Electron Microscopy of Intestinal Epithelial Cells of Piglets Infected with a Transmissible Gastroenteritis Virus

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ABSTRACT

An electron microscopic study of intestinal epithelial cells of neonatal piglets infected with transmissible gastroenteritis (TGE) virus revealed a unique parasite-host cell interaction. Entry of the TGE virus into intestinal epithelial cells of newborn piglets is mediated through a network of cytoplasmic tubules of plasmalemma origin. The tubules, called microcanaliculi, are morphologically distinct from endoplasmic reticulum and Golgi. In uninfected animals similar tubules appear to be responsible for the indiscriminate uptake of large quantities of macromolecules from colostrum during the first few days of life.

Thin-section profiles of plasmalemma invaginations resembled tubules or canals and frequently contained viral particles. TGE viral particles developed and accumulated within cytoplasmic vacuoles. Initially the vacuoles were continuous with the microcanaliculi formed by deep plasmalemma invagination. Mature viral particles were 60 to 85 mu in diameter with an electron dense doughnut-shaped nucleoid surrounded by a trilaminar membrane which resembled the vacuolar wall. Abundant evidence of viral effects was observed in absorbtive epithelial cells of the jejunum and ileum but not of the duodenum.

The ability of absorbtive intestinal epithelial cells to form deep cytoplasmic tubular invaginations is temporally related to the pathogenesis of TGE and may explain in part why pigs usually are fatally affected by TGE only during the neonatal period.

RÉSUMÉ

Une étude au microscope électronique des cellules épithéliales de l'intestin de porcelets nouveau-nés, infectés avec le virus de la gastro-entérite transmissible, a démontré une interaction parasite-cellule de l'hôte à caractère unique. La pénétration de ce virus dans les cellules épithéliales de l'intestin des porcelets naissants se fait par l'intermédiaire d'un réseau de tubules cytoplasmiques prenant naissance dans le plasmalemme. Ces tubules, appelés microcanalicules, sont morphologiquement distincts du réticulum endoplasmique et de l'appareil de Golgi. Chez les porcelets sains, des tubules similaires capteraient sans discernement une grande quantité de macromolécules du colostrum, au cours des premiers jours après la naissance.

Des profils de minces sections des invaginations du plasmalemme ressemblaient à des tubules ou à des canaux et contenaient souvent des particules virales. Les particules du virus de la gastro-entérite transmissible se développaient et s'accumulaient dans les vacuoles cytoplasmiques. Au début, les vacuoles étaient en continuité avec les microcanalicules formés par l'invagination prononcée du plasmalemme. Vues au microscope électronique, les particules virales parvenues à maturité mesuraient de 60 à 85 mu de diamètre et présentaient un nucléoïde dense, en forme de beigne et entouré d'une membrane trilaminaire ressemblant à la paroi vacuolaire. On observa une évidence incontestable des effets du virus dans les cellules épithéliales absorbantes du jéjunum et de l'iléon, mais non dans celles du duodénum.

L'aptitude des cellules épithéliales absorbantes de l'intestin à former des invaginations cytoplasmiques tubulaires profondes est temporairement reliée à la pathogénèse de la maladie et expliquerait partiellement la raison pour laquelle les porcs sont habituellement fatalement atteints par ce virus, seulement au cours de la période néo-natale.

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INTRODUCTION

Transmissible gastroenteritis (TGE) is a highly contagious viral disease of newborn pigs. Mortality approaches 100% in pigs infected during the first postnatal week. Mortality is reduced as pigs increase in age. Pigs over several weeks of age seldom die. Clinical signs of the disease appear within 12 to 36 hr after experimental inoculation. They include profuse diarrhea, vomition, dehydration and rapid weight loss.

The literature relevant to TGE research has been comprehensively reviewed by Leman (13) and Wagner (32). Electron microscopic studies of TGE virus in a variety of tissue culture systems have been reported (17,19,33). Also there have been reports on electron microscopic observation of isolated TGE virions visualized by negative staining (18,21,24,29,33).

There have been several reports of ultrastructural features of TGE in pig small intestine (4,30,34).

The purpose of this study was to identify high resolution fine structural features in absorptive cells of the small intestine of piglets experimentally infected with TGE virus.

MATERIAL AND METHODS

Both conventionally farrowed piglets and hysterectomy-derived and colostrum-deprived specific pathogen-free (SPF) piglets were used in this study. SPF piglets received a diet consisting of two parts of evaporated milk[1] and one part of water.

Conventional piglets, three or four days old, were obtained from herds with no prior history of TGE. Hence, the piglets were believed not to carry congenitally acquired immunity specific for TGE virus.

An Illinois strain of TGE virus was used as the inoculum. Inoculum was prepared by a method described previously (24). Piglets were inoculated per os with 1.0 ml of inoculum. In preliminary studies infectivity titres were determined to be 10^5 to 10^6 PID (pig infectious doses)/50 per ml (22).

Infected piglets generally vomited by 18 hr after inoculation with TGE virus. Diarrhea usually appeared several hours after vomiting. Piglets were killed by electrocution between 12 and 24 hr after inoculation.

Tissues were collected from various regions of the small intestine beginning in the duodenum around 10 cm from the pylorus and continuing at 25 cm intervals to an area approximately 10 cm from the ileo-cecal-colic junction. Tissues were fixed in two changes of 3% glutaraldehyde (25), post-fixed in 2% OsO_4, and embedded in an epoxy resin (14).

Ultrathin sections were cut with glass knives and a model 4800 LKB Ultratome. The sections were stained with both uranyl acetate and lead citrate (23,31). Specimens were examined with a Hitachi 11B electron microscope at 50 KV.

RESULTS

UNINOCULATED CONTROL PIGLETS

Electron microscopic examination of columnar absorbing epithelial cells from sections of the duodenum, jejunum and ileum of four uninoculated control piglets three to six days of age revealed ultrastructural features reminiscent of those observed by Mattisson and Karlsson (15, 16), and Staley et al (26-28). The most striking features of the jejunum and ileum were deep invaginations of the plasma membrane and the tubular structures in the apical cytoplasm limited by plasmalemma-like trilaminar membranes. Some of the plasma membrane invaginations continuous with the tubular and vesicular structures extended as far as the region of the nucleus generally located in the basilar region of the cell.

Plasmalemma invaginations and cytoplasmic tubular and vesicular structures were most abundant in absorptive cells of the jejunum and ileum. They were seen only occasionally in sections of duodenal absorptive cells. The appearance of tubular structures in the apical cytoplasm of absorbing epithelial cells of the small intestines of newborn pigs are well described in the works of Staley et al (26-28). In addition, their reports contain reviews of

literature relevant to the role of these tubules in absorption of colostrum and macromolecules during the early neonatal period.

Other ultrastructural features were common to columnar absorbing epithelial cells of many species. The luminal surface was lined by numerous straight and slender microvilli covered by extensions of the plasma membrane. The fine filamentous structure of the terminal web appeared to be continuous with the terminal bar at the lateral surface of the cell and the overlying matrix comprising the core of the microvilli.

Endoplasmic reticulum studded with ribonucleoprotein granules was found throughout the cytoplasm. Mitochondria were most
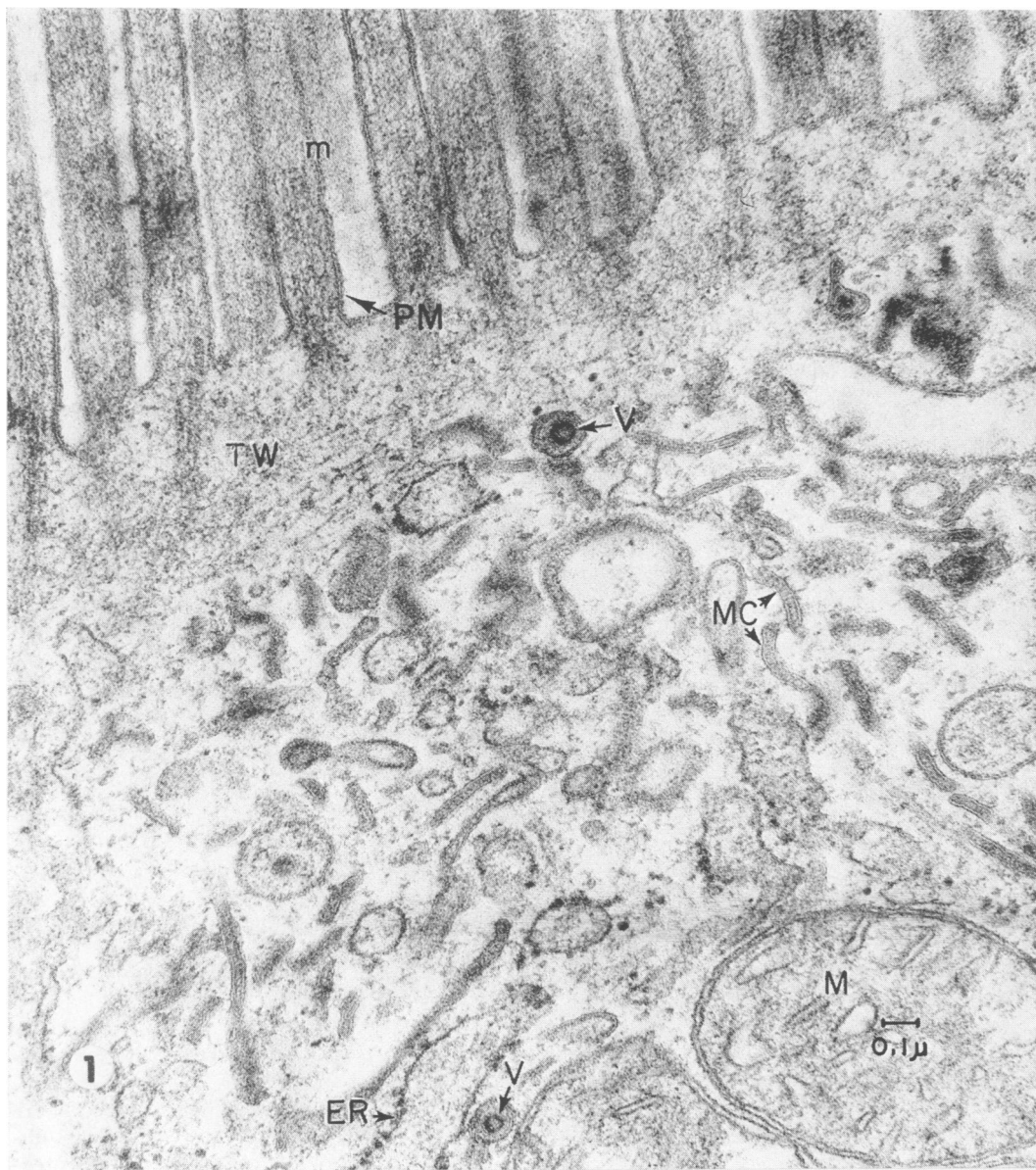

Fig. 1. Absorptive epithelial cell from jejunum of five day old piglet inoculated with TGE virus. The trilaminar membrane of the microcanaliculi (MC) believed to be of plasmalemmal (PM) origin is readily distinguished from the membrane of rough endoplasmic reticulum (ER). Viral particles (V) within vacuoles, mitochondria (M), microvilli (m) and the terminal web (TW) region of an absorptive epithelial cell are apparent. 41,000x.
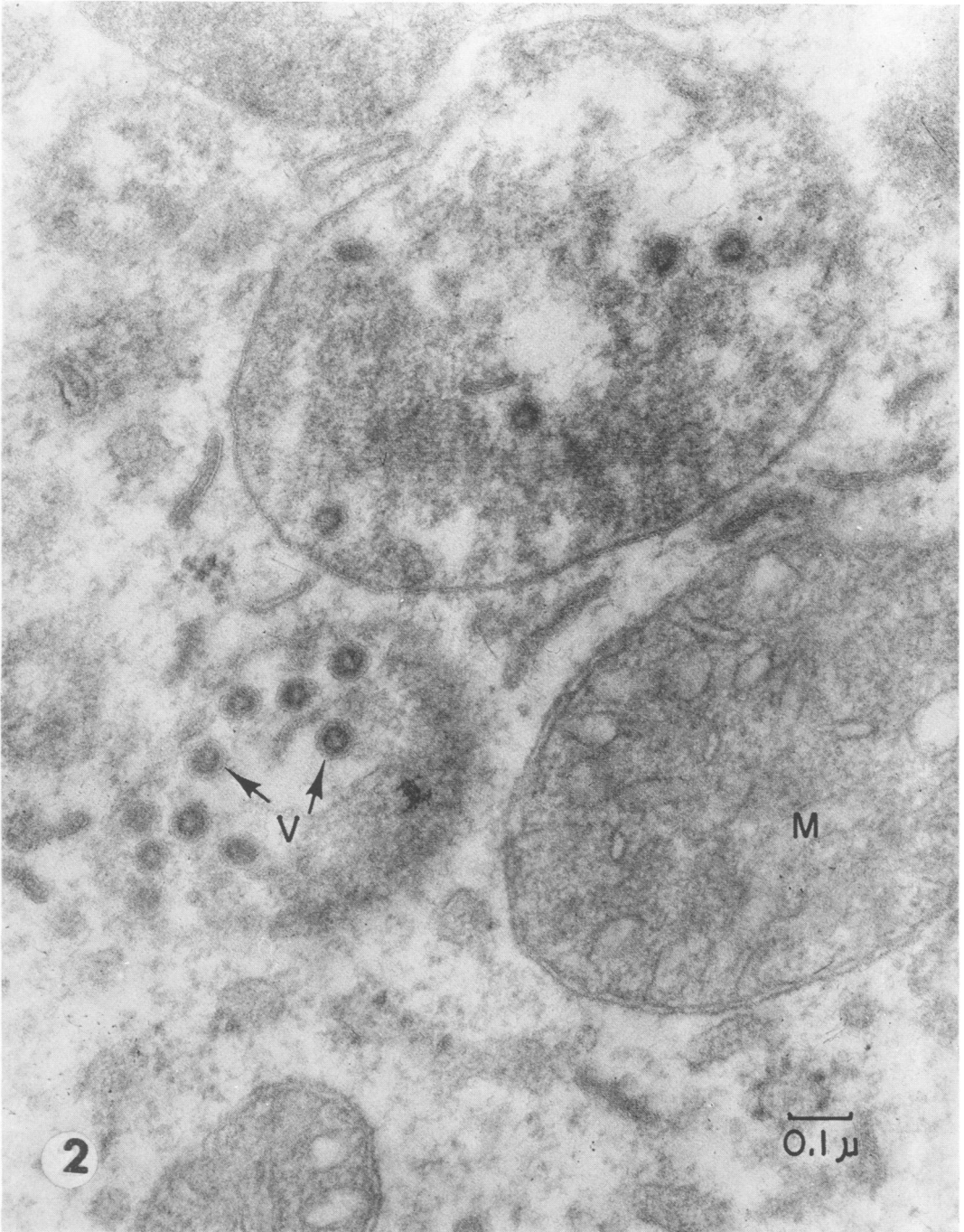
Fig. 2. Absorptive epithelial cell of six day old piglet inoculated with TGE virus. Numerous viral particles (V) are within cytoplasmic vacuoles. 84,000x

numerous in the apical cytoplasm just be-
neath the terminal web and the basilar cyto-
plasm midway between the basement mem-
brane and the nucleus. Nuclei were ar-
ranged in the basal portions of the cyto-
plasm about a third of the length of the
cells from the basement membrane. Most
nuclei had a single nucleolus.

INFECTED PIGLETS

Electron microscopic examination was
conducted on portions of the duodenum,
jejunum and ileum taken from 16 piglets
infected with TGE virus. To hold the num-
ber of sections for examination by electron
microscopy within manageable limits and
to increase the probability of finding cells
having undergone sufficient viral effects
to warrant detailed study, sections were
selected on the basis of changes observed
by light microscopy in adjacent tissues.
The extent of viral activity within intes-
tinal epithelial cells could be predicted by
the character of the cellular changes ob-
served with a light microscope. Few viral
particles were found in tall columnar epi-
thelial cells of infected piglets in areas of
intestinal mucosa which appeared to be
normal morphologically by light micro-
scopy; however, many tubular profiles
were observed. Numerous particles were
found in cells of those areas in which the
epithelial cells were altered to low columnar
or cuboidal form.

TYPE OF CELLS INFECTED

Only absorbtive intestinal epithelial cells
were observed to be infected with TGE
virus. Crypt epithelium, capillary and lym-
phatic endothelium, various cells in the
lamina propria and the smooth musculature
were not infected. Morphological changes
were not observed in epithelial cells of the
duodenum of piglets infected with TGE
virus, and only a few viral particles were
observed. Electron microscopic examina-
tion of sections of absorbtive epithelial
cells from the jejunum and ileum, how-
ever, revealed abundant evidence of viral
effect indicating that these are the target
host cells of TGE virus.

PLASMA MEMBRANE INVAGINATIONS, CYTO-
PLASMIC TUBULES AND VACUOLES

An extensive network of tubular struc-
tures was observed in the apical cytoplasm
of TGE infected absorbtive epithelial cells
of the jejunum and ileum (Fig. 1). The
character of the cytoplasmic network re-
sembled the tubular and vesicular struc-
tures observed in uninoculated control pig-
lets. Some of the tubular structures were
continuous with crypt-like invaginations
of the plasma membrane. Evidence of
branching and anastomosis was found in
other profiles of tubular structures. In
some sections, continuity was demonstrated
between cytoplasmic tubules and vacuoles
containing varying numbers of viral part-
icles. Trilaminar membranes limiting
tubules and vacuoles in the apical cyto-
plasm morphologically resembled the plasma
membrane and were believed to have had
their origin in the plasma membrane.

The character of the plasmalemma-like
membranes limiting the cytoplasmic tubules
and vacuoles was markedly different from
the membranes of endoplasmic reticulum,
Golgi apparatus and mitochondria (Fig. 2).
The membranes of the later cytoplasmic or-
ganelles were much less prominent. In
many lower magnification electron micro-
graphs they were resolved as single mem-
branes, whereas the membranes of the cyto-
plasmic tubules and vacuoles appeared as
trilaminar structures.

Maximal thickness of the trilaminar plas-
ma membranes covering microvilli as meas-
ured at ten sites in each of eight electron
micrographs was between 8 and 12 mu.
Microcanalicular membranes similarly
measured were 8 to 11 mu thick. The trila-
minar membranes of rough endoplasmic
reticulum, nuclear membrane and mito-
chondria were less than 7 mu thick and
generally measured around 6 mu, approxim-
ately half as thick as microcanalicular
membranes believed to be of plasmalemma
origin.

The prominent tubular structures ob-
served in these studies were morphologically
distinct from the microtubules described
as permanent cytoplasmic constituents of
a variety of mammalian cells by DeThe'
(8), and in plant cells by Ledbetter and
Porter (11). The microtubules described
by DeThe' (8) were 180 to 250 A in dia-
meter, considerably smaller than the 280
to 400 A of the plasmalemma invaginations
observed in glutaralydehyde fixed cells in
the present study. To avoid confusion with
a variety of previously described micro-
tubules and spindle tubules, we have used

the term microcanaliculi to identify the plasmalemma invaginations and the tubular structures which they form within the cytoplasm of intestinal epithelial cells. The network of anastomosing and branching canals which they form is referred to as microcanalicular system.

LOCATION OF VIRAL PARTICLES

Numerous structures identified as viral particles were observed in the cytoplasm of absorbtive epithelial cells of the jejunum and ileum. Viral particles were not found in the nucleus. The particles were observed most often in the apical cytoplasm between the nucleus and the region of the terminal web. The majority of viral particles were located in the deeper portions of the apical cytoplasm in vacuoles (Fig. 2) observed to be continuous with the microcanalicular system.

Particles resembling the virus but not clearly located within vacuoles were occasionally observed. Viral particles observed in the apical cytoplasm commonly occurred in microcanaliculi believed to originate from the luminal surface of the plasma membrane. Viral particles were frequently observed in the lumen of the small intestine between microvilli and within crypts formed by invagination of the plasma membrane at the base of microvilli. Masses of cellular debris free in the lumen of the intestine frequently contained both isolated viral particles and accumulations of particles in vacuoles.

Viral particles were not found in the basilar cytoplasm between the nucleus and basement membrane of infected epithelial cells. Viral particles and plasma membrane invaginations were not observed in the region of the lateral and basilar plasma membranes. Viral particles were not found between intact adjacent epithelial cells or in the basement membrane.

MORPHOLOGY OF VIRAL PARTICLES

From one to 40 viral particles were observed in cross-sections of single cytoplasmic vacuoles. Most vacuoles contained viral particles believed to be in different stages of maturation.

Viral particles containing less dense nucleoids with incomplete or partially limiting trilaminar membranes were believed to represent immature stages of particle differentiation.
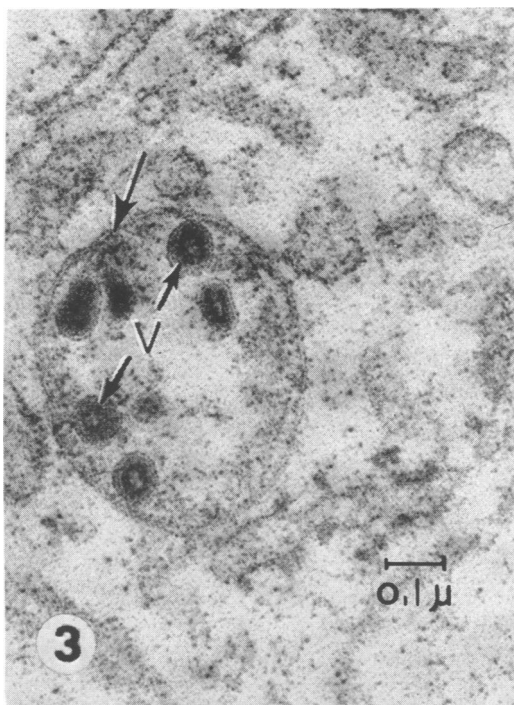

Fig. 3. Cytoplasmic vacupole in absorptive epithelial cell of piglet infected with TGE virus. Suggestions of tail-like membranous appendages between region of vacuolar wall and viral particles are designated by the large arrow. Other viral particles (V) may also have a connection with the vacuolar wall. 57,720x.

The trilaminar membrane surrounding some viral particles was continuous with the membrane of the limiting cytoplasmic vacuole. In the region immediately adjacent to the membrane comprising the cytoplasmic vacuole, the nuclei of some particles were more electron-lucent and presumably incomplete. Other viral particles had tail-like appendages composed of trilaminar membranes or areas of increased electron density, some of which extended from the particle to the region of the trilaminar membrane limiting the vacuole (Fig. 3).

Structures interpreted to be mature and complete viral particles were observed in greater number than a variety of pleomorphic and incomplete particles. The mature particles were from 60 to 85 mu in diameter with most particles measuring 70 to 75 mu. The particles were characterized by finely granular doughnut-shaped electron-dense nucleoids with electron lucent centers. The nucleoid was surrounded by an outer limiting trilaminar membrane which had morphological features resembling those of the limiting membrane of the cytoplasmic vacuole in which the particles de-
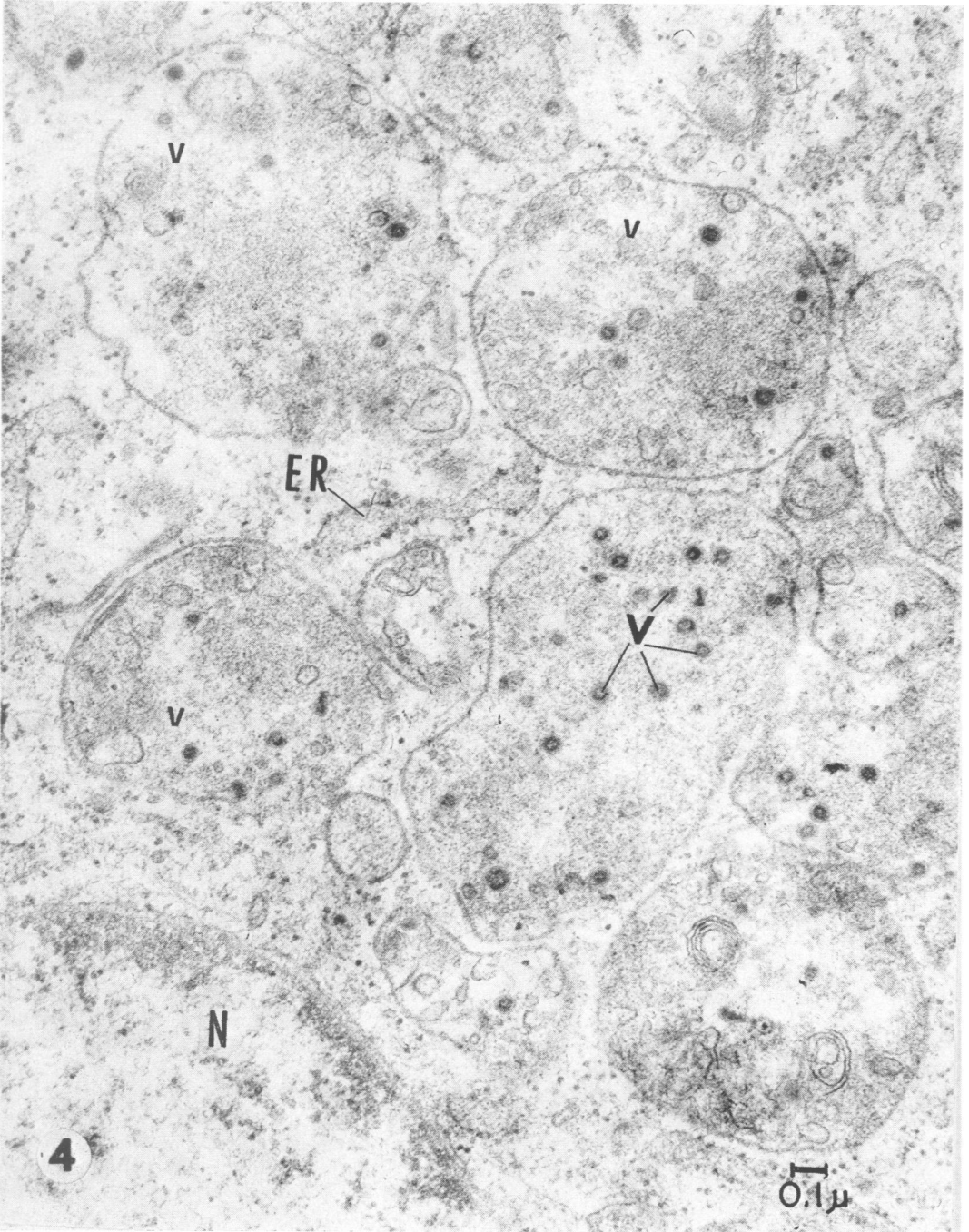
Fig. 4. Numerous vacuoles (v) containing viral particles (V), nucleus (N) and endoplasmic reticulum (ER) are apparent in the apical cytoplasm of absorptive epithelial cell from jejunum of five day old piglet 24 hr following inoculation with Illinois strain of TGE virus. 42,6000x

veloped. Between the electron-dense dough-nut-shaped nucleoid and the inner membrane of the limiting membrane there was a less electron-dense area comparable to that of the core of the nucleoid (Fig. 2).

CYTOPLASMIC VACUOLES

Vacuoles containing viral particles measured from 125 mu to several microns in diameter. The lumen contained a finely granular homogenous matrix interspersed occasionally by fine membranous structures. Large vacuoles each containing many viral particles occurred in the supranuclear region of the cytoplasm (Fig. 4). Smaller vacuoles containing fewer viral particles were located in the apical cytoplasm just beneath the terminal web and admixed with the microcanalicular system believed to be involved in viral invasion.

DISCUSSION

MICROCANALICULI, VACUOLES AND VIRAL INFECTION

Membranes forming microcanaliculi were interpreted as arising from the plasmalemma because of structural identity and morphological continuity with that membrane. In infected piglets, cytoplasmic vacuoles containing viral particles were believed to have a similar origin because of their observed continuity and structural identity with microcanaliculi.

Because of the tortuous course of microcanaliculi in the apical cytoplasm of infected cells, it was impossible to visualize the entire length of any one microcanaliculus. For this reason, it could not be determined whether all microcanaliculi maintained their association with the plasma membrane or whether some became separated from it in a process analogous to pinocytosis. A paucity of canal-like connections across the terminal web region suggested that pinching off from the plasma membrane with separation from microcanaliculi generally occurred. However, it is possible that the very tortuous course of lengthy microcanaliculi in the apical cytoplasm immediately below the terminal web resulted in the appearance of many more microcanalicular profiles than are apparent in the terminal web region.

The lumen of vacuoles observed to contain viral particles in infected piglets was presumed to have formed as a bulbous terminal expansion of the tubular invaginations of the plasma membrane. Hence, where pinching off in the terminal web region had not occurred, the vacuolar lumen may be considered as an extracellular space continuous with the lumen of the intestine. The amorphous material within the vacuoles is probably primarily of colostral or dietary origin (27).

A number of papers have appeared recently which describe a profuse system of plasmalemmal invaginations, tubules and vacuoles in the apical cytoplasma of absorbing epithelial cells of the duodenum (27), jejunum (30) and ileum (27) of normal neonatal piglets. These structures have not been observed in adult animals. Such structures have been observed in fetal rabbits (7) and in the ileum of a human fetus (2).

The tubules of the duodenum appeared following colostrum ingestion then disappeared 24 to 48 hr later (26). The tubules of the jejunum persisted for a longer period; however, they were absent at three weeks (28). Tubules and vacuoles in ileal absorptive cells persisted beyond six days but were not observed in pigs three weeks old (27). Our observations of very few viral particles in duodenal absorptive epithelial cells is in accord with observations by Clark and Hardy (5) of early "closure" following suckling in the duodenum and late "closure" (12 to 14 days) in the terminal ileum.

Leece et al (12) have demonstrated that protein absorption mechanisms in newborn pigs are qualitatively nonselective. Others (10,20) have demonstrated marked and steadily increasing concentrations of immunoglobulins in the serum of piglets during the first day of suckling. The immunoglobulins originated from the colostrum and were indiscriminately absorbed through the gut in large quantities essentially unaltered. There is close chronological correlation between the presence of numerous plasmalemmal invaginations and tubular and vesicular profiles in the cytoplasm and the ability of jejunal and ileal epithelial cells to absorb large quantities of macromolecules. It is during this same neonatal period that piglets most commonly are fatally affected by TGE viral infection. It appears that the marked transport potential of absorbing intestinal epithelial cells

of the jejunum and ileum of newborn piglets, presumably mediated through the microcanaliculi, may be essential to the highly fatal clinical syndrome of neonatal animals. Susceptibility to TGE virus appears to decrease as the number of microcanaliculi decrease and as intestinal epithelium differentiates and no longer forms microcanaliculi. While the age at which piglet intestinal epithelial cells lose their ability to form microcanaliculi has not been precisely established, our observations suggest that their progressively decreasing ability to develop microcanaliculi may relate directly to lower mortality of piglets exposed to TGE virus after they are ten days old. Another factor contributing to reduced mortality in older piglets may be the relative decrease in turnover time of villus epithelial cells in piglets after closure or the termination of macromolecular uptake (5). More rapid replacement of villar epithelial cells damaged by TGE would provide older pigs with increased protection from adverse effects of resident intestinal flora plus increased intestinal function.

The paucity of viral particles in duodenal epithelial cells indicates that these cells and/or their microenvironment do not constitute an optimal site for replication of TGE virus. These findings may be related to the observations of Staley *et al* (27) who observed that tubular profiles are present in duodenal epithelial cells of piglets for only a brief time following the ingestion of colostrum. Possibly, duodenal absorptive cells are not capable of interacting with the TGE virus to the extent of jejunal and ileal cells for reasons related to a reduced capacity for absorption of macromolecules. Proteins and colloidal materials administered orally to suckling rats and mice are ingested by columnar absorptive cells of the jejunum and ileum, but not of the duodenum (6).

Surgically isolated segments of the duodenum of piglets two to 18 days of age inoculated with TGE virus produced titres as high or higher than developed in similar segments of the jejunum (9). Our findings of minimal effects in intact duodenum of four to seven day old piglets is in contrast to the above study. The possibility that some of the piglets in the study referred to above may have been as young as two or three days of age may be an important consideration; i.e. the duodenum of very young piglets has more microcanaliculi and

is therefore more susceptible to TGE virus than that of older animals. Additionally, surgical isolation of portions of the small intestine (9) creates a regional starvation which may result in delayed closure and/or increased susceptibility to infectious agents. Also, surgical isolation would eliminate the flow of gastric secretions.

CYCLE OF DEVELOPMENT

We postulate that the cycle of replication of the TGE virus in small intestinal absorbtive epithelial cells of neonatal piglets involves use of the microcanalicular system for initial entry of infectious particles into the apical cytoplasm (Fig. 5). Following an eclipse period, viral replication occurs in the region of the innermost invaginations of the microcanaliculi which may or may not appear vacuolar at this time.

In a process resembling, but probably not identical to "budding" of oncogenic viruses, the developing nucleoid displaces the vacuolar membrane toward the interior of the vacuole. Nucleoid maturation proceeds within the vacuolar invagination. As the viral particle develops, it moves further into the vacuole. The viral-limiting membrane eventually encircles the nucleoid and becomes detached from the vacuolar wall. Vacuoles become larger and larger as viral particles accumulate within them.

The process by which the TGE virus is produced prior to its accumulation within cytoplasmic vacuoles of plasmalemmal origin was difficult to visualize in hundreds of ultrathin profiles examined. In several sections we visualized static structures which rather inconclusively suggested that the TGE virus may form by a "budding" process. Budding from the cell membrane is a phenomenon common to many viruses; for example, avian leukosis viruses, influenza viruses, vesicular stomatitis virus, mouse mammary virus and murine leukemia viruses. Absence of numerous "budding" forms in host cells infected with TGE virus can be interpreted as meaning that TGE virus has a method of replication somewhat different from typical oncogenic viruses or that the budding process occurs relatively quickly. We favor the former conclusion.

Witte *et al* (33) presumed that TGE virus particles detached from intracellular membranes and then migrated by way of the cytoplasmic canalicular systems to the

surface of tissue culture cells from where they are released. In our study of intestinal epithelial cells, microcanaliculi were believed to be intimately associated with the entry of TGE virus into susceptible cells but were not believed to function in the expulsion of viral particles from them. Viral particles are apparently released from vacuoles when sloughed or desquamated and degenerating infected cells are digested in the lumen of the intestine. Witte *et al* (33) also suggested the possibility of TGE viral maturation by budding at the cell surface. The budding process was never seen at the true cell surface of infected enterocytes in our study.

POSTULATED DEVELOPMENTAL CYCLE

OF TGE VIRUS
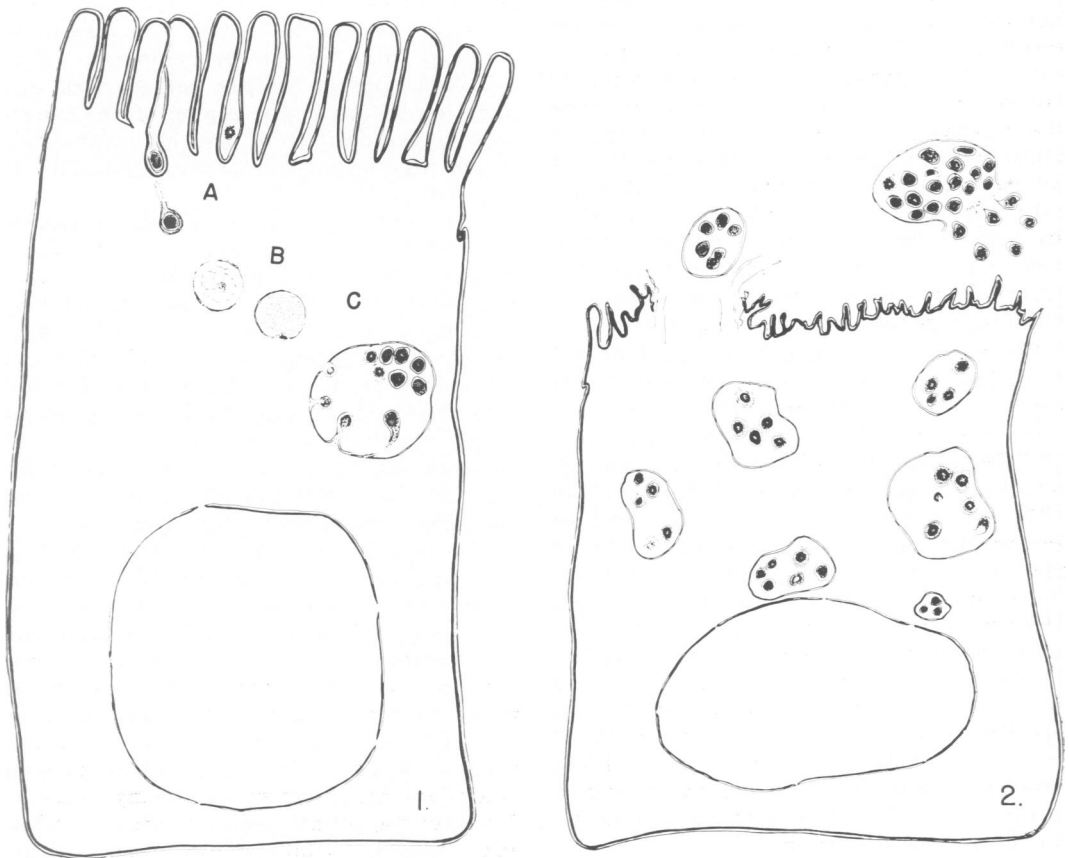
1. NORMAL INTESTINAL CELL

   A. PHASE OF VIRAL ENTRY

   B. " " " ECLIPSE

   C. " " " REPLICATION

2. INTESTINAL CELL DEGENERATION, RUPTURE, AND VIRAL RELEASE.

Fig. 5. Postulated cycle of development of TGE virus. A columnar absorptive intestinal epithelial cell (1) illustrates viral entry and replication. The sloughed and degenerative epithelial cell (2) has ruptured, releasing vacuoles containing TGE virus to the lumen of the intestine. Individual particles are released upon digestion and lysis of vacuoles.

The cycle of development of TGE virus is unique in its use of the highly specialized microcanalicular system of a neonatal animal to gain access to deeper portions of the cytoplasm of intestinal absorptive epithelial cells. To the best of our knowledge, no other viral agents have been described as utilizing the deep plasmalemmal invaginations peculiar to some neonatal animal species in their cycle of replication. However, this phenomenon has apparently not been extensively studied in intestinal epithelial cells of suckling animals of other species suffering from neonatal enteric infections. For example, TGE of swine and epizootic diarrhea of infant mice (EDIM) have similar clinical manifestations. Recent electron microscopic studies have revealed additional etiological similarities. Particle size of both agents is around 70 to 80 mu. It has been suggested that viral particles of both agents enter vacuoles by a process of budding. Both particles have been reported as occurring within cytoplasmic vacuoles and endoplasmic reticulum (1,17,33). Also, electron microscopic studies with both TGE and EDIM (1,33) resulted in interpretations that the viruses occur in the perinuclear space. Staley et al (28) have observed that coalescence of vacuolated tubular extensions of plasmalemmal invaginations created deep indentations into the apical surface of the nuclear membrane. In low magnification or low resolution electron micrographs, it may be difficult for investigators not cognizant of the microcanalicular system in neonatal animals to correctly distinguish vesiculated smooth endoplasmic reticulum from vacuolated microcanaliculi or dilated perinuclear spaces. Additional studies are needed to further clarify the etiological and clinical relationships of TGE and EDIM.

Contrary to findings of Okaniwa et al (17) and Witte et al (33) in tissue culture cells, we did not observe particles in granular and agranular endoplasmic reticulum or the perinuclear cisterna of absorptive enterocytes. Like Witte et al (33), we did observe particles within vacuoles bordered by double membranes. We interpreted these to be of plasmalemmal origin (28).

Based on morphological (3), physical and biochemical similarities of TGE virus to infectious bronchitis virus (IBV), it has been suggested that the TGE virus is a member of the coronavirus group (21, 29). Both viruses contain RNA, are ether sensitive and stable at pH 3. Both are of medium size (70 to 90 mu) and accumulate within cytoplasmic vacuoles and cisternae. Additionally, Phillip et al (21) have demonstrated an immunodiffusion identity line between TGE and known coronaviruses. We have also been impressed by ultrastructural similarities between TGE and IBV viruses; however, we are hesitant to speculate further on the matter of classification because of the diverse experimental milieu involved in studies of IBV and TGE.

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