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ELECTRON MICROSCOPY OF THE INTESTINE OF GNOTOBIOTIC PIGLETS INFECTED WITH PORCINE ROTAVIRUS

By

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INTRODUCTION

Porcine rotavirus is an enteropathogen for neonatal piglets (Fukusho, Shimizu and Ito, 1981; Lecce and King, 1978; McNulty, Pearson, McFerran, Collins and Allan, 1976). Mortality is high in pigs infected during the first 3 days after birth, but it is reduced as the pig increases in age (Pearson and McNulty, 1977). The pathogenesis of porcine rotavirus in gnotobiotic piglets was studied by light microscopy (LM), fluorescent antibody technique (FA), scanning electron microscopy (SEM) and transmission electron microscopy (TEM), and it was demonstrated that clinical signs were correlated with viral replication and with induced virus lesions in the small intestine (McAdaragh, Bergeland, Meyer, Johnshoy, Stotz, Benfield and Hammer, 1980; Narita, Fukusho, Konno and Shimizu, 1982; Pearson and McNulty, 1979; Theil, Bohl, Cross, Kohler and Agnes, 1978; Torres-Medina and Underdahl, 1980). However, there are as yet no reports about the penetration of rotaviruses into the epithelial cells.

The present paper describes the morphological changes in infected intestinal cells of 2-day-old piglets inoculated orally with porcine rotavirus and discusses the penetration of virus into the absorptive cells.

MATERIALS AND METHODS

Virus

This was prepared as previously described (Narita *et al.*, 1982). Briefly, a faecal suspension was prepared as a bacteria-free 20 per cent intestinal contents filtrate after one gnotobiotic piglet passage. No virus other than rotavirus was detected in the suspension by electron microscopic examination. Piglets were inoculated orally with 2 ml of the faecal filtrate.

Animals

Five 2-day-old gnotobiotic piglets were used. They were kept at 26 °C in individual metal cages with positive pressure ventilation. Each was given 2 ml of the rotavirus suspension and they were killed at 6, 12, 18, 24 and 48 h after inoculation.

Electron Microscopic Examination

Samples of the small intestine were removed under general anaesthesia from 5 sites adjacent to those from which samples had been taken for histopathology. Samples

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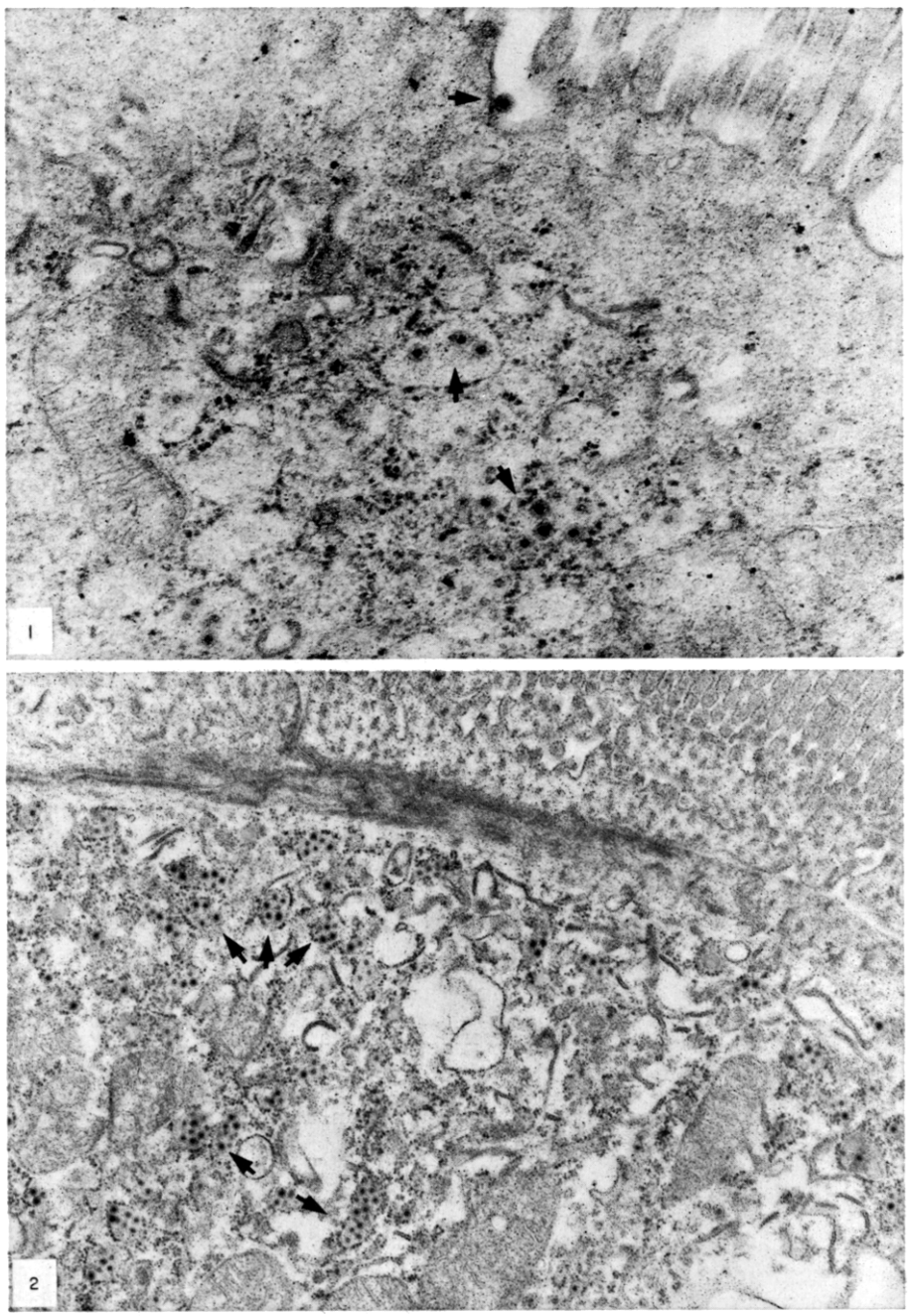

Fig. 1. Virus particles (↓) in absorptive cells of the jejunum 12 h after infection. ×40 000.
Fig. 2. Single and small groups of virus particles (↓) inside vesicles in the upper region of the apical cytoplasm of absorptive cells of the jejunum 12 h after infection. ×19 000.

were fixed in 2·5 per cent glutaraldehyde in 0·1 M phosphate buffer, pH 7·2, for 2 h at 4 °C. They were post-fixed in 1 per cent osmium tetroxide in phosphate buffer for 1 h at 4°C. The tissues were dehydrated in a graded series of ethanol and embedded in Epon 812. Sections were cut with glass knives, stained with uranyl acetate and lead citrate, and examined with a Nihon Denshi TEM-100CX electron microscope.

RESULTS

Duodenal, jejunal and ileal epithelial cells from piglets 6 and 12 h after inoculation showed no morphological alteration detectable by ultrastructural examination; there was no sign of detachment or desquamation of the villi. Microvilli were intact and contained a prominent nucleus, abundant mitochondria, and rough endoplasmic reticulum (RER). However, virus particles ranging from 65 to 75 nm in diameter were detected in a few absorptive cells of a sample of jejunum collected at 12 h. Particles were observed on the microvillar border or between the microvilli where they were often located in rows (Fig. 1). Individual or small numbers of particles were found inside vesicles located in the terminal web area or in the upper region of the apical cytoplasm (Fig. 2).

Eighteen hours after infection most of the absorptive epithelial cells in the jejunum and ileum were slightly swollen. Most of the microvilli of the brush border were normal in length, but some were short, thick and irregular. These infected cells had lost most of their staining density and contained many virus particles within the distended RER. In some sections, effete cells containing viral particles were found in the process of being destroyed (Figs 3 and 4). A few goblet cells also contained virus particles (Fig. 5).

At 24 h after infection, some of the absorptive epithelial cells were becoming degenerate. In the jejunum, the degenerating cells were rounded, swollen, and had lost some of their staining intensity. They had large lipid droplets and an RER distended by vacuoles. These degenerated cells contained few viral particles (Fig. 6). In some sections, the infected cells appeared to be detaching from adjacent epithelial cells and being shed into the lumen (Fig. 7). Many viral particles were seen within the distended RER. They were classified as 3 types; enveloped, non-enveloped particles and nucleoids. Enveloped particles were 70 to 80 nm in size and some appeared to have acquired an outer shell by budding through the endoplasmic reticulum. Non-enveloped particles were 50 to 65 nm in size. Both types of viral particles had an electron-dense core measuring 20 to 30 nm. In the cytoplasm of the infected cells, non-membrane bound, granular matrices containing an electron-dense virus core were present (Fig. 8). In the ileum, most of the epithelial cells were desquamated from the villi and a small number of neutrophils was observed in the submucosa.

At 48 h after infection, disruption and desquamation of the cells were still present in a few areas of villi. At this time the short villi were covered with a type of cell which was ultrastructurally different from any of those seen on the villi in the earlier stages. They were flat to cuboidal, contained oval nuclei lying parallel to the axis of the villi and were closely applied to the basement membrane. Their microvillar border was very uneven, short and incomplete

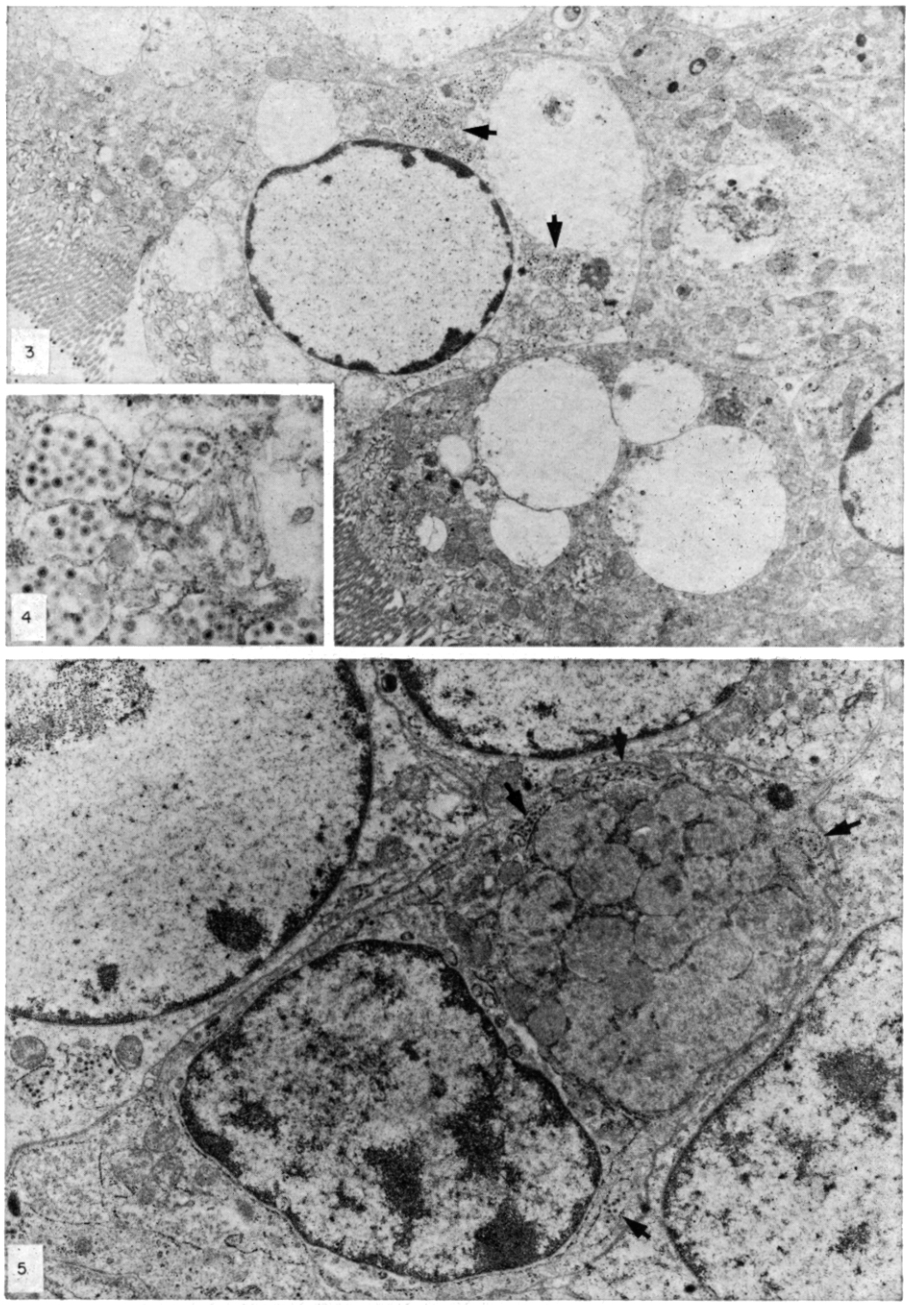

Fig. 3. Absorptive cells containing virus particles (↓) in process of destruction in the jejunum 18 h after infection. ×5000.
Fig. 4. Higher magnification of virus particles shown in Fig. 3 within RER 18 h after infection. ×26 000.
Fig. 5. Goblet cell in the jejunum containing virus particles (↓) 18 h after infection. ×9000.

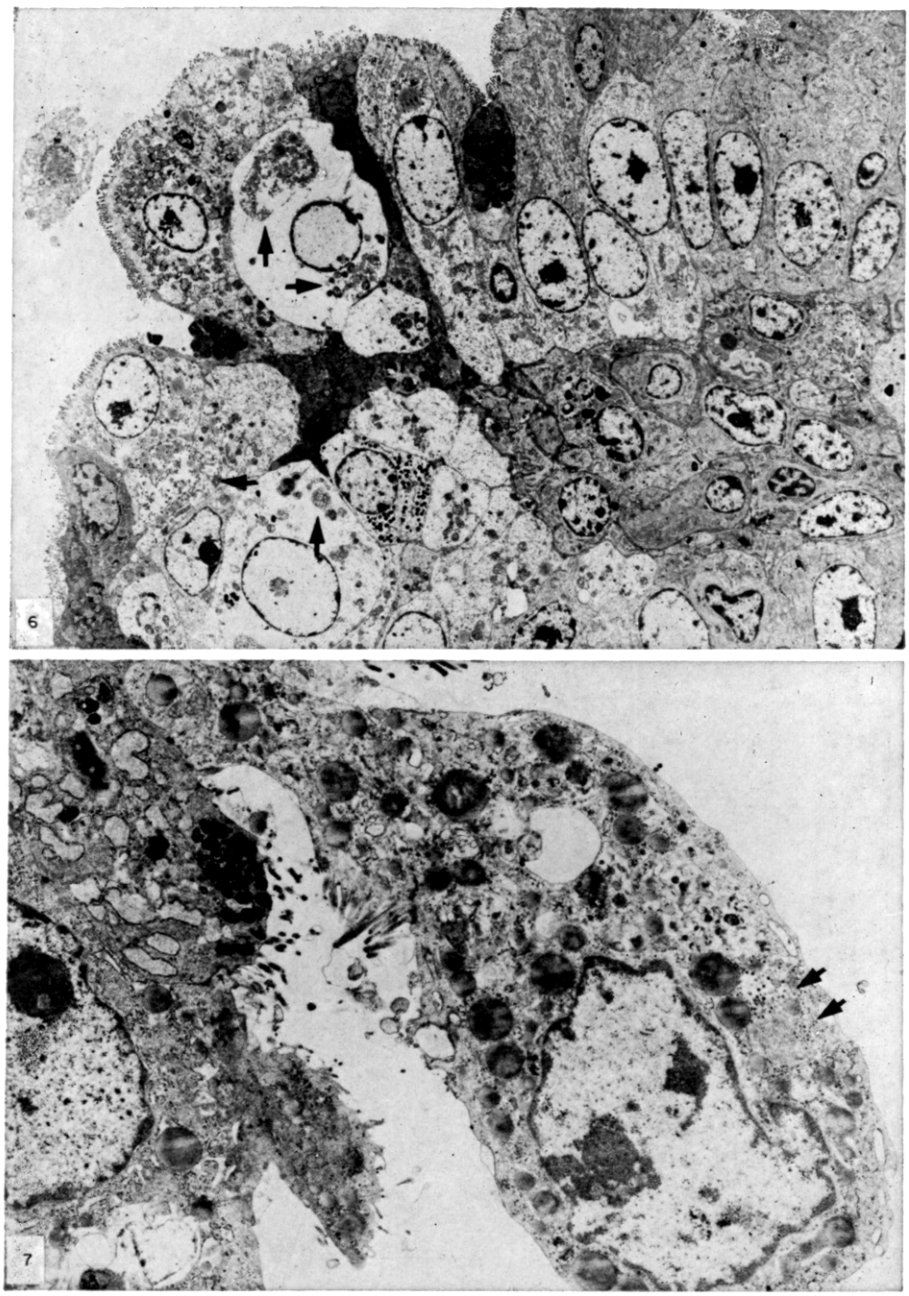

Fig. 6. Almost all the infected cells in the jejunum are rounded, and have large lipid droplets. These infected cells have a small number of virus particles (↓). 24 h after infection. ×2000.
Fig. 7. Infected epithelial cells in the process of being shed from the ileal epithelium. Virus particles are seen within distended RER (↓). 24 h after infection. ×6600.

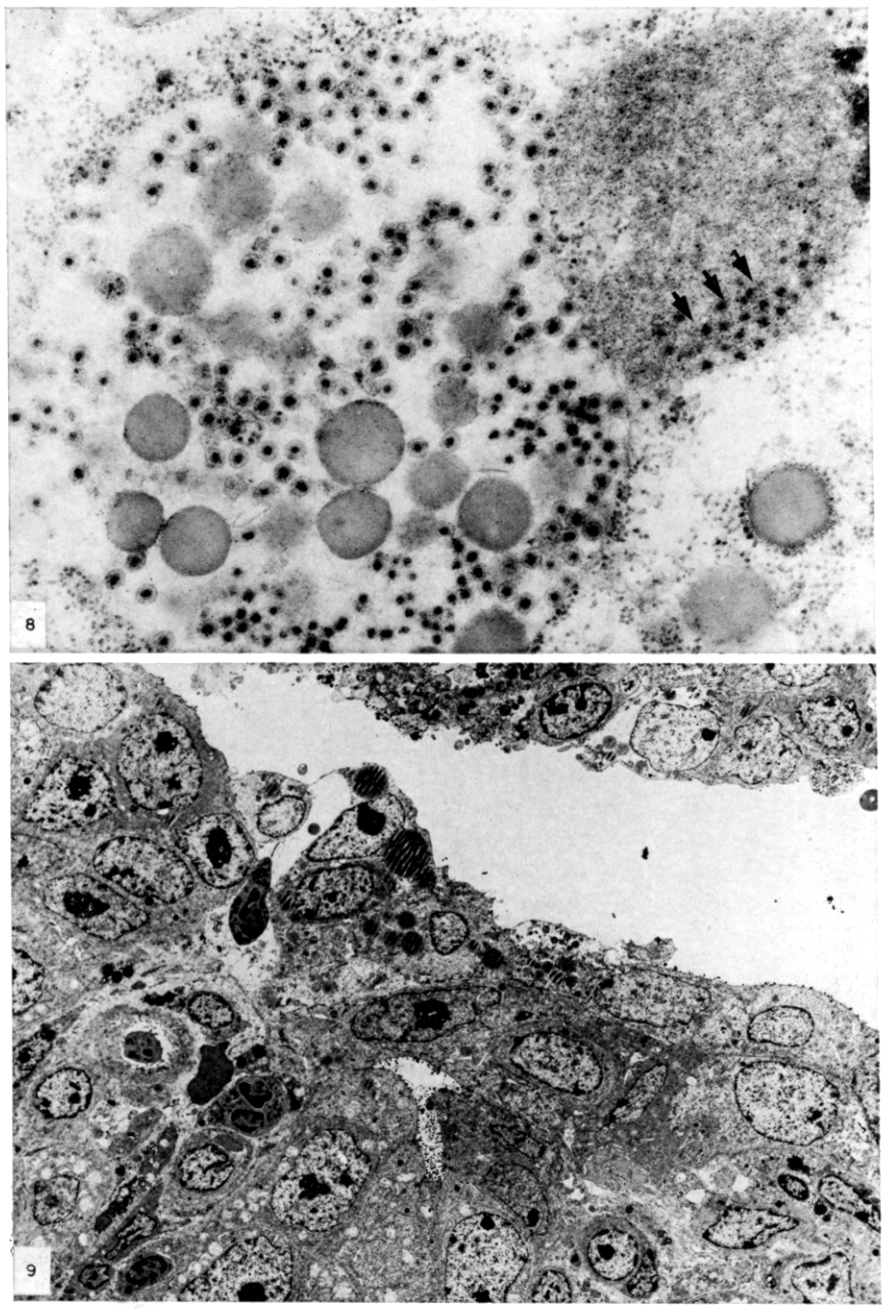

Fig. 8. Non-enveloped and enveloped virus particles within RER. Viroplasm containing an electron-dense core (↓) is seen in adjacent cytoplasm. 24 h after infection. ×32 000.
Fig. 9. Cuboidal villous epithelial cells. The microvillal border is uneven, short and incomplete. 48 h after infection. ×1500.

(Fig. 9). Viral particles were not detected in these apparently newly produced and undifferentiated cells.

DISCUSSION

The present EM study has established that the porcine rotavirus becomes localized within the epithelial and goblet cells of the small intestine of piglets. The viral particles, in the form of double-shelled or enveloped particles (70 to 85 nm), single-shelled particles (60 to 70 nm), and small cores or nucleoids (25 to 30 nm), were readily seen in the epithelium of the piglets killed 18 h after infection. Thereafter, infected cells were only rarely observed. These observations agreed with previous reports by Adams and Kraft (1976), Chasey (1977), Esparza, Gorziglia, Gil and Römer (1980), Pearson and McNulty (1979) and Saif, Theil and Bohl (1978), but detection of virus within goblet cells is a new finding.

At low magnification the infected cells were visible, with many viral particles present in the RER and the apical cytoplasm. The least severe and probably the earliest changes consisted of shortening and loss of the microvilli. At a more advanced stage the infected cells were rounded, had large lipid droplets and an RER distended with vacuoles. Subsequently, most of the absorptive cells appeared to be lost due to either cell desquamation or disruption. Therefore, the interaction of virus and intestinal cells, and the resulting absorptive cell degeneration, indicates that rotavirus is pathogenic for the epithelial cells of the small intestine. These TEM changes correlated well with those previously found by LM, SEM and FA examination by Lecce, King and Mock (1976), McAdaragh *et al.* (1980), Pearson and McNulty (1977), Theil *et al.* (1978) and Torres-Medina and Underdahl (1980).

Concerning the entry of the virus into cells, Pensaert, Haelterman and Hinsman (1970) and Wagner, Beamer and Ristic (1973) reported that transmissible gastroenteritis (TGE) virions are taken up by pinocytosis and then transported into the epithelial cytoplasm. Electron micrographs of cells taken 12 h after inoculation with rotavirus revealed viral particles between microvilli and in the apical tips, and vesicles in the terminal web areas of some otherwise apparently normal epithelial cells. These findings resemble those with TGE virus infection and therefore they strongly suggest that porcine rotavirus is also taken up by pinocytosis.

The present EM observations have provided additional information on the mode of virus release from host cells into the lumen. There seem to be 2 processes involved; one is the desquamation of the infected cells of the villous epithelial lining, and the other is the destruction of the luminal plasma membrane, with consequent discharge of cytoplasmic contents. These processes are commonly seen in small intestine infected with adenovirus (Takeuchi and Hashimoto, 1976), TGE virus (Chandler, Derbyshire and Smith, 1969; Larson, Morehouse, Solorzano and Kinden, 1979; Morin and Morehouse, 1974; Thake, 1968), and canine coronavirus (Takeuchi, Binn, Jervis, Keenan, Hildebrandt, Valas and Bland, 1976; Vandenberghe, Ducatelle, Debouck and Hoorens, 1980). It would therefore appear that the porcine rotavirus shares

with other enteric viruses the same release mechanisms from the epithelial cells into the lumen.

SUMMARY

Five gnotobiotic piglets inoculated orally with porcine rotavirus developed an enteric lesion.

Electron microscopy of the mucosal epithelium 12 h after inoculation showed that the virus penetrates into the absorptive cells between microvilli, possibly by a pinocytic mechanism. Afterwards, virus particles were most often seen within dilated cisternae of the rough endoplasmic reticulum (RER). These infected cells showed a range of changes, such as disruption of the microvilli, loss of cytoplasmic density and deposition of lipid droplets. Subsequently, most of the epithelial cells were desquamated from the villi. The interaction of virus and intestinal cells thus indicates that rotavirus is pathogenic for the epithelial cells.

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