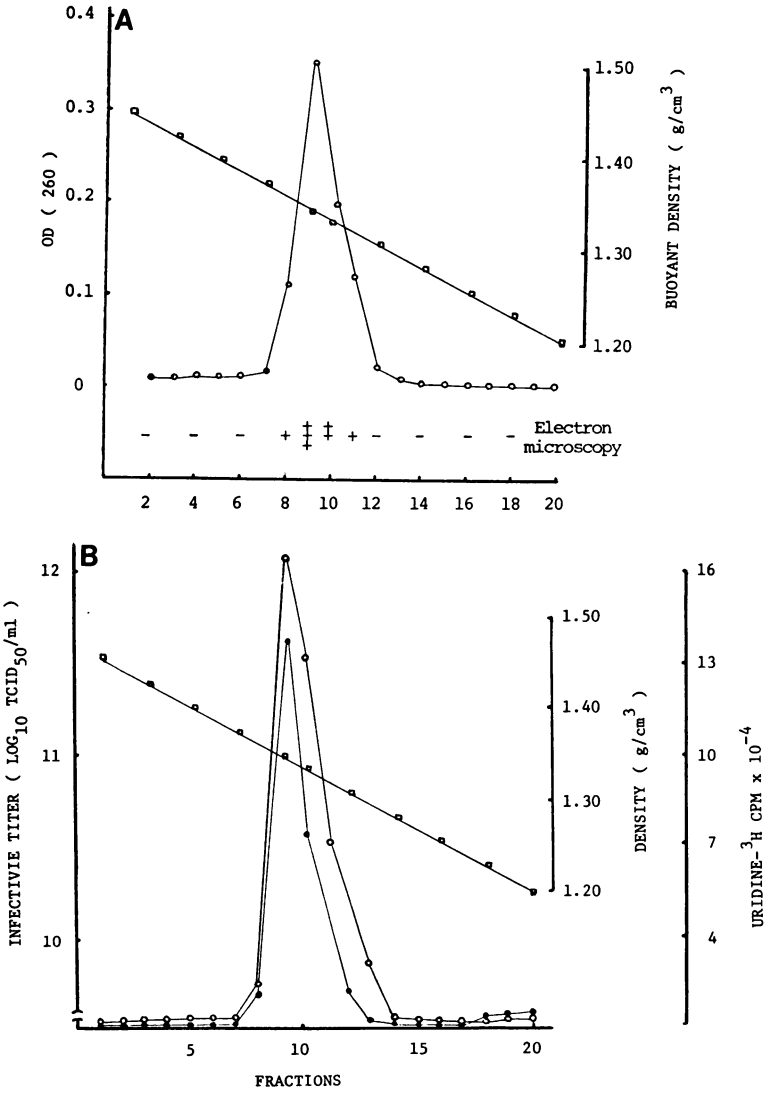

FIG. 1. *(A) Isopycnic sedimentation profiles of IBD virus in CsCl. Viral material was obtained from the BF of chickens inoculated with virulent strain GBF-1. The virus was centrifuged at 30,000 rpm for 24 h at 4 C in a Hitachi 55 P model ultracentrifuge with an RPS 40 rotor. The direction of the centrifugal force is from left to right. Symbols: ○, OD; □, buoyant density; and +, number of viral particles. (B) Sedimentation profiles of IBD virus in CsCl. Viral materials were obtained from culture fluids of chicken embryo fibroblast cells inoculated with nonvirulent egg-adapted strain 1/PV. The centrifugation of [^{3}H]uridine-labeled virus at 4 C was for 24 h at 30,000 rpm with the RPS 40 rotor in the Hitachi 55 P model ultracentrifuge. The direction of the centrifugal force is from left to right. Symbols: ○, viral infectivity; ●, [^{3}H]uridine counts/min; and □, buoyant density.*

labeled IBD virus (nonvirulent, egg-adapted strain 1/PV) also yielded a single component (Fig. 1B). The peaks of radioactivity corresponded to those of virus infectivity at a density of 1.34 g/cm^3.

Shape and size of the virions. Negatively stained preparations of the banded virus samples (1.34 g/cm^3) are presented in Fig. 2. Both an egg-adapted nonvirulent strain of virus (Fig. 2A) and a virulent strain (Fig. 2B) were employed for comparison. The virus particles were present in a majority of unaltered particles within the respective bands. The majority of the virus particles appeared uniform in shape and size and clearly showed a nonenveloped, hexagonal profile in outline; they probably represent an icosahedral configuration viewed along an axis of two- or threefold symmetry. The parti-

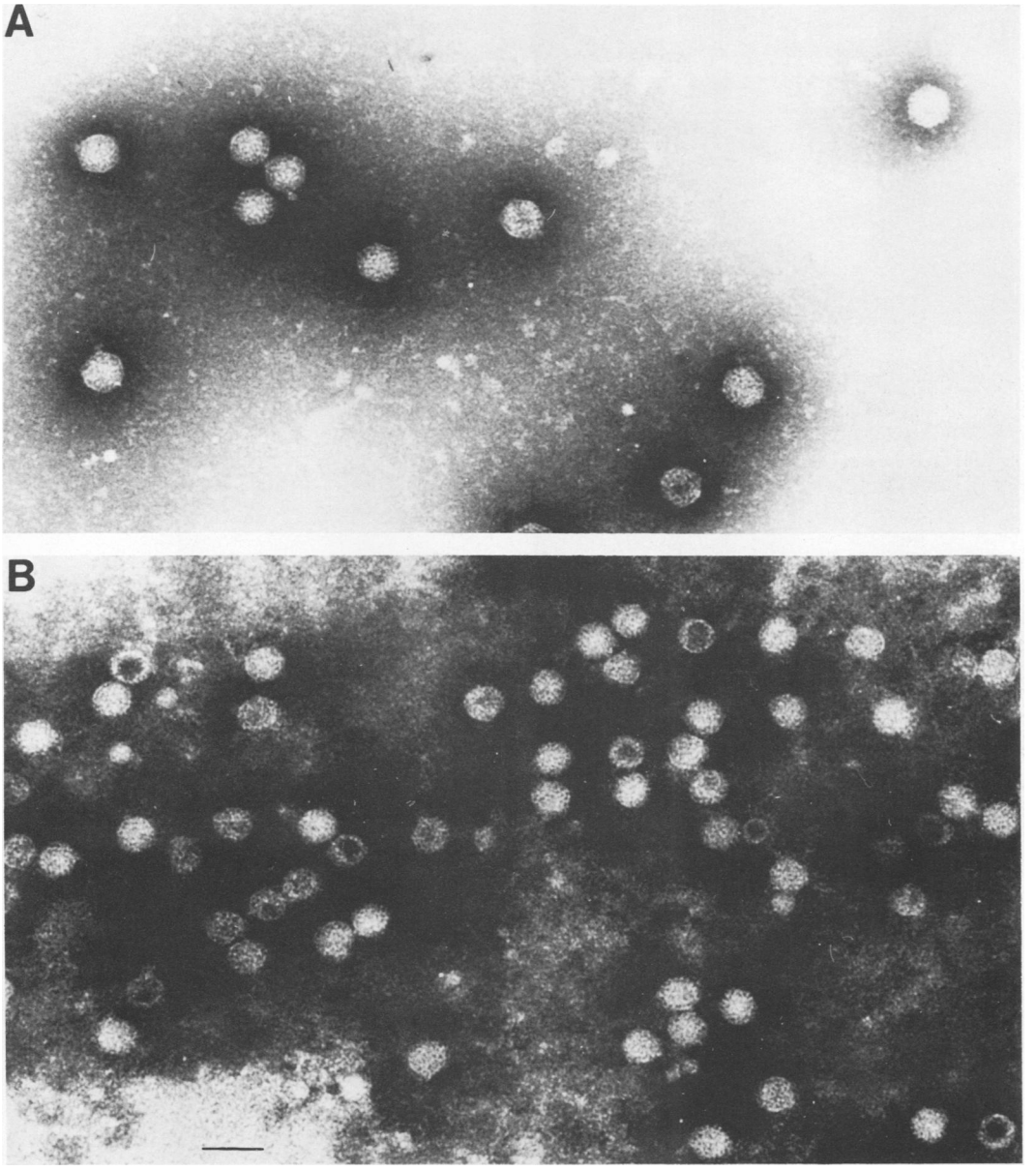

FIG. 2. *Electron micrographs of negatively stained preparations of CsCl-banded virus. It is 55 nm in average diameter. (A) IBD virus particles from the nonvirulent strain (1/PV; ×99,900). (B) A field of IBD virus particles from the virulent strain (GBF-1; ×86,400). Each bar represents 100 nm.*

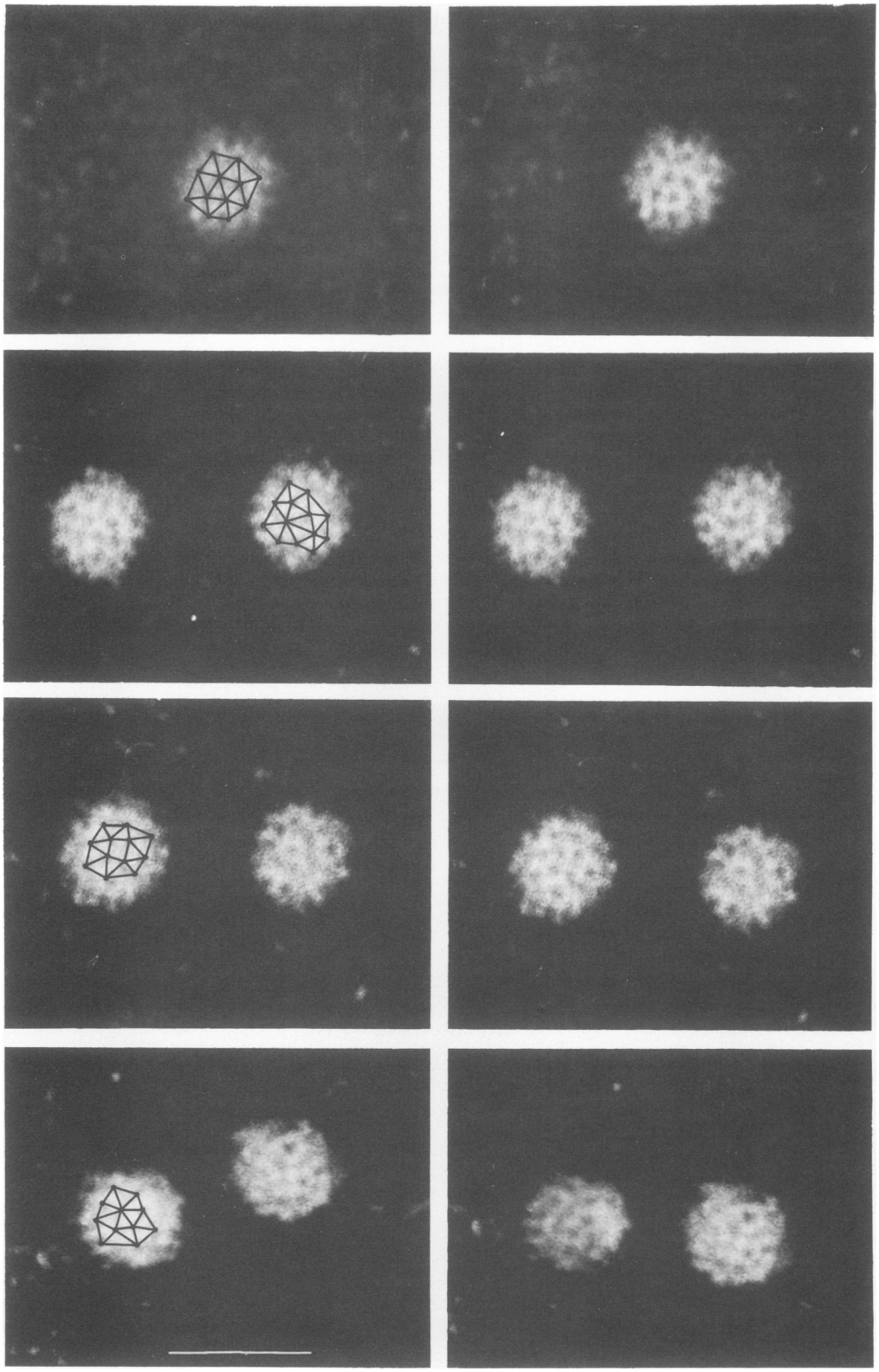

FIG. 3. *Pairs of electron micrographs of IBD virus particles are shown where part of the icosahedral surface lattice T=3 has been drawn onto some of the particles (×273,000). The bar indicates 100 nm.*

cles lacked the readily discernible inner-layer structure characteristically present in all reoviruses (15, 16, 19, 28). The mean diameter of 100 particles measured was 55 nm.

Arrangement of capsomeres. In all the preparations, we frequently observed a doughnut-shaped structure on the surface of the virions. These structures were observed to have a diameter of 8 to 12 nm, with a hole of 3 to 5 nm in diameter. In a few particles, a capsomere was observed to consist of smaller structural units in a regular pentagonal or hexagonal array (Fig. 3A and 5B). In an attempt to fulfull the criteria established by Casper and Klug (7) and Hosaka (12) for icosahedral symmetry, the surface lattice has been drawn onto a few particles to indicate the possible position of the coordinate points (Fig. 3). Capsomere arrangement displayed hexamer-pentamer models having icosahedral symmetry with T=3. Therefore, it may be concluded that the IBD virus particle is an icosahedron with T=3 (32 capsomeres).

Degradation of the virions. When the virus particles were stored at 4 C for 7 days, more than 90% of them had characteristics of intact virions; the remainder were coreless particles of a similar size. After 3 weeks of storage at 4 C, the coreless particles increased in number (Fig. 4), and a portion of the particles had degraded with missing segments (Fig. 5). In these particles, it was also noted that the doughnut-shaped structure corresponded closely in size, as measured in electron micrographs of the intact virions. In a few cases, a hexamer-pentamer array of capsomeres could also be seen. The majority of particles treated with 0.01% sodium dodecyl sulfate for 15 min produced partial or complete disruption. A long string-like structure consisting of the partially degraded capsomeres could be seen (Fig. 6). When the particles were treated with 0.1% sodium dodecyl sulfate, the string-like structure was not observed.

DISCUSSION

The evidence obtained from this study indicates that the structure of IBD virus consisted of a single layer of probably only 32 capsomeres with a hexamer-pentamer structural unit in accordance with icosahedral symmetry (the T=3 icosahedral surface lattice), and that the virion measured about 55 nm in diameter and

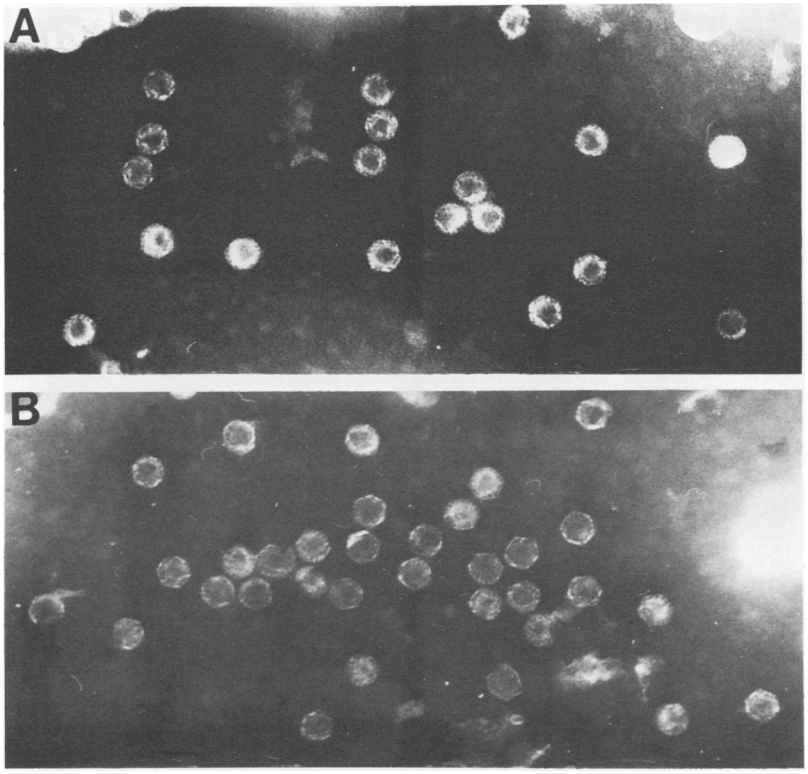

FIG. 4. *Coreless particles observed after 3 weeks of storage at 4 C with similar size and shape of intact virions* (×78,000). *The bar represents 100 nm.*

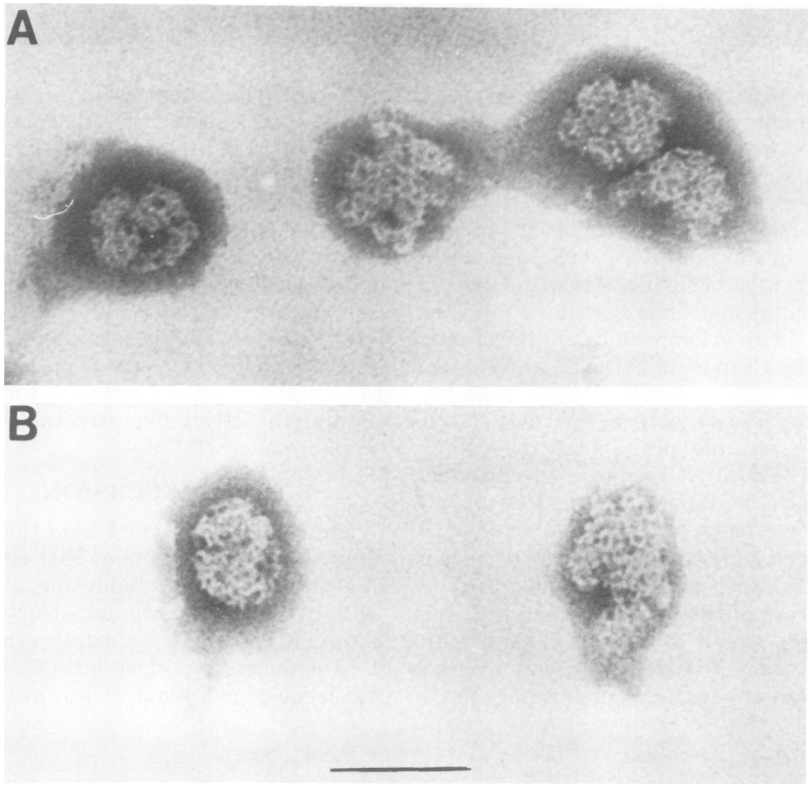

FIG. 5. *The partially disrupted IBD virus particles often found in the preparations after storage at 4 C for 3 weeks (×180,000). The bar represents 100 nm.*

had no envelope.

The size and capsomere arrangement of the IBD virus were indistinguishable from those of bluetongue virus (6, 10, 20, 25). Els and Verwoerd (10) concluded that the bluetongue virus particle is an icosahedron with T=3 (32 capsomeres).

Morphologically, a distinct division can be made between the IBD virus and the reovirus. Reovirus is 75 nm in diameter and possesses a double-layered capsid. The outer layer was generally thought to consist of 92 capsomeres, but it is likely to have a different type of capsid structure with a trimer or dimer arrangement of structural units (2, 28). IBD virus particles appear to be different from the double capsid type of structure found in reovirus particles.

The replication of the IBD virus was not inhibited by 5-iodo-2'-deoxyuridine (14, 22), and the radioactivity peaks of [^{3}H]uridine-labeled IBD virus corresponded to those of virus infectivity as presented here, indicating that the viral nucleic acid is a RNA virus. Petek et al. (22) observed the brownish-green color in the cytoplasm of cells infected with IBD virus when stained with acridine orange. They suggested that IBD virus may be a double-stranded RNA virus.

There are some notable physicochemical differences between IBD virus and bluetongue virus. IBD virus is pH- and thermostable (3, 11, 22), whereas bluetongue virus is very unstable (13, 21, 26, 27). IBD virus is absolutely resistant to ether and chloroform (5, 9, 11), whereas bluetongue virus is only relatively resistant to both (20, 24, 27, 29).

There are also some physicochemical differences between IBD virus and reovirus. It is known that reovirus produces cytoplasmic inclusion bodies and polycaryocites in infected cells, whereas no such properties have been found in the case of IBD virus (8, 11, 22). IBD virus has been reported to be more resistant than Crawley reovirus to photodynamic inactivation and UV irradiation (22).

Andrewes and Pereira (3) provisionally included IBD virus in the reovirus group, as it resembled reoviruses morphologically in thin

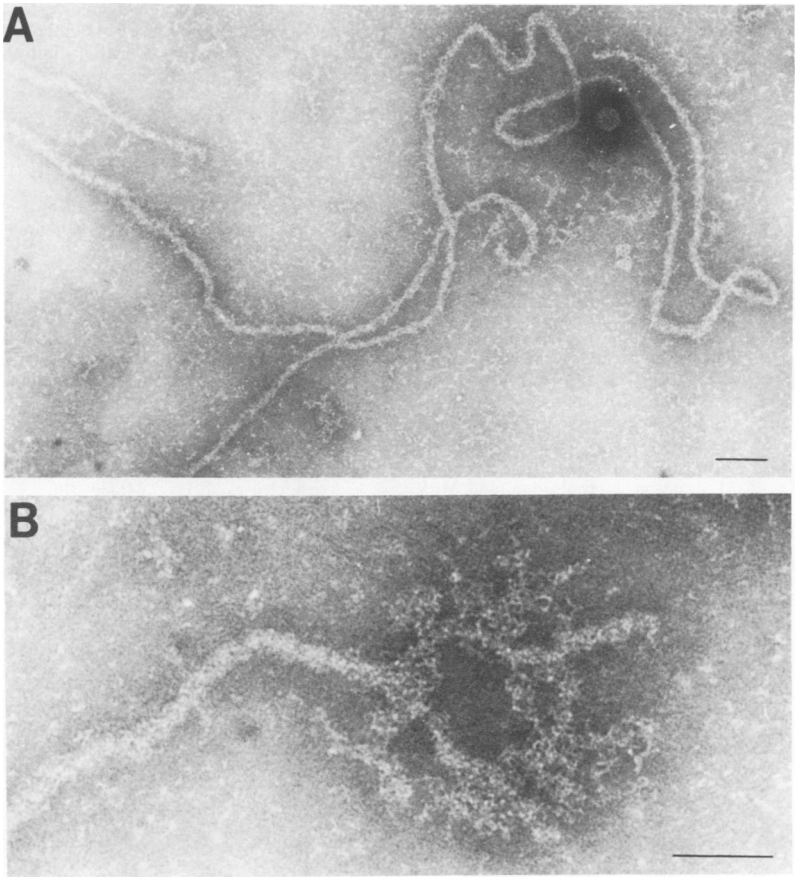

FIG. 6. *IBD virus particles were treated with 0.01% sodium dodecyl sulfate for 15 min prior to staining with phosphotungstic acid. (A) Electron micrograph showing a long string-like structure (×60,000). (B) The string-like structure consisting of the partially degraded capsomeres (×150,000). Each bar represents 100 nm.*

sections and in other basic properties. However, evidence was not then available as to whether IBD virus RNA was double stranded like that of reovirus and bluetongue virus. Thus, further investigation on the classification of IBD virus remains to be done, so as to clarify the double strandedness of the viral RNA, the structural proteins of the virus capsid, and other basic features. Studies are now in progress to elucidate these properties of the IBD virus.

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