PHYSICAL AND MORPHOLOGICAL CHARACTERIZATION OF TRANSMISSIBLE ENTERITIS VIRUS OF TURKEYS

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SUMMARY

The virus of transmissible enteritis of turkeys was shown to be an enveloped pleomorphic particle with an average diameter of 110 nm (range 60–250 nm). The surface bore tubular projections about 12 nm long. The internal component was very fragile, and could not be demonstrated in situ. It appeared as a helix, with a diameter of approximately 7 nm. Hemagglutination could not be demonstrated, but the virus had a strong tendency to adhere to other material during isopycnic centrifugation in sucrose. The complete virus had a density of 1.24 g/cm³.

INTRODUCTION

Transmissible enteritis is an acute enteric disease of considerable economic importance in turkeys. The causal virus is about 100 nm in diameter and is chloroform-labile (6,7). It is restricted primarily to the intestinal tract and is host-specific for the turkey (7). Although the disease has been studied for several years (10), it has not been possible to isolate the virus in cell culture (6,7), and it has been grown in avian embryos only under special circumstances (1).

Thin-section electron microscopy of embryos infected with transmissible enteritis virus revealed virus-like particles which did not appear to fall into any of the major virus groups (4). These particles have been purified, and studied further, to define their position better.

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Fig. 1. Density-gradient centrifugation of transmissible enteritis virus for 18 hours (A), and for 40 hours (B), as described in Materials and Methods. Sedimentation was from right to left. Absorbance at 280 nm, $A_{280}$, and infectivity for pouls (broken line), expressed as weight loss in grams over a period of 3 days postinfection.
MATERIALS AND METHODS

Virus. The Minnesota strain of transmissible enteritis virus (7) was cultivated in the amnionic sac of 24-day-old turkey embryos. Intestines from infected embryos were ground in phosphate-buffered saline and centrifuged, and the supernatant fluid diluted 1:100 before inoculation into other embryos. This material had a titer of $10^6$ turkey-infective-doses/ml. The embryonating eggs were obtained from a flock free of known eggborne pathogens. The virus was assayed by measurement of weight loss over a period of 3 days in turkey poults which had been inoculated per os with the material to be tested, as described previously (1).

Virus purification. The intestines were removed from infected embryos just prior to hatching. Intestines were opened, and each was washed in 1 ml of buffer containing 0.1M NaCl, 0.01M cysteine, 0.005M ethylenediaminetetraacetate (EDTA), and 0.0075M Tris (hydroxymethyl)-aminomethane (Tris) (pH 6.8). The washings were centrifuged at 8,700 × g for 10 minutes, and the supernatant fluid was then subjected to rate-zonal sedimentation through a linear 20–60% sucrose gradient in buffer at 4 °C, in an SW-27 rotor at 80,000 × g for 2 hours. The virus band was collected with a hypodermic syringe, diluted in buffer, and pelleted at 50,000 × g for 60 minutes. The pellet was resuspended in 0.5 ml of buffer.

Density-gradient centrifugation. Virus thus purified was layered in 0.2-ml amounts onto preformed linear gradients of 20–60% sucrose in buffer, and centrifuged at 65,000 × g in SW-65 Ti rotor at 4 °C for 18 or 40 hours. After centrifugation, three-drop fractions were collected by punching the bottom of the tube, and absorbance was determined at 280 nm. Six 0.9-ml fractions were collected from one tube for infectivity trials in poults. Densities were calculated from refractive indices of the sucrose of each fraction.

Electron microscopy. Pellets of purified virus were resuspended in distilled water, placed on grids, coated with parlodion and carbon, and negatively stained with potassium phosphotungstate at pH 6.7. Some preparations were shaken with 5% chloroform before pelleting. Grids were examined at × 30,000–40,000 with a Hitachi HS-8 electron microscope.

Hemagglutination. Erythrocytes from various species were collected in Alsever’s solution, washed three times, and suspended to a 5% concentration in phosphate-buffered saline, pH 7.2. Unpurified infective intestinal washings, with a titer of $10^5$ embryo-infective-doses/ml, were used undiluted and at 2-fold dilutions in saline through 1:160. Tests with human and chicken erythrocytes were
also carried out with purified virus. Tests were incubated at both 25 and 4°C and examined at 30-minute intervals for 2 hours and at 18 hours.

RESULTS

A titer of $10^5$ turkey-infective-doses/ml of transmissible enteritis virus was maintained for 15 passages in embryos.

**Centrifugation.** After rate-zonal centrifugation of intestinal washing in a sucrose gradient for 2 hours, a band was observed one third of the way down the tube. Transmissible-enteritis infectivity for poults was associated primarily with this band. Electron-microscope observation of this fraction with negative staining revealed typical virus particles. After 18 hours of centrifugation in a sucrose gradient, three peaks of ultraviolet absorbance were observed (Fig. 1A). The upper peak, with a density of 1.12 g/cm³, was seen by electron microscopy to consist primarily of cellular debris. A central peak, density 1.18 g/cm³, contained predominantly empty virus envelopes, and a lower peak, density of 1.23 g/cm³, was seen to con-

![Graph showing density and absorbance](image-url)

Fig. 2. Density-gradient recentrifugation of lower peak from Fig. 1, and expressed as in Fig. 1.
tain whole virus. Infectivity for poults was associated with all three peaks (Fig. 1A).

After 40 hours of centrifugation in a sucrose gradient, three peaks were observed, as before, but infectivity for poults was associated primarily with the lowest peak (Fig. 1B). Small amounts of infectious virus were present in all the fractions. When the lowest peak was recentrifuged in a sucrose gradient for 18 hours, peaks equivalent to the middle (1.18 g/cm$^3$) and lowest (1.24 g/cm$^3$) peaks were again formed (Fig. 2). This material had lost its infectivity for poults.

**Electron microscopy.** Negative staining revealed pleomorphic particles varied in size between 60 and 250 nm (average diameter 110 nm). Observed occasionally were very large particles up to 450 nm in diameter. These seemed to contain other virus particles. Also observed were some small particles, complete with projections (Fig. 3).

Fig. 3. Negatively stained preparation of transmissible enteritis virus particles. Note the wide range of sizes. $\times89,700$. 
Fig. 4. Filamentous form of transmissible enteritis virus with dilations. Surface projections may be observed. ×109,600.

Fig. 5. Branching filamentous form of transmissible enteritis viruses, from a low egg passage. ×66,300.
For the first few passages in the turkey embryo, there was a proportion of filamentous forms, up to 1.75 μm in length. The filaments were beaded terminally, and occasionally at intervals throughout their length (Fig. 4). A few branched filaments were observed (Fig. 5).

The particles were covered by an envelope which was easily distorted into a variety of shapes during staining (Figs. 6, 7). The envelope bore large tubular projections approximately 13 nm long and 9 nm wide. The projections were not very close together and were regularly spaced, but were not arranged in any pattern (Figs. 6, 7, 8). The envelope could be disrupted by chloroform and, in some particles, by freezing. The envelope was not permeable to the stain, so it was not possible to observe any internal component in situ.

In preparations of ruptured virus, an internal component was observed. It was very fragile and generally disintegrated into very short lengths as it was being released (Fig. 9). Pieces which remained intact tended to aggregate side by side, as short rigid rods. They had a diameter of approximately 7 nm, and a helical substructure could be observed (Fig. 10).
Hemagglutination. No hemagglutination was observed with erythrocytes of the following species: goose, duck, turkey, chicken, mouse, guinea pig, sheep, ox, and human type O.

DISCUSSION

Although the virus could not be demonstrated to hemagglutinate, it had a strong tendency to adhere to other cellular components. After 20 hours of centrifugation, infectious virus was found in all peaks. This behavior could not be modified by altering the buffer. After 40 hours of centrifugation, infectivity had concentrated in the band which, on electron microscopy, contained whole virus. All fractions, however, contained small amounts of infectious virus. Study of this aspect was hampered by the cumbersome infectivity assay system.

Results obtained show that the virus has some morphological similarities to influenza virus, including overall size, narrow diameter of internal component, and the formation of virus filaments in the early passages. However, transmissible enteritis virus has a very wide range of size, the surface projections were more widely spaced, and it did not hemagglutinate. Furthermore, transmissible enteritis virus buds from the Golgi apparatus, while most myxoviruses form by budding from the cell surface. In these respects the virus resembles some of the paramyxovirus group, such as rinder-
Characterization of transmissible enteritis virus

Fig. 8. Transmissible enteritis virion which has lost some surface projections. No internal structure is apparent. ×121,000.

Fig. 9. Transmissible enteritis virions ruptured by freeze-thaw. Pieces of internal component may be observed at the point of rupture (arrow). ×120,750.
pest virus. Transmissible enteritis virus has a greater buoyant density than most myxoviruses. It must be concluded that transmissible enteritis virus does not fall into any virus group described so far.

While some of the objects pictured could be interpreted as a virus or as a mycoplasma, it is believed that the agent of transmissible enteritis is not a mycoplasma because it cannot be visualized by the light microscope (2), it is not susceptible to tylosin and broad-spectrum antibiotics (2), it passes a 0.22-μm filter (6), it has a density of 1.24 g/cm³ compared with about 1.69 for several mycoplasma tested (9), and cannot be propagated in media which support mycoplasmas. Observations by thin-section electron microscopy revealed small enveloped virus-like particles budding into membrane-lined sernae in the cytoplasm of epithelial cells from both infected pouls and embryos (4). Furthermore, seen in the negatively stained preparations were objects resembling the internal component of myxoviruses. These characteristics are unlike those of mycoplasmas.

It has been shown that if undiluted infective transmissible enteritis material is passaged in avian embryos, the titer diminishes and disappears after a few passages (1). Dilution of the inoculum
in these experiments allowed a high infective titer to be maintained for 15 passages. This suggests an auto-interference phenomenon. A very wide range of size of virus particles was noted by electron microscopy. It is possible that the different-sized particles have different amounts of genetic material, and that the smaller particles observed are incomplete. These could interfere with multiplication of the normal particles. Such a phenomenon may account for the difficulty that has been experienced in isolating this virus in cell culture.

Transmissible enteritis virus is only one of a number of enteric viruses which have proven difficult to isolate and classify. Evidence suggests that other pathogenic intestinal viruses remain to be isolated, in species including man (5,8). It is hoped that knowledge gained in studies on viruses such as this may assist investigations in other areas. The recent isolation of transmissible enteritis virus in intestinal organ cultures (3) may help in this regard.

REFERENCES


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