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## **Three-dimensional Sequential Study of the Intestinal Surface in Experimental Porcine CV 777 Coronavirus Enteritis**

By

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*With 9 figures*

*(Received for publication April 13, 1980)*

### **Introduction**

Viral enteritis in piglets is usually associated with Transmissible Gastro-enteritis Virus or with Porcine Rotavirus. In 1978, a new coronavirus-like agent was detected in the intestinal contents of piglets with diarrhoea (11). The agent was shown to differ antigenically from the porcine coronaviruses Transmissible Gastro-enteritis Virus and Haemagglutinating Encephalomyelitis Virus. The clinical aspects of the disease and the virological aspects of the causative agent have been described (2). The morphogenesis of the virus is also characteristic for a member of the coronaviridae family (15). Histopathological, histochemical (COUSSEMENT et al., to be published), ultrastructural (DUCATELLE et al., to be published) and immunofluorescence studies (DEBOUCK et al., to be published) of the intestine in caesarean-derived, colostrum-deprived piglets have been done. These studies have shown that the lesions and the distribution of the viral antigen are similar to Transmissible Gastro-enteritis Viral infection, although with some restrictions: the lesions are slightly less severe, and the virus also replicates in the colonic epithelium but without causing any histological lesions.

The purpose of the present study is to describe the alterations of the intestinal surface and to relate them to possible mechanisms of diarrhoea, by comparing with known enteritic conditions in piglets.

## Material and Methods

A three-dimensional study was done by stereo-microscopy, histology of longitudinal and transverse sections, and scanning electron microscopy (SEM). The middle of the jejunum was used as a reference point because it has proven to have the most striking lesions.

*Animals:* Eighteen caesarean-derived and colostrum-deprived piglets were used in this study. The animals were kept individually in Horsfall type units and fed sterile cow's milk.

*Virus:* The corona virus isolate CV 777 (11) was used for experimental infection. The animals were inoculated oro-nasally with  $10^4$  to  $10^6$  pig infective doses of a virus stock that was made as described (2).

*Experimental design:* 16 animals were inoculated oro-nasally at two or three days. Two animals were kept as controls. The animals were killed sequentially at 12 h, 18 h, 24 h, 30 h, 32 h, 36 h, 38 h, 41 h, 48 h, 60 h, 72 h, 96 h, 120 h, 122 h after inoculation.

At autopsy specimens were taken from the duodenum, the middle of the jejunum, the ileum and the colon.

*Processing of the material:* For stereo microscopy the fresh specimens were immersed in aqua destillata.

For histology the specimens were fixed in phosphate buffered formalin and dehydrated and embedded according to routine procedures.  $4\ \mu$  sections were cut from longitudinally and transversely embedded specimens and stained with haematoxylin and eosin.

For SEM the specimens were not washed. They were fixed immediately in glutaraldehyde 2.5%, formaldehyde 2% in 0.1 M cacodylate buffer. The specimens were postfixed in osmium tetroxide, dehydrated through a graded concentration of ethanol and acetone, and dried by the critical-point method. The specimens, coated with gold, were examined in a Philips 501 scanning electron microscope.

## Results

### Controls

In these animals the villi in the middle of the jejunum were long, slender and regular, and moderately densely packed, as observed by stereo-microscopy. With SEM most of the villi were equal in length, with more or less parallel circular transverse grooves (Fig. 1). The villi were mostly round in cross-section. Occasional smaller villi were seen. Mucus clumps were seen at the orifices of goblet cells, scattered on the villous surface. The villous tips often had a somewhat irregular appearance. At higher magnification these irregularities seemed to correspond with individual epithelial cell apices which were elevated, especially in the extrusion zone. Very small nodules with a regular pattern, representing the microvillous tips, could be observed in many areas on the villi. The histology of the epithelial cells is described elsewhere in detail (COUSSEMENT et al., to be published). The morphology of the intestinal surface in the duodenum was similar to that in the jejunum.

In the ileum the villi varied in length. Above Peyer's patches they were very short and blunt, whereas elsewhere they were long and slender.

In the colon the intestinal surface was almost flat. Goblet cells releasing their mucus were scattered throughout the epithelium. The mouths of the crypts were connected by narrow grooves (Fig. 2). The colonic mucosal surface of the controls was thus comparable to published data (3).

### Incubation period

This was the period prior to the first clinical signs (usually vomiting). It varied from 24 to 38h. In this period the intestinal surface still looked normal under the stereomicroscope. Also histologically no alterations were observed.

With SEM the intestinal surface still looked normal in the early incubation period (Fig. 3), but it was somewhat irregular towards the end of the incubation period. A number of villi were shorter and thicker than in the con-

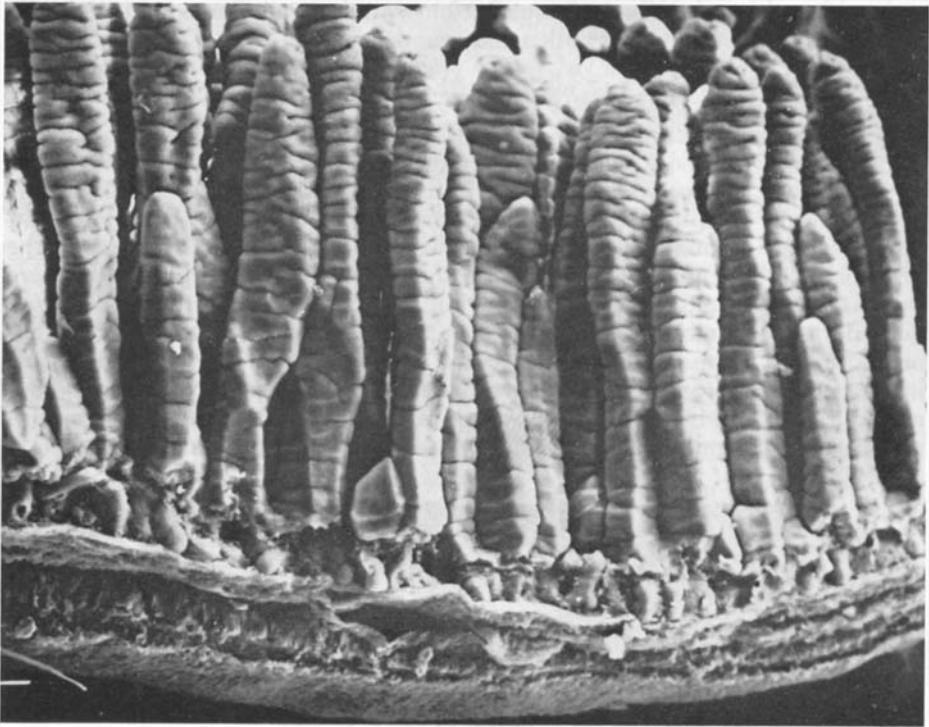


Fig. 1. Middle jejunum of control piglet. The villi are long and slender, with transverse folds (bar = 100  $\mu$ m.)

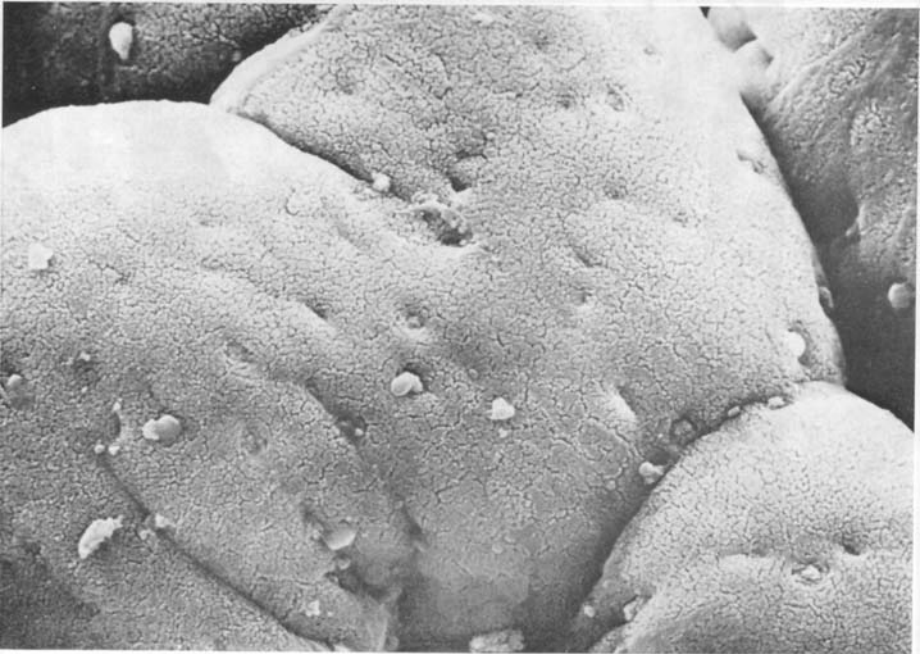


Fig. 2. Colon of control piglet. The crypts are connected by narrow grooves. Note mucus plugs at the mouths of the goblet cells scattered on the epithelial surface (bar = 10  $\mu$ m.)

trols, although no significant desquamation was observed. Some epithelial cells on the tips of the villi were swollen and rounded.

*First 6h after the onset of clinical signs*

At the onset of clinical signs the villi of the middle of the jejunum appeared shortened and more rigid under the dissecting microscope. Severe changes of the jejunal surface were observed histologically. There was an important desquamation of epithelial cells and release of cell debris into the intestinal lumen. Desquamation could also be seen with SEM, leaving small areas on the villi completely denuded (Fig. 4). Large amounts of cell debris and mucus were present between and adhering to the villi. The villi were severely distorted and shortened, almost completely losing their transverse foldings. At higher magnification of the desquamated areas, pores could sometimes be observed in the basal membrane (Fig. 5). In the duodenum, ileum and colon lesions were not seen with SEM at the time of onset of diarrhoea. Also later on the duodenum had no significant lesions, except some villous shortening. In the ileum

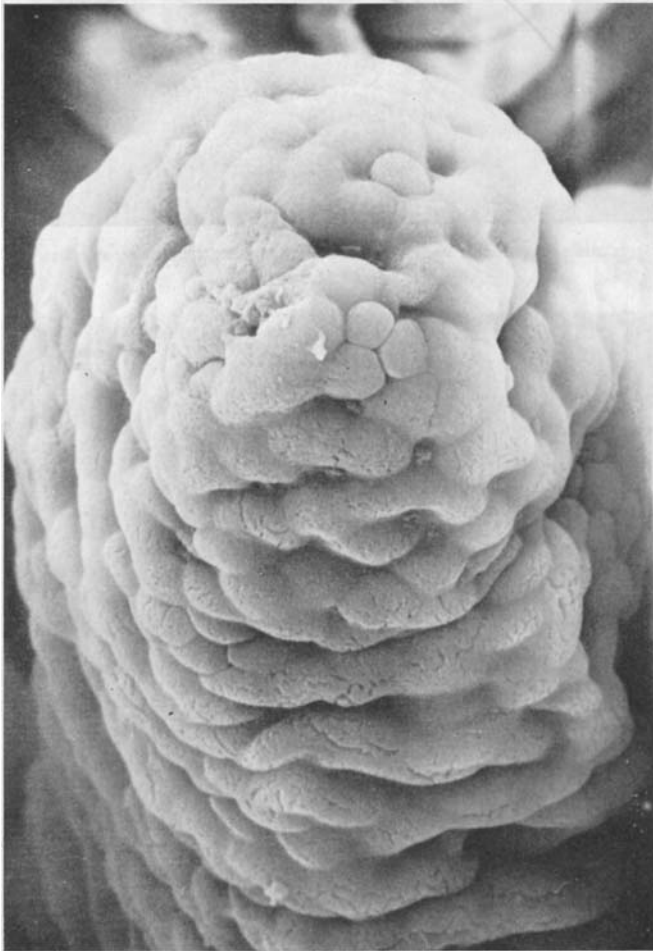


Fig. 3. Ileal villus in the early incubation period with a normal epithelial surface. Some irregularity is seen at the extrusion zone on the tip of the villus (bar = 10  $\mu\text{m}$ .)

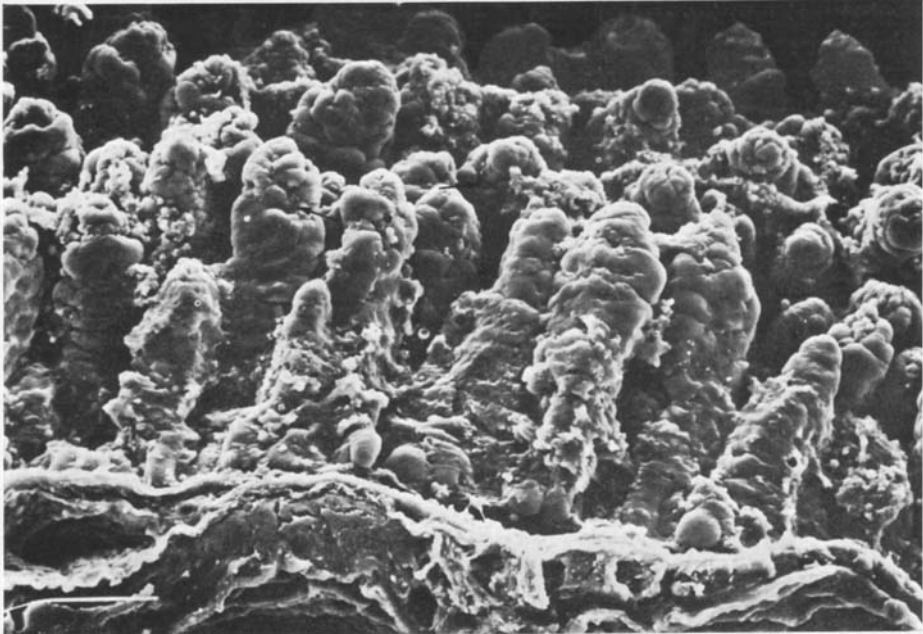


Fig. 4. Middle jejunum at the onset of clinical signs. The villi are short and irregular, and they have lost most of their transverse folds. Debris are present between the villi. Small epithelial defects can be seen on the upper half of the villi (arrows) (bar = 100  $\mu$ m.)



Fig. 5. Epithelial defect on the top of a villus in the middle of the jejunum at the onset of clinical signs. Note pores in the basal membrane (arrows) (bar = 10  $\mu$ m.)

moderate shortening and stunting of villi was seen about 6 h after the onset of clinical signs. Desquamation could sometimes be observed, leaving occasional denuded areas on the villous surface (Fig. 6). The lesions were less and were of variable intensity as compared to the middle of the jejunum. In the colon alterations of the mucosal surface were not observed. Nevertheless at 6h after the onset of clinical signs, cellular debris, occasional erythrocytes, and small pseudomembranes could be seen covering the colonic epithelial surface (Fig. 7).

*From 6h till 90h after the onset of clinical signs*

In this period the jejunal villi were moderately long and irregular. Desquamation was rare. Little cell debris were seen between the villi by SEM. Pores and clefts between the epithelial cells, especially in the upper half of the villi, were seen. Histologically the villous epithelial cells frequently were low cuboidal, with a narrow brush border.

From 24h after the first clinical signs onwards, fusion of villi was observed. At first occasionally only two or three villi were fused, and this fusion was usually not complete, leaving deep infoldings of the fused surface. Later

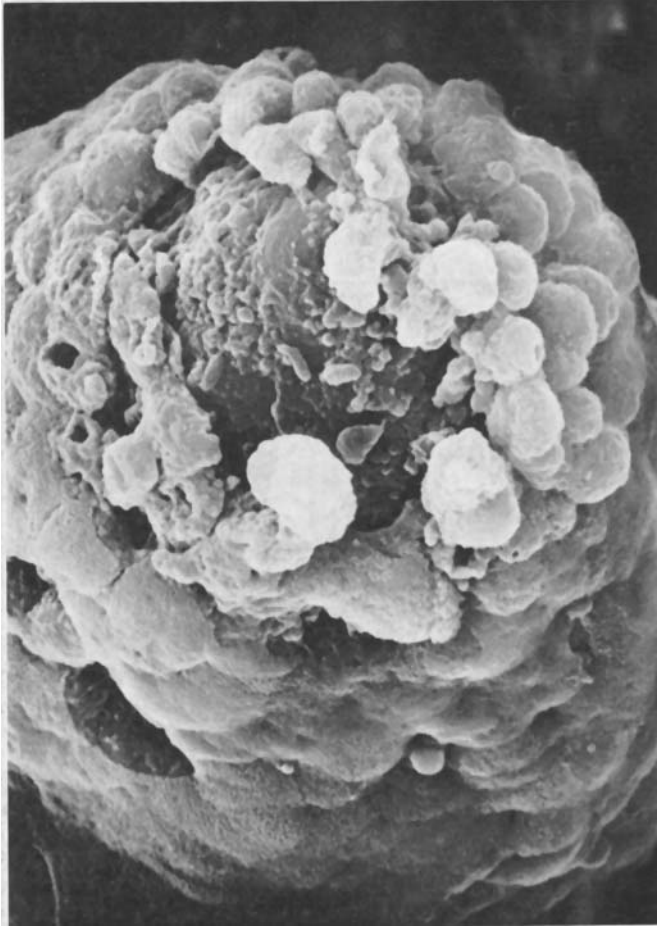


Fig. 6. Ileal villous tip, 6 h after the onset of clinical signs, with an epithelial defect (bar = 10  $\mu$ m.)

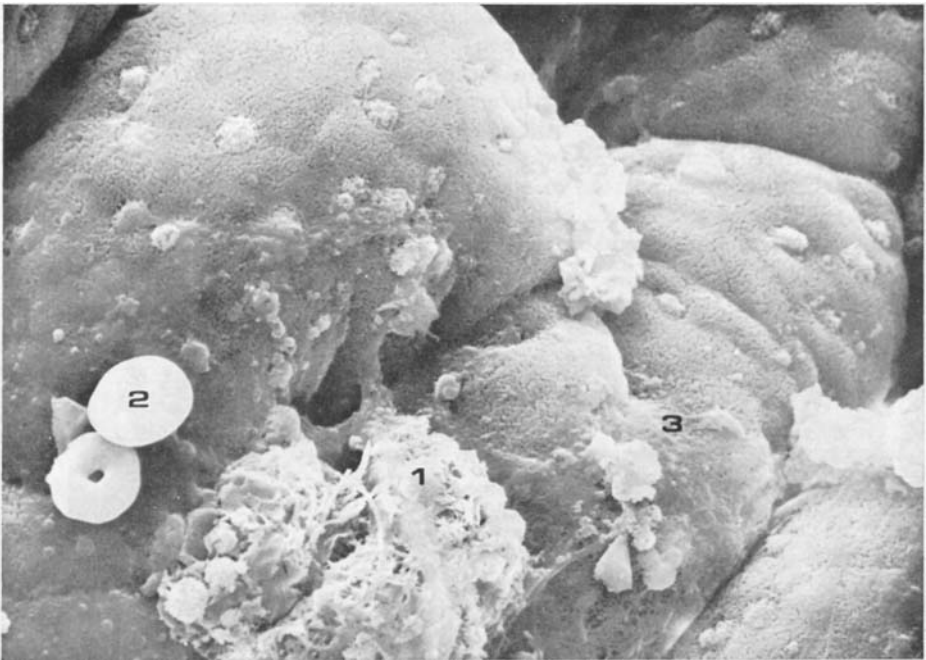


Fig. 7. Colonic mucosal surface, 6 h after the onset of clinical signs. Debris (1), erythrocytes (2) and pseudomembranes (3) lie on the epithelium (bar = 10  $\mu$ m.)

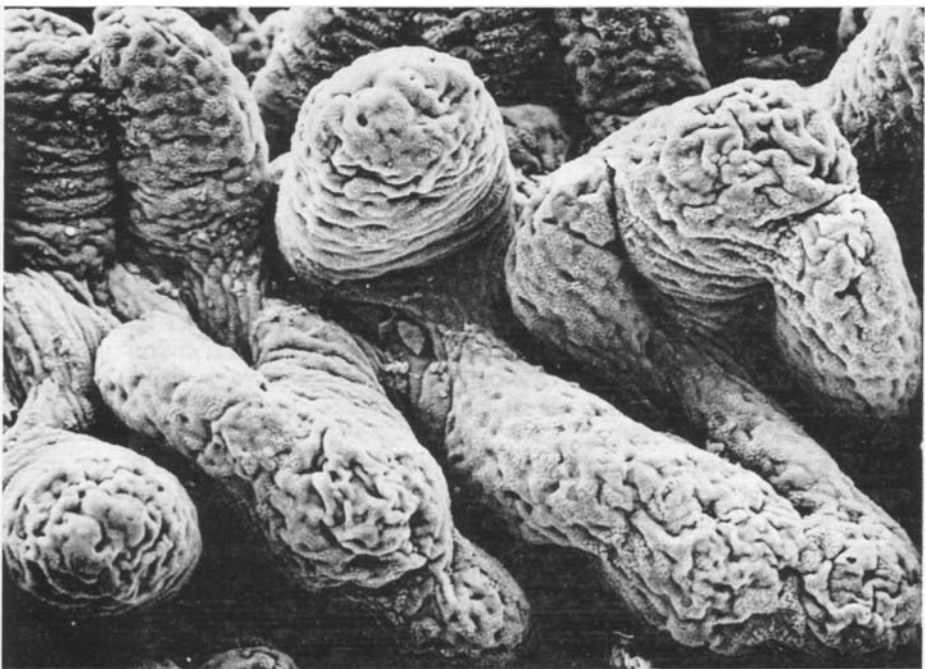


Fig. 8. Extensive villous fusion in the middle of the jejunum at 30 h after the first clinical signs (bar = 10  $\mu$ m.)

on fusion became more extensive, involving sometimes up to six villi, to form either "ridge-shaped" or "leaf-shaped" villi (Fig. 8), comparable to the description given for White Scours in three-weeks-old piglets (9). In this period large amounts of mucus regularly interfered with the SEM examinations.

In the ileum moderate villous shortening and occasional rounded or partly released epithelial cells were seen near the villous tips in this period. In both the duodenum and the colon the intestinal surface appeared normal.

At 90h after the onset of clinical signs, villous fusion again became rare in the jejunum. Only occasional narrow bridges between two or three villi were seen (Fig. 9).



Fig. 9. Narrow bridges between villi (arrows) at 90 h after the first clinical signs (bar = 10  $\mu$ m.)

### Discussion

The intestinal mucosal surface of the control piglets was similar to descriptions of normal pig intestine (6, 8, 9, 16). No irregularity or flattening of the villi was observed in the small intestine except in the villi covering Peyer's patches of the ileum. The microvillous tips were not always visible, possibly because they were covered with an enteric surface coat or microvillous fuzz (13).

Villous shortening and swelling of epithelial cells at the end of the incubation period as demonstrated with SEM is in accordance with the histological measurements (COUSSEMENT et al., to be published), and with lesions seen in porcine rotavirus infection (14), but the SEM seemed to be the more sensitive technique for the detection of villus distortion.

Cell desquamation and villous atrophy were significant in the first 6h after the onset of clinical signs. This is in accordance with histological (COUSSEMENT et al., to be published), immunofluorescence (DEBOUCK et al., to be published) and transmission electron microscopic (DUCATELLE et al., to be published) findings. The presence of large amounts of cell debris and detritus



between the villi in the early phase of diarrhoea is in contrast with the findings in porcine rotavirus infection (6). The presence of this cell debris probably reflects the *in vivo* situation more closely, since in the present study the intestine was not washed out during the preparation of the tissue specimens. Also these masses of debris in the intestinal lumen may account in part for the "explosion-like" distribution of immunofluorescence-positive material in the first 6 h after the onset of diarrhoea (DEBOUCK, personal communication).

Shortening of the villi was not as severe as in Transmissible Gastro-enteritis (16), but was more universal than in porcine rotavirus infection (6). The rough and irregular appearance of the stunted villi has been attributed to irregularity of epithelial cells (6).

Bridging of small intestinal villi has been described occasionally in the intestines of normal individuals (15, 16). It was not observed in the present control material from newborn colostrum-deprived piglets. Also, in the period around 60h after the onset of clinical signs, villous fusion in the jejunum was very severe, taking an aspect similar to the description of Three Weeks Enteritis of piglets (8). The morphogenesis of these fused villi could not unequivocally be established in the present sequential study. Nevertheless, some indications were found that they were initiated as a narrow side-by-side synechia between adjacent villi, as has been suggested in man (12). It was not possible to check whether this initial fusion was complete, as is suggested in colibacillosis of calves (10), or whether it started as a bridge, as it appears in light microscopy.

Desquamation of epithelial cells is probably the principal defect accounting for the malabsorption and leading to diarrhoea here, as was suggested for Transmissible Gastro-enteritis (4). Pores between epithelial cells, as they were observed in later stages of the disease, may also contribute to increase the permeability of the intestinal mucosa. This is also a known possible mechanism in the pathogenesis of diarrhoea (7).

The pseudomembranes and debris in the colon probably merely derive from the small intestinal lesions, since the colonic mucosal surface appeared unaffected.

### Summary

The intestinal surface of piglets, experimentally infected with the CV777 coronavirus, was studied at different time intervals using stereo-microscopy, histology and scanning electron microscopy. Desquamation of epithelial cells was most severe on jejunal villi at the time of onset of clinical signs. It was also seen at 6h after the onset of clinical signs in the ileum. Duodenum and colon remained normal. From 24h after the first clinical signs onwards, villous fusion was seen. The alterations of the mucosal surface are compared to those seen in other enteropathogenic conditions of pigs.

### Acknowledgements

We are indebted to the Virology Laboratory (Dir. Prof. M. PENSART) for preparing the infectious material. We thank Mr. C. PUTTEVILS for technical assistance.

### Zusammenfassung

#### Rasterelektronen- und stereomikroskopische Untersuchung der Darmoberfläche nach experimenteller Infektion mit dem porcinen Coronavirus CV777

Die Darmoberfläche von experimentell mit CV 777 infizierten Ferkeln wurde nach bestimmten Zeitintervallen mit Hilfe der Stereo- und einfachen

Lichtmikroskopie sowie mit dem Raster-Elektronenmikroskop untersucht. Bei Auftreten der ersten klinischen Symptome sowie sechs Stunden danach war die Desquamation der Epithelzellen des Jejunums am ausgeprägtesten. Ab 24 Stunden nach der Infektion wurde eine Verschmelzung der Zotten untereinander beobachtet. Die Veränderungen der Schleimhautoberfläche werden mit denen bei anderen enteropathogenen Prozessen verglichen.

### Résumé

#### Recherche au microscope électronique à balayage et au stéréomicroscope de la surface intestinale après une infection expérimentale avec le Coronavirus CV 777 du porc

On a examiné la surface intestinale chez des porcelets infectés avec CV 777 à intervalles déterminés au stéréomicroscope, au microscope optique et au microscope électronique à balayage. La desquamation des cellules épithéliales du Jejunum fut la plus marquée au début des premiers symptômes cliniques et six heures après. On a observé un amalgame des villosités dès 24 heures suivant l'infection. Les modifications de la surface de la muqueuse sont comparées à celles rencontrées dans d'autres processus entéropathogènes.

### Resumen

#### Estudios con el microscopio electrónico de trazados y con el estereo de la superficie intestinal tras la infección experimental con el virus corona porcino CV 777

Se examinó la superficie intestinal de lechones infectados experimentalmente con CV 777 tras ciertos intervalos de tiempo con ayuda de la estereomicroscopía y del microscopio óptico simple, así como con el microscopio electrónico de trazados. Al aparecer los síntomas clínicos primeros y seis horas después era muy manifiesta la descamación de las células epiteliales en el yeyuno. A partir de las 24 horas tras la infección se observó una coalescencia de las vellosidades entre sí. Las modificaciones en la superficie mucosa se comparan con las de los otros procesos enteropatógenos.

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