

## Ultrastructural Changes in the Small Intestinal Epithelium of Suckling Pigs Affected with a Transmissible Gastroenteritis (TGE)-Like Disease

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With 9 Figures

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### Summary

The small intestine of piglets collected during a sudden outbreak of diarrhoeal disease resembling transmissible gastroenteritis (TGE) was examined by light and electron microscopy. The principal histopathological changes were moderate infiltration by mononuclear cells in the lamina propria of the villi and cytoplasmic vacuolation. These were most pronounced in the epithelial cells covering the villous tips. By scanning electron microscopy, the intestinal villi were swollen and the transverse furrows disappeared. Microvilli were reduced in number leaving denuded areas on the brush border of the villous epithelial cells.

The ultrastructural changes were restricted to the cytoplasm of affected villous epithelial cells. The cell organelles were missing in rounded areas leaving cleared areas in the cytoplasm. Parallel fascicles and bundles were seen in these areas. Viral particles with an average diameter of 70 nm were found within the dilated apical tubulo-vesicular system, free in the cytoplasm, among the microvilli or lying free in the intestinal lumen. Viral particles surrounded a non-membrane bound viroplasm in some cases. The negatively stained particles showed a typical coronavirus morphology. These particles were found to be distinct from the known coronaviruses of swine, TGE virus and hemagglutinating encephalomyelitis virus by immune electron microscopy.

### Introduction

Transmissible gastroenteritis (TGE) is a highly contagious enteric disease of swine of all ages causing important economic losses, especially in dense swine populations. The disease was first described by DOYLE and HUTCHINGS (9) in the United States, in 1946. In Hungary, SZENT-IVÁNYI *et al.* (20) reported the occurrence of TGE in 1964. Following the elaboration of methods for the routine

laboratory diagnosis of the disease (7), CSONTOS and BENYEDA (6) turned their attention in 1970 to a new TGE-like epizootic diarrhoea of swine in which the causative role of TGE virus was excluded on the basis of virological examinations. Clinical observations and the experimental reproduction of diarrhoea in colostrum-deprived pigs with bacteria-free intestinal material have also supported the viral etiology of this diarrhoeal disease (1, 11).

In the past few years, an increased frequency of newborn pig diarrhoea caused by rotavirus was reported (3, 8, 12, 13, 16, 22, 24). However, in a number of instances, the significance of rotavirus could not be established (23). Finally, in Belgium PENSART and DE BOUCK (15) and in England CHASEY and CARTWRIGHT (4) detected coronavirus-like particles in the feces and intestinal epithelium of pigs affected with a TGE-like epizootic diarrhoea.

The present report describes the ultrastructural changes in the small intestinal epithelium and the detection of a virus morphologically similar to the coronaviruses in the intestinal content and epithelium of pigs affected with a TGE-like disease, which has occurred periodically in Hungary since 1970.

## Materials and Methods

### *Animals*

Three to seven days old piglets were collected from 3 large Hungarian swine-breeding farms during sudden outbreaks of diarrhoeal disease affecting animals of all ages. All the pigs showed a watery diarrhoea. The etiological role of TGE coronavirus was excluded by the negative result of direct immunofluorescence tests carried out on scrapings of the small intestinal mucosa, by unsuccessful virus isolation attempts on secondary swine thyroid cell cultures and by the absence of TGE virus-neutralizing antibodies in the acute- and convalescent-phase sera (14). Direct immunofluorescence (21), counter-immunoelectrophoresis (19), electron microscopic (18) and immune electron microscopic examinations (17) for rotavirus were also negative and no rotavirus antibodies were found by counter-immunoelectrophoresis (5) in acute- and convalescent-phase sera from the recovered animals.

### *Histopathology*

Intestinal segments were removed from six different regions of the small intestine of sacrificed piglets and fixed in buffered formalin. The sections were stained with hematoxylin-eosin (HE).

### *Scanning Electron Microscopy*

Disc-shaped portions of intestinal wall, approximately 1.5 cm in diameter were stretched on a dental wax sheet. They were fixed in 2.5 per cent glutaraldehyde at pH 7.3 and postfixed in 1 per cent phosphate-buffered osmium tetroxide, pH 7.3. They were dehydrated in graded acetone and vacuum-dried. Finally, tissue blocks were vapor coated with gold in an Edwards' vacuum-evaporator. The preparations were examined under a JEOL SM 15 scanning electron microscope using an acceleration potential of 21 kV.

### *Electron Microscopic Methods*

Small segments were removed from six sites of the small intestine, adjacent to those taken for histopathology. The segments were fixed in Karnovsky's paraformaldehyde or in 2.5 per cent glutaraldehyde at pH 7.3 and post-fixed in 1 per cent phosphate-buffered osmium tetroxide at pH 7.3. Tissue blocks were dehydrated in graded ethanol and embedded in Durcupan ACM. The sections were cut with a Reichert Om U3 ultramicrotome. Semi-thin sections were stained with toluidine blue and thin sections with uranyl acetate and lead citrate.

Intestinal contents were diluted 1 to 5 (v/v) in phosphate-buffered saline (PBS), pH 7.2 and clarified by centrifugation at  $3000 \times g$  for 30 minutes at  $4^\circ \text{C}$ . The supernatant was filtered through a 450 nm Millipore filter and centrifuged at  $100,000 \times g$  for 90 minutes at  $4^\circ \text{C}$  in an MSE SS 50 ultracentrifuge. The resulting pellet was resuspended in a few drops of distilled water, placed on 100 mesh, formvar-coated grids and stained with 2 per cent phosphotungstic acid.

For direct immune electron microscopy (IEM), 2 ml of clarified and filtered intestinal content was mixed with 2 ml of properly diluted convalescent serum or specific antiserum. The mixture was incubated at  $37^\circ \text{C}$  for 30 minutes, then kept at  $4^\circ \text{C}$  for 18 hours. The sample was centrifuged at  $31,000 \times g$  for 60 minutes at  $4^\circ \text{C}$ . The pellet was resuspended in one drop of distilled water, placed on 100 mesh formvar-coated grids and stained with 2 per cent phosphotungstic acid.

Grids were examined under a Philips 201 CS electron microscope operating at an acceleration potential of 60 kV.

#### *Antisera*

Porcine anti-TGE serum was prepared in an SPF piglet orally exposed to the virulent Miller-3 strain of TGE virus (2). Porcine anti-hemagglutinating encephalomyelitis virus (HEV) serum was obtained from Dr. M. B. PENSART, University of Ghent, Belgium. The sera were heat-inactivated and stored frozen until used. The neutralizing antibody titre of TGE serum was 1:2560. Its optimal dilution for IEM was 1:50. The neutralizing antibody titre of HEV antiserum was 1:12,288 and its optimal dilution for IEM was 1:400. The optimal antibody dilution for IEM was considered to be that which produced the largest virus-antibody aggregates.

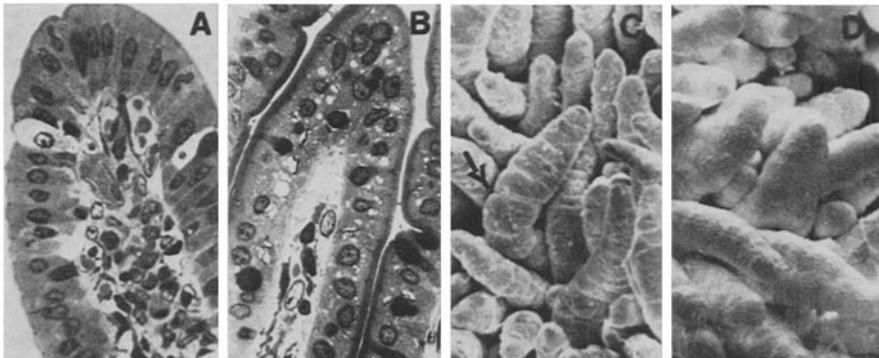


Fig. 1. Histopathology and scanning electron micrographs (SEM) of villi from the distal part of the jejunum. *A* Semithin section of a jejunal villus of a non-exposed pig. Toluidine blue stain.  $\times 370$ . *B* Jejunal villus from a pig affected with TGE-like disease. Vesicles and vacuoles are in the cytoplasm of the columnar epithelial cells. Toluidine blue stain.  $\times 370$ . *C* SEM of villi from a non-exposed pig. These are long and slender with prominent transverse furrows (arrow).  $\times 115$ . *D* SEM of the jejunum from a pig affected with TGE-like disease. The villi are swollen and the transverse furrows are missing.  $\times 115$

## Results

### *Histopathology of Intestine*

In HE stained sections, moderate infiltration by mononuclear cells and dilated capillaries were found in the lamina propria of the villus. In semi-thin sections stained with toluidine blue, vesicles and vacuoles were seen in the cytoplasm of columnar epithelial cells. Bundles (fascicles) showing a deep blue col-

oration were present in some of them (Fig. 1B). These alterations were most pronounced in the epithelial cells covering the tips of the villi but they were missing in those lining the crypts of Lieberkühn.

#### *Scanning Electron Microscopy*

Examination of small intestines of normal pigs revealed long, finger-like villi of various lengths and configurations. The surfaces of most villi were interrupted by transverse furrows of various depths (Fig. 1C). In the small intestines of the affected pigs, villi were swollen and the transverse furrows disappeared. Microvilli were reduced in number leaving denuded areas on the brush border of the epithelial cells (Fig. 1D).

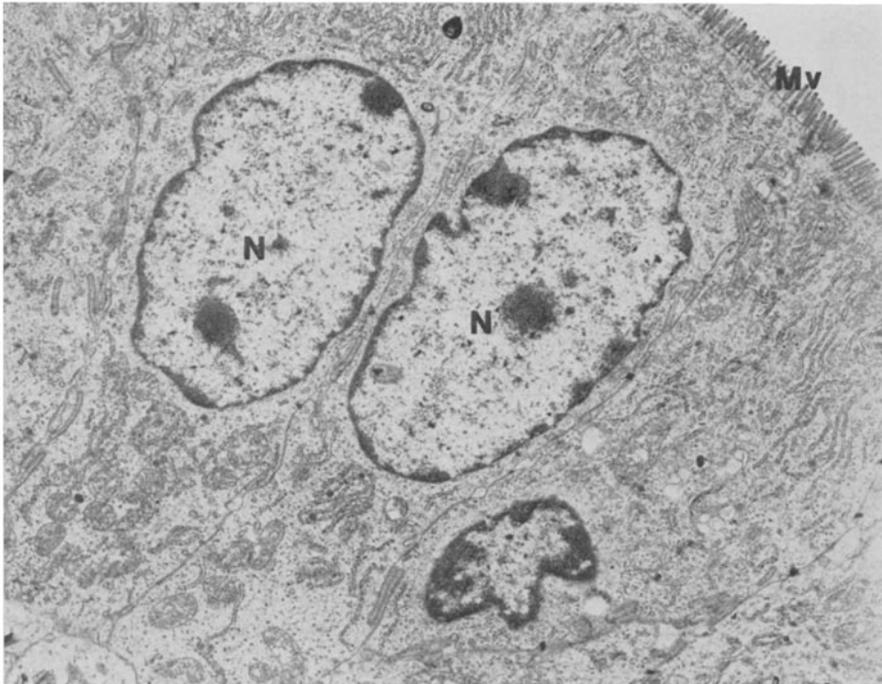


Fig. 2. Columnar villous epithelial cells of the jejunum from a non-exposed pig. *Mv* Microvilli. *N* Nucleus.  $\times 9200$

#### *Ultrastructural Changes*

Principal ultrastructural changes were found in the cytoplasm of the villous epithelial cells of the small intestine. The cell surface and the nucleus were generally intact (Fig. 3). In some of the affected cells, the cell organelles were missing in rounded areas leaving cleared areas in the cytoplasm (Fig. 4). Viral particles were present along the border adjacent to the intact areas of cytoplasm (Fig. 4). Sometimes, viral particles surrounded an electron-dense, coarsely granulated viral precursor material or viroplasm (Fig. 4). The virions consisted of a central electron-opaque core surrounded by an intermediary electron translucent zone and

by an electron-opaque outer coat (Fig. 5). Their average diameter was 70 nm. Viral particles were also present in the dilated apical tubulo-vesicular system, free in the cytoplasm (Fig. 6), among the microvilli (Fig. 5), or lying free within the dilated cisternae of rough endoplasmic reticulum (Fig. 7).

Most viral particles were seen in the epithelial cells covering the tips of villi in the jejunum. Their number decreased gradually approaching the inlets of the crypts of Lieberkühn and no viral particles were found in the epithelial cells lining the crypts. Likewise, viral particles were not found within the nucleus. Sporadically, parallel bundles and fascicles were seen in the cleared areas of cytoplasm (Figs. 3 and 4). Disruption of microvilli of the brush border and the separation of infected epithelial cells were also demonstrated occasionally.

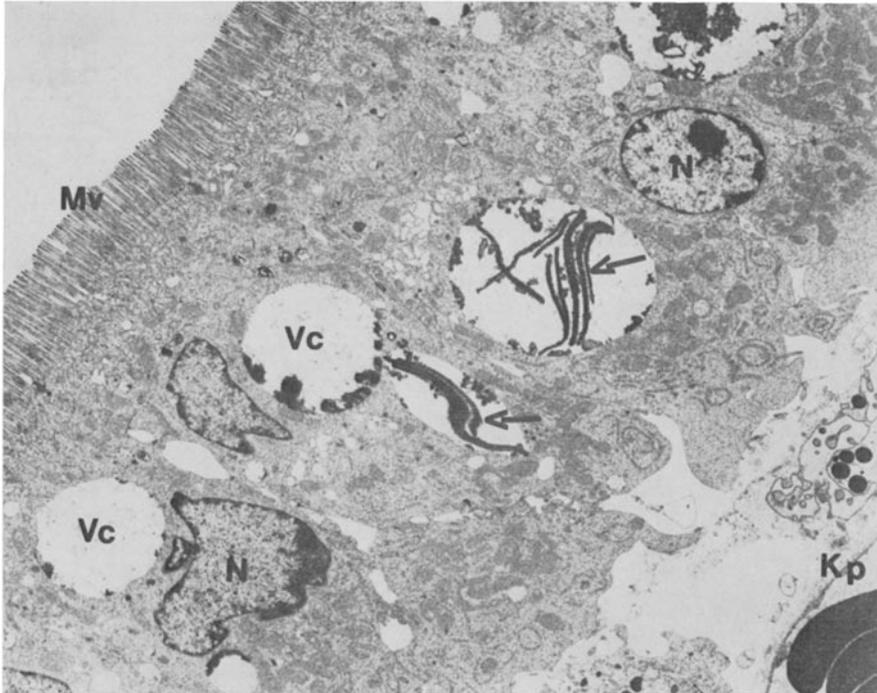


Fig. 3. Differentiated villous epithelial cells of the jejunum from an affected pig. Microvilli (*Mv*) are intact. The cytoplasm contains numerous vacuoles (*Vc*) with characteristic fascicles (arrow). The nucleus (*N*) is intact. The nuclear membrane is irregular *Kp* Capillary vessel.  $\times 3900$

#### *Electron Microscopy and Immune Electron Microscopy of Intestinal Content*

Coronavirus-like particles were found in negatively stained preparations of the intestinal contents of all piglets. The particles were pleomorphic with short surface projections. The projections formed a single fringe radiating from the electron-opaque central core. The diameter of the particles ranged between 100 and 122 nm. The length of the surface projections was approximately 20 nm (Fig. 8).

The direct IEM for TGE virus and HEV was negative.

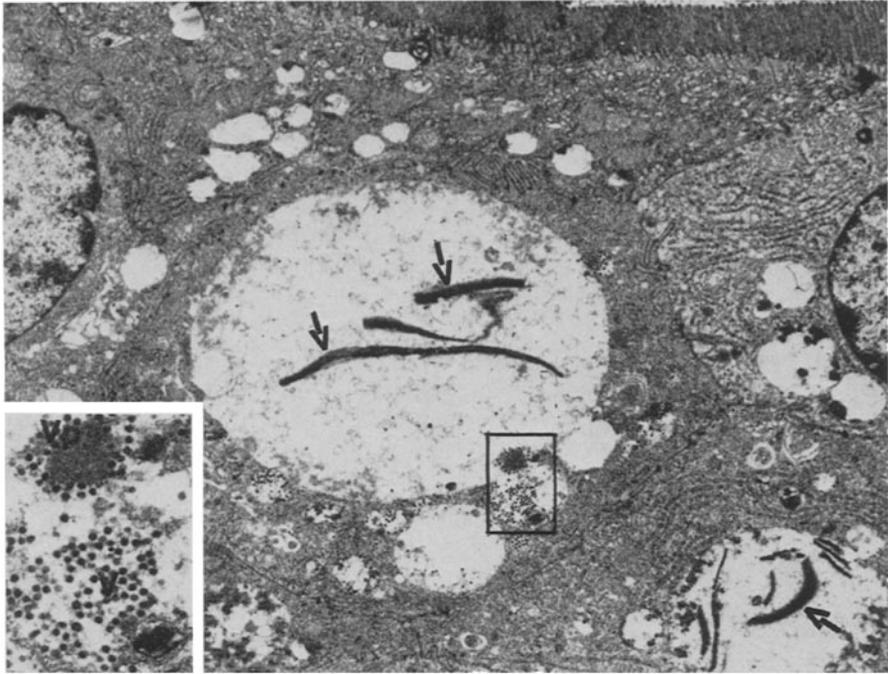


Fig. 4. A section from the duodenum of the same pig. The cell organelles are missing in the cleared areas of cytoplasm. Characteristic bundles and fascicles (arrow) in the cytoplasm.  $\times 6100$ . The outlined area presented with higher magnification. Viral particles (*V*) surround a viral precursor material, the so-called viroplasm (*Vp*) located in the cleared areas in the cytoplasm adjacent to the intact area.  $\times 21,300$

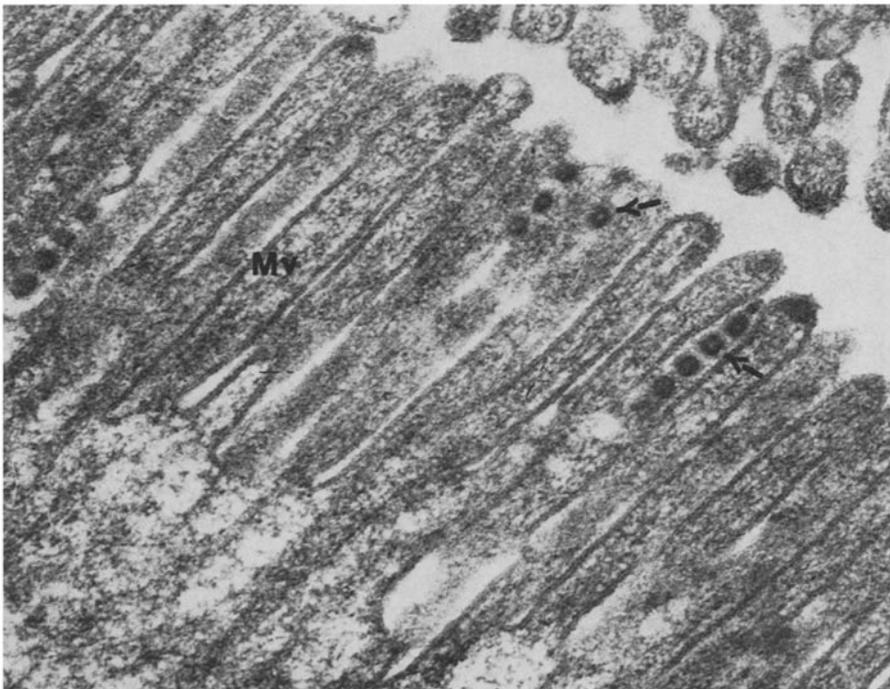


Fig. 5. Viral particles (arrow) between the microvilli (*Mv*) of a columnar epithelial cell from the ileum showing a regular arrangement.  $\times 65,700$

### Discussion

Previous observations and experimental results have assumed the viral etiology of this TGE-like disease. Experimental reproduction of diarrhoea in colostrum-deprived pigs with bacteria-free fecal samples of sick pigs (1, 11) and the explosive spread of diarrhoea within all age groups have equally led to this assumption.

The results of the present study proved the viral etiology of this TGE-like disease. Virions with an average diameter of 70 nm were found in the intestinal

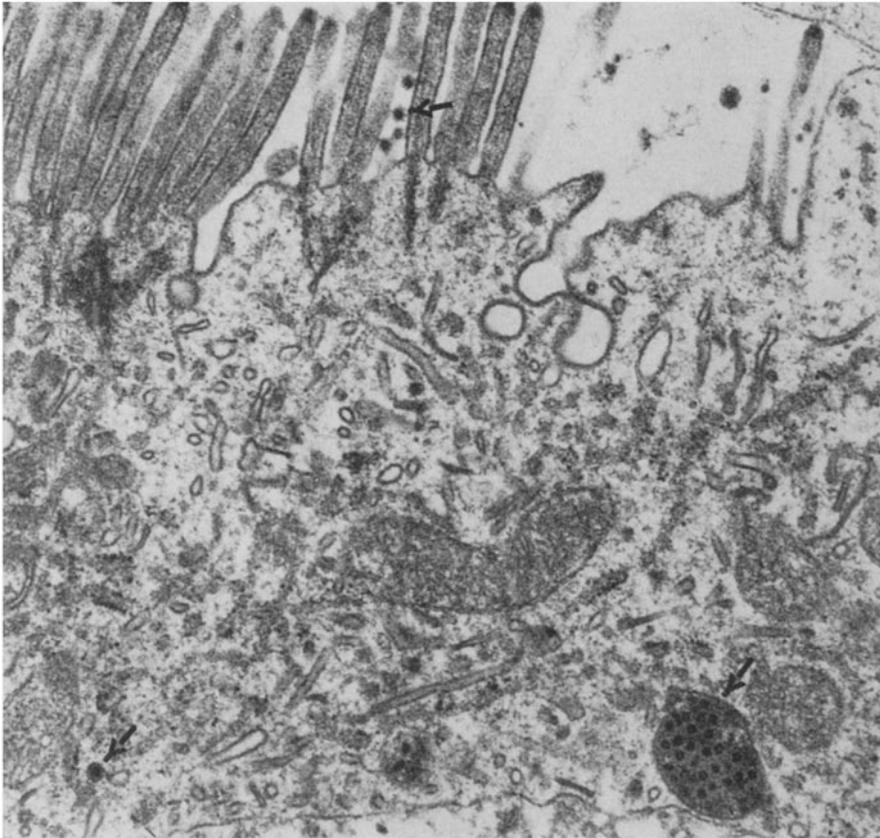


Fig. 6. Dilated tubulo-vesicular system characteristic for suckling pigs that—besides macromolecules—can also promote the intake of infectious agents. Viral particles (arrow) locating intra- and extracellular. Microvilli of the brush border are disrupted.  $\times 27,900$

villous epithelial cells of naturally infected piglets or free in the intestinal lumen. In the infected epithelial cells, viral particles were located mainly within the dilated cisternae of the rough endoplasmic reticulum. In addition, viral particles were found free in the cytoplasm or surrounded by a unit membrane. The location of viral particles closely parallels that of TGE virus.

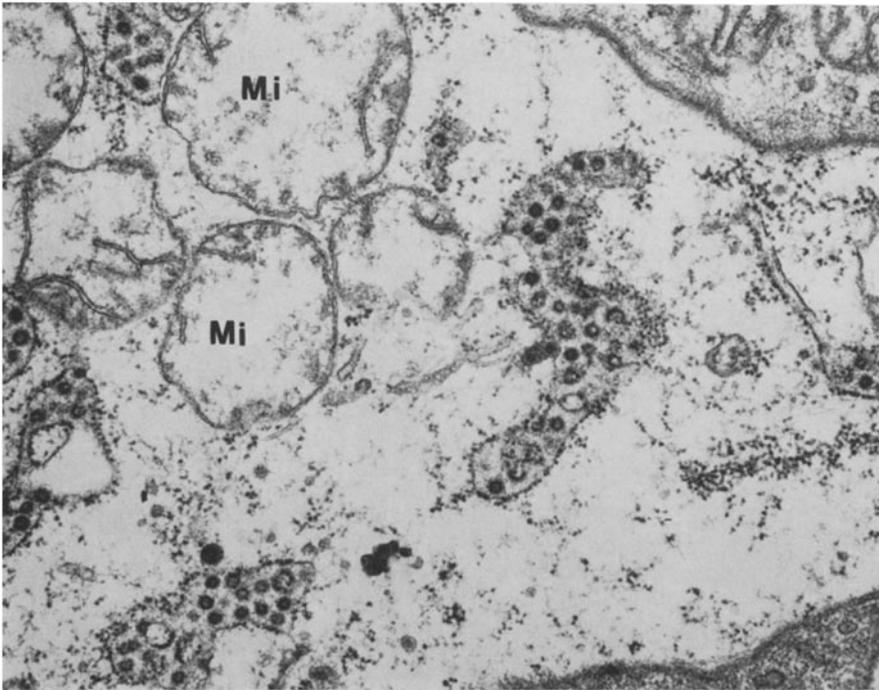


Fig. 7. The cytoplasm of an infected villous epithelial cell of the jejunum. Virus particles in the dilated lumen of the rough endoplasmic reticulum. Some of the mitochondria (*Mi*) are swollen.  $\times 42,600$

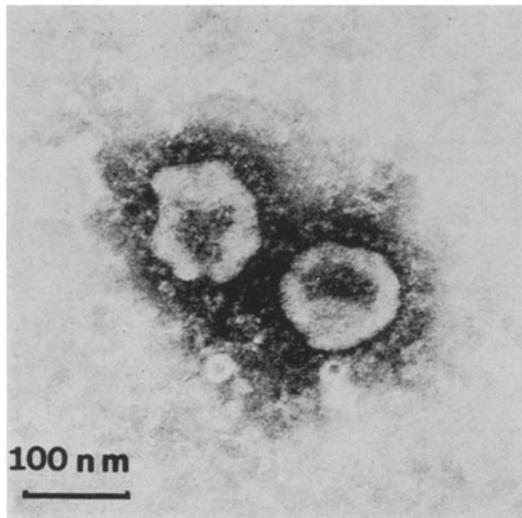


Fig. 8. Negatively stained pleomorphic coronavirus-like particles with short surface projections from the intestinal content of a pig affected with TGE-like disease. Bar represents 100 nm

The negatively stained particles showed a typical coronavirus morphology. These coronavirus-like particles were morphologically indistinguishable from TGE virus and HEV particles in similar preparations. However, by direct IEM, they were distinct from these two coronaviruses of swine. The size and morphology of the particles were similar to those of the recently recognized coronaviruses reported by PENSABERT and DE BOUCK (15) and by CHASEY and CARTWRIGHT (4) in epidemic diarrhoea of pigs.

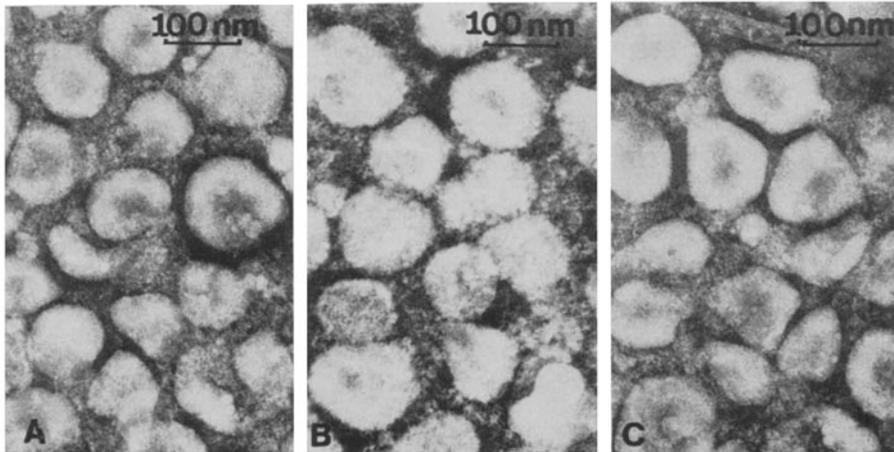


Fig. 9. Immune electron micrographs. *A* Hemagglutinating encephalomyelitis virus (HEV) treated with antiserum to HEV. *B* Intestinal content of a pig affected with TGE-like disease treated with the convalescent serum from a recovered pig. *C* Transmissible gastroenteritis (TGE) virus treated with antiserum to TGE virus. Bars represent 100 nm

As mentioned above, TGE virus was excluded as the etiological cause of diarrhoea on the original farms. The histopathology of the intestine and the ultrastructural changes in villous epithelial cells also differed from those caused by TGE virus. In the case of TGE, the marked shortening of villi of the small intestines that HOOPER and HAELTERMAN (10) referred to as villous atrophy proved to be a highly significant lesion. These morphological changes affect the entirety of the villi; they are not restricted to the absorptive cells. Furthermore, simultaneously with the regressive alterations, a marked cell proliferation can be demonstrated in the crypts of Lieberkühn presumably replacing the destroyed and sloughed epithelial cells.

In the case of TGE-like diarrhoea, the morphological changes are restricted to the cytoplasm of the differentiated villous epithelial cells. In most cases, the cell surface was intact, even if the cytoplasm showed marked pathological changes. In addition, parallel bundles and fascicles were seen in vacuoles in the cytoplasm that were observed neither in normal (Figs. 1 *A* and 2) nor in TGE virus-infected pigs.

All attempts to isolate and propagate the virus in cell cultures have so far been unsuccessful.

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