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COMPARATIVE VIRULENCE OF PORCINE ROTAVIRUS SEROTYPES 1 AND 2 IN GNOTOBIOTIC PIGS

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Stuart D Sleight
Major professor

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COMPARATIVE VIRULENCE OF PORCINE ROTAVIRUS SEROTYPES 1 AND 2 IN GNOTOBIOTIC PIGS

By

James Edward Collins

A DISSERTATION

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ABSTRACT

COMPARATIVE VIRULENCE OF PORCINE ROTAVIRUS SEROTYPES 1 AND 2 IN GNOTOBIOTIC PIGS

BY

JAMES EDWARD COLLINS

The purpose of the present study was to compare serotypes 1 (Ohio State University strain) and 2 (South Dakota State University strain) of porcine rotavirus to determine if specific clinical signs, mortality rates, virus shedding patterns, virus antigen distribution, or lesion distribution and severity were related to the serotype of rotavirus with which pigs were infected.

Forty hysterotomy derived gnotobiotic pigs were inoculated orally at 3 days of age with 2 ml of homogenate containing 10^5 pig-infectious $dose_{50}$ of either the Ohio State University (OSU) strain or the South Dakota State University (SDSU) strain of porcine rotavirus. Controls were inoculated with media only. Mortality, clinical signs, and body weights were monitored daily. Five pigs in each of the virus inoculated groups and 4 control pigs were killed at 24 and 168 hours after inoculation. Four pigs in each of the 3 experimental groups (OSU, SDSU, and control) were

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killed 72 hours after inoculation. Specimens were collected at necropsy for serum chemistry, histologic, immunofluorescent, and scanning electron microscopic examinations.

All of the pigs inoculated with the OSU or SDSU strains survived. Control pigs remained healthy throughout the study. Pigs inoculated with the OSU or SDSU strains developed diarrhea 19-48 hours and 24-54 hours after inoculation, respectively. Vomiting was observed in 5 of 14 (36%) of the pigs infected with the OSU strain whereas pigs inoculated with the SDSU strain did not vomit. Pigs inoculated with the OSU or SDSU strains had reduced weight gain compared to control pigs, but there was no difference in weight gain between pigs given the OSU or SDSU strains. Results of immunofluorescent examinations were similar for pigs given either rotavirus strain except that at 24 hours after inoculation, viral antigen was detected at the duodenal-jejunal flexure in 5/5 pigs given the OSU strain and in 1/5 pigs given the SDSU strain. Villous atrophy and fusion were more severe at the duodenal-jejunal flexure of pigs inoculated with the SDSU strain when compared to pigs inoculated with the OSU strain. Although the OSU and SDSU strains did show differences in the occurrence of vomiting and distribution of villous atrophy, these strains were equally virulent for 3-day-old gnotobiotic pigs.

Dedicated, with love, to my wife, Barbara,

and our sons, Brian and Daniel.

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INTRODUCTION

Rotaviruses were first identified in the feces of a healthy vervet monkey and in intestinal washings from sheep and cattle at a slaughterhouse (Malherbe and Strickland-Cholmley, 1967). However, it was not until Mebus et al (1969) associated a rotavirus with diarrhea in calves in Nebraska that the significance of these viruses was recognized. Rotaviruses are now considered important causes of neonatal gastroenteritis in many mammalian and avian species (Mebus et al, 1971b; Bishop et al, 1973; Bohl et al, 1978; McNulty et al, 1978). In children, rotavirus is the single most important etiologic agent of acute infantile gastroenteritis throughout the world and has been estimated to account for one million diarrheal deaths annually (Vesikari et al, 1983).

Initially, all viruses with rotavirus morphology were thought to possess a common group antigen located on the inner capsid layer of the virus (Woode et al, 1976a; Yolken et al, 1978a). Recently, several rotaviruses which are morphologically similar to but antigenically distinct from the conventional rotaviruses have been described. These viruses have been referred to as

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pararotaviruses (Bohl et al, 1982), rotavirus-like viruses (Rodger et al, 1982), and atypical rotaviruses (Snodgrass et al, 1984). To distinguish among these isolates, Pedley et al (1983) proposed a scheme in which typical and atypical rotaviruses are classified based on the presence of distinctive group specific antigens into groups designated A, B, C, and recently D and E (Pedley et al, 1986).

Group A rotaviruses have been further classified based on serotype and subgroup specific antigens. Serotype specific antigens are located on the outer capsid layer of the virus (Bridger, 1978), and are detected by plaque reduction and fluorescent focus neutralization assays (Sato et al, 1982; Bohl et al, 1984; Hoshino et al, 1984). Subgroup specific antigens are located on the inner capsid layer (Woode et al, 1976a) and are detected by enzyme-linked immunosorbent, immune-adherence hemagglutination, and complement fixation assays (Struker et al, 1979; Zissis and Lambert, 1980). By using these assays, multiple serotypes and subgroups of mammalian rotaviruses have been identified.

Recent reports have indicated differences in clinical signs, viral antigen distribution, and lesion severity for different rotavirus serotypes, subgroups, and groups. An avian group A rotavirus, studied by McNulty et al (1983) had a predilection for the duodenal mucosa whereas a non-group A avian rotavirus replicated

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best in the mid-small intestine. In gnotobiotic pigs, a group B porcine rotavirus caused mild clinical signs and lesions (Theil et al, 1985) whereas a group A porcine rotavirus (Ohio State University strain) caused severe diarrhea and extensive intestinal damage (Theil et al, 1980). Four strains of rotavirus isolated from diarrheic calves caused different amounts of villous atrophy when inoculated into ligated intestinal loops in colostrum deprived calves (Carpio et al, 1981b). Differences in the clinical symptoms associated with infection by two subgroups of rotavirus in humans have been reported by some investigators (Uhnoo and Svensson, 1986), but these findings are in disagreement with the findings of others (White et al, 1984).

Two serotypes of porcine rotavirus have been identified by plaque reduction neutralization tests, cross protection studies in gnotobiotic piglets, and electrophoresis of rotaviral double-stranded RNA (Bohl et al, 1984; Hoshino et al, 1984). The purpose of the present study was to determine if specific clinical signs, mortality rates, virus shedding patterns, and lesion distribution and severity were related to the serotype of porcine rotavirus with which gnotobiotic pigs were infected.

LITERATURE REVIEW

Classification

Rotaviruses derive their name from the Latin word "rota" meaning wheel, which they resemble in appearance (Flewett et al, 1974). Initially, they were referred to as "orbivirus", "duovirus", "reovirus-like agent" and "infantile gastroenteritis virus" (Mebus et al, 1971b; Bishop et al, 1973; Kapikian et al, 1975).

Rotaviruses, reoviruses, and orbiviruses have many similarities. They are approximately the same size (65 to 75 nm), have double-shelled capsids, have similar buoyant density in cesium chloride and possess a double-stranded RNA genome (Palmer et al, 1977). Rotaviruses can be distinguished from orbiviruses and reoviruses by the presence of 11 segments of the RNA genome; orbiviruses and reoviruses possess only 10 segments (Palmer et al, 1977). On the basis of these similarities and differences, rotaviruses are classified with reoviruses and orbiviruses within the family reoviridae (Matthews, 1979).

Rotavirus Serotypes and Subgroups

Most rotaviruses from mammalian and avian species share a common antigen that is associated with the inner

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capsid layer (Woode et al, 1976a). Serotype specific antigens are distinct from the common antigen and are located on the outer capsid (Bridger, 1978). When rotaviruses from several mammalian species became adapted to replicate in cell culture, plaque reduction and fluorescent focus neutralization assays were developed for the antigenic comparison of different rotaviruses. These assays led to the recognition of several serotypes of rotaviruses from different mammalian and avian species (Sato et al, 1982; Murakami et al, 1983; Bohl et al, 1984; Hoshino et al, 1984).

In addition to neutralization assays, rotaviruses have been studied by enzyme-linked immunoassays, complement fixation, immune-adherence hemagglutination, and other techniques (Struker et al, 1979; Zissis and Lambert, 1980). The use of these assays led to much confusion in the literature about the serologic classification of rotaviruses because it was assumed that the above techniques gave identical results to conventional neutralization assays. It has been shown, however, that antigens detected by enzyme immunoassays, immune-adherence hemagglutination, and complement fixation are distinct from those detected by plaque reduction or fluorescent focus neutralization (Kapikian et al, 1981).

"Serotypes" as determined by neutralization tests depend on differences in polypeptides located on the

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outer capsid whereas "serotypes" as determined by enzyme-immunoassays, complement fixation, and immune-adherence hemagglutination are based on differences in the major inner capsid polypeptide (Kalica et al, 1981). To avoid confusion, Kapikian et al (1981) proposed that the term "serotype" be reserved for designation of neutralization specificity, and the term "subgroup" be used in place of serotype, to indicate differences detected by enzyme immunoassays, complement fixation, and immune-adherence hemagglutination (Kapikian et al, 1981).

A comprehensive study of the serotypic similarity and diversity of human and other mammalian and avian rotaviruses has been reported recently (Hoshino et al, 1984). Sixteen different strains of rotavirus derived from seven mammalian species and two avian species were studied by plaque reduction neutralization. Seven antigenically distinct serotypes were established on the basis of a greater than 20-fold difference between neutralizing titers of homologous and heterologous antiserum. In this study, three strains of porcine rotavirus (Gottfried, SB-1A and SB-2), and one strain of human rotavirus (St. Thomas No. 4) were of the same serotype designated type 4. Porcine rotavirus (OSU) and an equine rotavirus (H-1) made up a possible fifth serotype. The serotype 4 rotaviruses as described by Hoshino et al (1984) represent an example of shared

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serotype specificity between rotaviruses of human origin and those from other animals.

The Gottfried strain of porcine rotavirus and the OSU strain of porcine rotavirus were found to have subgroup II and subgroup I specificities, respectively. Strain SB-2 of porcine rotavirus was found to be subgroup I, whereas the Gottfried strain, as mentioned above, was subgroup II. This pair of isolates represents the first example of two rotaviruses from one animal species that are of the same serotype, but differ in subgroup specificity.

Bohl et al (1984) studied seven strains of rotaviruses isolated from intestinal contents of piglets with diarrhea. Two serotypes of porcine rotavirus were identified by plaque reduction neutralization tests, cross-protection studies on gnotobiotic piglets, and electrophoresis of rotaviral double-stranded RNA. The Ohio State University (OSU) porcine isolate was suggested as the prototype serotype 1 rotavirus. The Gottfried (G) strain porcine rotavirus was suggested as the prototype serotype 2 rotavirus. Serotypes 1 and 2 porcine rotaviruses as described by Bohl et al (1984) correspond to serotypes 5 and 4, respectively, as described by Hoshino et al (1984). The classification system of Bohl et al (1984) for porcine rotavirus strains will be used in the remainder of this dissertation.

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Rotavirus Electropherotypes

In addition to subgroup and serotype classification systems, there has been an effort to classify rotaviruses based on the mobility of their RNA segments during polyacrylamide gel electrophoresis. Lourenco et al (1981) have proposed a scheme for rotaviral electropherotype classification in which the 11 RNA segments are divided into four groups. RNA segments 1-4 are denoted as group I, bands 5 and 6 as group II, bands 7-9 as group III, and bands 10 and 11 as group IV. Differences in the relative migration of RNA segments within a group are designated by a small letter (example Ia, IIa, IIIb, IVc). This system allows rapid classification of RNA patterns when standard methods of conducting the test are employed.

The migration of RNA segments 10 and 11 of human rotavirus strains has been shown to correlate with subgroup specificity (Kalica et al, 1981). Human rotaviruses with slow migrating segments 10 and 11 belong to subgroup I, whereas those with fast migrating segments 10 and 11 belong to subgroup II. Such a correlation of subgroup specificity with RNA electropherotype does not apply to animal rotaviruses. Comparison of RNA electropherotypes has proved useful in the identification of new groups of rotaviruses that are antigenically distinct from the classical rotaviruses (Bohl et al, 1982; Bridger et al, 1982; Snodgrass et al, 1984).

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Classification Systems

A unified serological classification for rotaviruses, similar to the universal classification scheme for influenza virus, has not yet been achieved. Graham and Estes (1985) recently have proposed a serologic classification system which utilizes a cryptogram to convey the information necessary to distinguish and identify new rotavirus isolates from any source. However, this classification system may already need modification. Greenberg et al (1983) demonstrated that neutralization of a simian rotavirus is mediated by at least two gene products of rotavirus; those of gene 8 or 9 and gene 4. Rotavirus genes 8 or 9 code for the major neutralization antigen (VP7) whereas gene 4 codes for a minor neutralization antigen (VP3). Hoshino et al (1984) suggested that future systems of rotaviral classification may need to indicate two, instead of one, distinct serotypic specificities for each isolate of rotavirus.

Atypical Rotaviruses

Initially, all viruses with rotavirus morphology from different animal species were thought to possess a common group antigen or antigens located in the inner capsid layer of the virus (Woode et al, 1976a; Yolken et al, 1978). In 1980, Saif et al detected, by electron microscopy, virus particles that were morphologically indistinguishable from rotaviruses but which did not

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cross-react with antisera to porcine, bovine, or human rotaviruses or to reovirus type 3. Furthermore, previous infection of gnotobiotic pigs with the rotavirus-like particles failed to protect them from challenge with serotype 1 porcine rotavirus (strain OSU). These findings suggested the presence of a "new" rotavirus type. The pathogenesis of rotaviral diarrhea caused by this "new rotavirus" was detailed in a subsequent report (Bohl et al, 1982).

Recently, several additional atypical rotaviruses which lack the conventional rotavirus group specific antigen have been described. These viruses have been referred to as pararotaviruses (Bohl et al, 1982), rotavirus-like viruses (Rodger et al, 1982), and atypical rotaviruses (Snodgrass et al, 1984).

As these viruses were studied in greater detail, it became evident that several atypical rotaviruses were antigenically distinct from and biochemically unrelated to each other. Therefore, it became necessary to develop a classification scheme to distinguish among these viruses. Pedley et al (1983) proposed a classification scheme in which typical and atypical rotaviruses were classified in the genus as rotaviruses but further subclassified, based on the presence of distinctive group specific antigens into groups designated A, B, and C. Additional atypical rotaviruses that do not possess group specific antigen characteristic of groups A, B, or C have

been identified indicating the need for additional groups (Snodgrass et al, 1984; Vonderfecht et al, 1984; Pedley et al, 1986).

Detection of samples which are positive for rotavirus particles by electron microscopy but negative for group A rotavirus in fluorescent antibody or enzyme-linked immunoassay tests is suggestive of a nongroup A rotavirus. But further testing, using antisera which reacts with only the subgroup in question, is required to confirm the presence of atypical rotaviruses (Pedley et al, 1983).

Electrophoresis of viral RNA in polyacrylamide gel also is helpful in distinguishing rotavirus groups (Pedley et al, 1983). The major difference in the RNA electrophoretic profiles of groups A, B, and C is that group A rotavirus bands 7, 8, and 9 migrate as a triplet, whereas in groups B and C, this triplet is replaced by a doublet.

At present, the importance of atypical rotaviruses in diarrheal diseases of mammalian and avian species is unknown. It appears that the prevalence of atypical rotaviruses in cattle and swine is quite low (Chasey et al, 1984; Theil et al, 1985). Atypical rotavirus are identified more frequently than group A rotaviruses in feces from diarrheic turkey poults and lambs (Chasey and Davies, 1984; Saif et al, 1985) so they are of importance in those species.

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Physicochemical Properties

The structure of the rotavirion has been the subject of several investigations (Martin et al, 1975; Stammond and Schoub, 1977). Although there is no general agreement about the number of capsomeres, the rotavirion does have a definite capsomere structure. It is generally agreed that rotaviruses have two main particle types: single- and double-shelled (see Morphogenesis). Negatively stained double-shelled virus particles, as viewed with the transmission electron microscope, have a smooth outer layer. In contrast, the orbiviruses have a "fuzzy" outer layer, and the outer capsid of reovirus is almost featureless (Palmer et al, 1977). Double-shelled rotavirus particles are approximately 70 to 75 nm in diameter and have a buoyant density of 1.36 g/ml in cesium chloride. Single-shelled particles are approximately 65 nm in diameter with a density in cesium chloride of 1.38 g/ml (Bridger and Woode, 1976; Rodger et al, 1975).

The physicochemical properties of rotaviruses have been discussed in detail (Flewett and Wood, 1978a). Rotaviruses are stable to non-ionic detergents, lipid solvents, heat, extremes of pH and high salt concentrations. Such stability is central to the survival of rotaviruses in the intestinal tract. Simian rotavirus SA-11 is rapidly inactivated when heated at 50°C in the presence of 2 M $MgCl_2$, but is stabilized by

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heating in 2 M $MgSO_4$ (Estes et al, 1979). Calcium ions also stabilize rotavirus infectivity (Shirley et al, 1981). Chelators such as EDTA may be used to destabilize rotavirions by removing divalent cations such as calcium from the outer coat of the virus particle, thereby rendering them more permeable (Cohen, 1977).

Rotaviruses are resistant to many chemical disinfectants and antiseptics commonly used in research and chemical laboratories (Sattar et al, 1983). Ethanol (75%) and formaldehyde (3.7%) are somewhat effective in reducing the amount of virus even in the presence of organic matter, but these chemicals are impractical for widespread use in a farm environment. Snodgrass et al (1977) found a rotavirus purified from the intestinal contents of a lamb resistant to iodine-based disinfectants. Because of the resistant nature of rotaviruses, it is unlikely that disinfection under farm conditions will be completely successful.

Cultivation in Cell Culture

Rotaviruses grow extremely well in their natural habitat, the intestinal tract, but have been difficult to adapt to cell culture. The first rotaviruses cultured, SA-11 and the "O" agent, were isolated from an asymptomatic vervet monkey and slaughterhouse waste, respectively. These viruses were not recognized as rotaviruses until much later (Malherbe and Stickland-Cholmley, 1967). Interest in the cultivation

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of rotaviruses intensified when neonatal calf diarrhea virus (NCDV), a cause of bovine calf diarrhea, was successfully isolated in cell culture (Mebus et al, 1971a).

Excluding bovine rotavirus, serial propagation of these viruses in cell culture proved difficult (Woode et al, 1976b; McNulty et al, 1976a). Porcine rotavirus was successfully passaged in cell culture by treatment of viral suspensions with pancreatin prior to inoculation onto cell monolayers (Theil et al, 1977). In a subsequent paper, Theil et al (1980) reported that rotavirus infectivity for porcine kidney cells (PK-15) was enhanced by incorporation of pancreatic endopeptidases into the cell culture maintenance medium. These important findings were widely applied and led to the successful cultivation of rotaviruses derived from many mammalian and avian species (McNulty et al, 1979; Wyatt et al, 1980; Tajima et al, 1984; Makabe et al, 1985). It has not proved possible, however, to culture all rotaviruses by these methods.

Rotaviruses vary in their requirement for trypsin in the culture medium. The SA-11 and bovine rotavirus isolates (NCDV) were cultured without trypsin whereas human rotavirus has only been isolated with culture media containing trypsin (Wyatt et al, 1980; Sato et al, 1981). Feline, canine, and simian rotavirus strains were found to be less dependent upon trypsin than human, bovine,

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porcine, chicken, and turkey rotaviruses (Hoshino et al, 1981). Although trypsin treatment of bovine rotavirus is not required for the isolation of some bovine rotavirus strains, it can effectively enhance viral replication in cell culture, thus producing high titer stocks of the virus (Clark et al, 1979).

The molecular basis for the proteolytic enhancement of rotavirus infectivity has been studied (Espejo et al, 1981; Estes et al 1981). Trypsin cleaves a major non-glycosylated polypeptide of the outer capsid (VP3) yielding two polypeptides with molecular weights of approximately 60,000 and 28,000. It is proposed that the latter polypeptides contain hydrophobic regions that, once exposed, aid in viral penetration into the host cell.

Primary kidney cell cultures from a variety of species have been the cell-type most frequently used to culture rotaviruses. At present, a number of cell lines are being used successfully, including MDBK (Madin-Darby bovine kidney), PK-15 (porcine kidney), BSC-1 (green monkey kidney), LLC-MK2 (Rhesus monkey kidney), CV-1 (green monkey kidney), and MA-104 (Rhesus monkey kidney) (Wyatt and James, 1982). Primary cells appear to support virus growth more efficiently than continuous cell lines and therefore are more sensitive for rotavirus isolation (Ward et al, 1984).

Rotavirus-specific antibodies are prevalent in the

sera of domestic and laboratory animals (Sato et al, 1981), so for cultivation investigators must avoid the use of adult animal sera containing rotavirus antibody. Rotavirus antibodies have been detected in fetal bovine serum and in purified serum albumin preparations and may interfere with diagnostic assays for rotavirus antigens or antibodies (Offit et al, 1984). This finding, in part, explains why human rotavirus has been isolated only in serum-free media.

Cytopathic effect (CPE) caused by rotavirus in cell culture depends on the cell-type used, the presence of trypsin, and the strain of rotavirus. Neonatal calf diarrhea virus produces an easily discernible CPE when inoculated at high concentrations onto cell cultures (Welch and Twiehaus, 1973). Cytopathic effect is detectable by 24 hours after inoculation, and by 48 hours, some areas of the cell culture may be devoid of cells. Infected cells may appear granular and finely vacuolated, and contain single or multiple eosinophilic inclusions. Some cells become thin, elongated, and crescent-shaped and remain attached to the monolayer only by a single process. Those that completely detach are small, rounded or elongated and have pyknotic nuclei. The cytopathic effect has been produced in a wide variety of cell culture systems (Fernelius et al, 1972). Another bovine rotavirus strain produces similar CPE when inoculated onto MDBK kidney cells, except a larger

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portion of the monolayer is destroyed, and cytoplasmic inclusions are uncommon (McNulty et al, 1977).

In contrast to neonatal calf diarrhea virus, porcine rotavirus strains produce minimal to moderate CPE in cell culture unless the strain has been passaged several times in vitro. The detection of rotavirus strains that produce minimal CPE in cell culture has been improved by the development of plaque assays (Bohl et al, 1984). Plaque assays utilize pancreatin or trypsin in an overlay medium. In this system, porcine rotavirus, as well as other rotavirus strains, will produce plaques after inoculation onto MA-104 cells. By use of this technique, a plaque reduction neutralization test has been developed for assaying neutralizing antibody and has been used widely for the detection of antigenic diversity among rotavirus strains (Bohl et al, 1984; Hoshino et al, 1984).

Morphogenesis

The morphogenesis of rotavirus has been studied by electron microscopic examination of cell cultures (Chasey, 1977; Saif et al, 1978; Quan and Doane, 1983; Suzuki et al, 1984) and intestinal epithelial cells (Chasey, 1977; Pearson and McNulty, 1979). The morphogenetic stages of rotaviruses isolated from different animal species is similar both in vitro and in vivo. Because of these similarities as well as the early unsuccessful attempts to isolate human rotavirus strains,

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tissue-culture-adapted simian rotavirus SA-11 became a model system for the study of rotavirus morphogenesis (Quan and Doane, 1983).

Study of the morphogenesis of SA-11 virus in cell culture revealed the following sequence of events: (a) virions enter the cell by receptor-mediated endocytosis; (b) in some instances, the virus is taken up directly into lysosomes, but in others, the virus particles are found first in endosomes and then lysosomes; (c) as early as 6 hours after infection, immature virus can be seen in cytoplasmic aggregates of viroplasm and at the edge of viroplasm budding into rough endoplasmic reticulum; (d) these particles become enveloped during budding, but the envelope, which is not required for infectivity, is lost, thus forming a mature virion which is released by cell lysis.

Despite the many reports about rotavirus morphogenesis, some aspects concerning early stages of the replication cycle are controversial. Suzuki et al (1985) have found that the mode of viral entry into MA 104 cells is affected by trypsin. With trypsin pretreatment, viral nucleoids passed directly into the cytoplasm within 5 minutes after inoculation through dissolution of viral capsid and cell membrane. In the absence of trypsin, phagocytosis occurred in which virions were sequestered into lysosomes 20 minutes after virus attachment to cell membranes. After sequestration,

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uncoating of rotavirus virions within lysosomes was seen, but it did not result in the release of the viral genome. Therefore, Suzuki et al (1985) discounted the theory that phagocytosis is related to viral replication.

The capsid of simian rotavirus SA-11 consists of at least 5 protein classes. Three of these proteins (VP1, VP2, VP6) make up the inner layer, whereas the other two (VP3 and VP7) form the outer layer (Espejo et al, 1981; Estes et al, 1981). Immunocytochemistry studies have identified VP2 and VP6 associated with viroplasmic inclusions (Petrie et al, 1982). Therefore, it is believed that the core and inner capsid layer are assembled in the viroplasmic inclusions and that the outer shell glycoprotein is added during the budding process (Petrie et al, 1982). Tubules, fibrils, and multivesicular bodies have been associated with rotavirus morphogenesis, but their origin and function are unknown (Suzuki et al, 1984).

Multiple rotaviral particle types have been identified (Saif et al, 1978; Pearson and McNulty, 1979, Petrie et al, 1981). Double-shelled particles and single-shelled particles are released from infected cells, but only the smooth double-shelled rotavirus particles have been found to be infectious (Bridger and Woode, 1976). Two additional particle types in cytoplasmic organelles have been described by Petrie et al, 1981. The first is a subviral particle which is the

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Epidemiology

Serologic studies on the prevalence of rotavirus antibodies indicate a widespread distribution in many mammalian and avian species (Petri et al, 1978; McNulty et al, 1978; Bohl et al, 1984; Bridger and Brown, 1985). Bohl et al (1984) found that 94% of 274 porcine serum samples and 100% of 75 herds in the United States were serologically positive to the OSU strain of porcine rotavirus. A high prevalence of antibodies to rotavirus also has been found in swine in other countries (Utera et al, 1984; Bridger and Brown, 1985). The prevalence of antibody to the atypical rotaviruses (groups B and C) is common in pigs in the United Kingdom (Bridger and Brown, 1958), but Theil et al (1985) found a much lower prevalence (23%) of antibody to group B rotaviruses in Ohio swine sera. Results of serologic surveys also indicate that some serotypes of human rotavirus are more

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prevalent than others (Yolken et al, 1978). Although at least two serotypes of porcine rotavirus have been distinguished (Bohl et al, 1984), epidemiologic surveys comparing the prevalence of infection by porcine rotavirus types 1 and 2 have not been reported.

There are seasonal variations in the prevalence of many infectious diseases. Rotaviral diarrhea in people is more prevalent in the fall and winter months (Kapikian et al, 1976). Although the cause of the seasonal pattern is not known, low relative humidity in the homes has been suggested as a factor influencing rotavirus survival. Moe and Shirley (1982) found that rotaviruses in feces on environmental surfaces survive for days or weeks at high or low relative humidity, but not intermediate humidity. A seasonal increase in the prevalence of rotavirus infections in animals during the winter has been suggested (Woode and Bridger, 1975a), but this may be a reflection of seasonal variation in livestock numbers.

To assess the importance of interspecies transmission of rotaviruses and to make antigenic comparisons between rotavirus strains isolated from different species, numerous experimental cross-species infection studies have been done (Mebus et al, 1977; Tzipori et al, 1980; Bridger and Brown, 1984). Some strains of rotavirus isolated from children with diarrhea readily infected pigs (Middleton et al, 1975). Woode and

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Bridger (1975a) reported that calves were refractory to infection by a strain of human rotavirus, but other investigators successfully infected calves with a rotavirus isolated from people (Mebus et al, 1977).

Many of the investigations of cross-species infection by rotaviruses were completed prior to the recognition of multiple rotavirus serotypes. Thus, discrepancies in the literature concerning the ability of human or other animal rotaviruses to infect experimental animals may be related to the use of different serotypes in these experiments. Bridger and Brown (1984) recently have shown that the ability of bovine rotaviruses to infect pigs depends on the antigenic relationship to porcine rotaviruses. Only a bovine rotavirus (strain PP-1) that was antigenically closely related to porcine rotavirus caused disease. However, a close antigenic relationship does not imply similarities in virulence because, in the same report, another strain of bovine rotavirus (CP-1) which was indistinguishable from PP-1 by cross-neutralization tests, was not pathogenic for pigs.

Subgroup characteristics also influence the ability of rotaviruses to infect atypical host species. Zissis et al (1983) have shown that colostrum-deprived piglets are susceptible to several human-origin strains of rotavirus, except those belonging to subgroup I.

Although rotaviruses are widely distributed in almost every species, there is no evidence that under

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natural conditions, animal rotaviruses infect people. Cross-species infection is important, however, because it has enabled the development of many laboratory animal models for the study of rotavirus infections.

Simultaneous infection by more than one electropherotype of human or porcine rotavirus has been detected (Spencer et al, 1983; Rodriguez et al, 1983; Bohl et al, 1984). It has been postulated that during such "mixed" infections, genetic reasssortment, a well known property of rotaviruses (Greenberg et al, 1981) may occur. This genetic reassortment could lead to antigenic variation among rotaviruses, thus enabling their continued persistence within a population. Constant antigenic variation by influenza viruses which, like rotaviruses, have a segmented genome, is well recognized (Gething et al 1980), and evidence of "antigenic drift" among rotavirus isolates from human neonates has been reported recently (Coulson et al, 1985).

Clinical Signs

The clinical signs of rotavirus infection have been described in conventional, colostrum-deprived, and gnotobiotic animals of many species (Theil et al, 1978; McAdaragh et al, 1980; Johnson et al, 1983; McNulty et al, 1983; Gillespie et al, 1984). The first clinical signs, anorexia and reluctance to move, are seen in gnotobiotic piglets within 12-36 hours after inoculation (Crouch and Wood, 1978; Theil et al, 1978). Similar

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incubation periods have been reported in several other species including people (Yolken et al, 1978b). Anorexia is usually resolved by 72 hours after inoculation at which time piglets resume eating.

Vomiting by piglets infected with porcine rotavirus has been reported inconsistently (Theil et al, 1978; McAdaragh et al, 1980; Torres-Medina and Underdahl, 1980). Variations in the serotypes of porcine rotavirus used to experimentally infect the piglets might explain the discrepancies in reports of vomiting. For example, vomiting in people with rotaviral gastroenteritis has been reported to be influenced by the serotype or subgroup of rotavirus involved. A Type 2 human rotavirus isolate caused vomiting in 9 of 16 patients whereas Type 1 rotavirus caused vomiting in only 3 of 11 (Yolken et al, 1978b). Uhnoo and Svensson (1986) recently reported that clinical symptoms (vomiting and diarrhea) were more severe in people infected with subgroup II than subgroup I rotaviruses. However, White et al (1984) found no differences in the occurrence of fever or vomiting among children shedding different subgroups of rotavirus.

There has been no clear evidence that the gastric mucosa is infected by rotavirus (Pearson and McNulty, 1977). Apparently, injury to the small bowel mucosa is sufficient to initiate a reflex resulting in vomiting. Vomiting is usually not a feature of field cases of porcine rotavirus infection (Bohl et al, 1978) a finding

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that is sometimes useful in differentiating this disease from transmissible gastroenteritis. Fever is usually not seen in rotaviral infection in pigs (Pearson and McNulty, 1977) but has been associated with rotaviral infections in human infants and young children (Carr et al, 1976).

Rotavirus infection in gnotobiotic pigs may cause acute loss of body weight (10-40%) over the first 2-3 days followed by rapid recovery. At 5-7 days after inoculation, pigs may still be below (20-43%) their expected weight (Woode, 1979).

Mortality rates caused by porcine rotavirus are variable. When gnotobiotic piglets were inoculated with contents of the same vial of porcine rotavirus in two experiments, mortality rates varied from 0-13% and this did not correlate with the dose of virus inoculated (Woode et al, 1976b). In field cases of porcine rotavirus infection, mortality rates of 7-15% were reported (Bohl et al, 1978). Mortality rates are affected by environmental, nutritional, and other management factors. For example, Crouch and Woode (1978) found that, under natural or experimental conditions, a drop of 10 to 20°C in ambient temperature increased the mortality associated with the disease in pigs. It appears that if pigs are kept warm, dry, and well fed, mortality can be reduced.

Subclinical infections by rotavirus are common (Crouch and Woode, 1978; Banatvala et al, 1978), and the

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severity of clinical signs in experimental rotavirus infections are quite variable. This variation is, in part, age-dependent. Woode et al (1976b) noted up to 100% mortality in piglets infected at 0-4 days of age, 10% mortality at 5-7 days, and no mortality at 28 days of age. The clinical signs of rotavirus infection in mice also are age-dependent. Mice greater than 10-14 days of age do not develop clinical signs of rotavirus infection (Sheridan et al, 1983). Subclinical rotavirus infections also occur in adult swine (Benfield et al, 1982) and cattle (Crouch and Acres, 1984). Human rotaviruses, in some instances, may affect adults causing severe gastroenteritis (Echeverria et al, 1983), but in other instances only mild infections occur (Wenmann et al, 1979).

Pigs have a remarkable age-resistance to clinical disease caused by transmissible gastroenteritis infection. A number of explanations have been offered to explain this age-resistance. Cornelius et al (1968) found young pigs more vulnerable to dehydration than adults because of changes in body water content and renal electrolyte regulation with age. The colon of older, but not younger, pigs can compensate for the increased fluid loss from the small intestine by increased fluid absorption (Argenzio et al, 1984). Other explanations include the greater involvement of the upper small intestine and the slower proliferation of the intestinal

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crypts resulting in slower replacement of damaged aborptive epithelial cells in younger pigs (Moon, 1971). One or more of these explanations may apply to the age-resistance shown by rotavirus infection. The age-dependence of rotavirus infection may also reflect a difference in the number of virus-specific receptors on enterocytes of different maturity (Wolf et al, 1981). Riepenhoff-Talty et al (1982a) found fewer rotavirus receptors on enterocytes in the intestine of adult mice than in mice less than 11 days of age. However, this does not completely explain the difference in age susceptibility. Although mice greater than 14 days of age are refractory to clinical disease, they are infected, and the extent and distribution of antigen is similar to that seen in 7-day-old mice with rotaviral diarrhea (Eydelloth et al, 1984). Thus, the ability of mice to resist disease is not simply related to the ability of the virus to infect certain portions of the intestinal tract.

Rotavirus antibody is present in the serum, milk, and colostrum of most, if not all, adult sows (Corthier, 1981; Bohl et al, 1984). Thus the clinical signs in nursing piglets will be influenced by the immune status of the lactating sow, a situation similar to that found with transmissible gastroenteritis (Hooper and Haltermann, 1966). As expected, removal of nursing piglets from the influence of "lactogenic" immunity, such

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Clinical Pathology

Serum biochemical alterations associated with rotaviral diarrhea have not been studied in great detail (Mouwen et al, 1972; Tallett et al, 1977; Rodriguez et al, 1977). Rodriguez et al (1977), in a study of 72 children hospitalized with rotavirus gastroenteritis, found isotonic dehydration, compensated metabolic acidosis, and elevations in blood urea nitrogen (BUN) and the specific gravity of urine. Hypertonic dehydration also was reported, although much less commonly. In a study of 27 children hospitalized with acute diarrhea caused by rotavirus, most had mild compensated metabolic acidosis associated with decreased plasma bicarbonate (Tallet et al, 1977). Serum sodium concentration in most of these children was normal, but in 6 instances mild hypernatremia was detected. Additional alterations in serum chemistry included mildly decreased potassium, increased chloride, and elevated, normal, or reduced BUN. These laboratory findings reflect the high frequency of vomiting and dehydration associated with rotaviral illness in people.

Some of the biochemical aspects of "white scours" in piglets were investigated before rotavirus was recognized as the cause of this condition (Mouwen et al, 1972). The effect of "white scours" on serum lipids indicated

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increased neutral fat and decreased total cholesterol and phospholipid. These investigators noted that serum lipid alterations resembled those found in sprue in people.

The pathophysiology of TGE of swine is similar to, but more severe than that of rotaviral enteritis. The serum biochemical changes associated with TGE have been studied more extensively (Reber and Whitehair, 1955; Cornelius et al, 1968; Drolet et al, 1984). When 2-day-old piglets were challenged with TGE virus, those that became moribund had increased packed-cell-volumes and total proteins which were attributed to the loss of fluids associated with vomiting and dehydration (Drolet et al, 1984). Elevations in packed-cell-volume and total protein secondary to TGE have been reported by others (Cornelius et al, 1968), but are in disagreement with those reported by Reber and Whitehair (1955). Additional serum biochemical changes associated with TGE were metabolic acidosis, increased BUN, elevated chloride, hypoglycemia, and unchanged sodium and potassium (Cornelius et al, 1968; Drolet et al, 1984). Drolet et al (1984) considered hypoglycemia a major feature of TGE that may be responsible for the high rate of neonatal mortality with that disease. Piglets 5 to 6 days of age or older are refractory to hypoglycemia even if fasted from 6 to 7 days (Sampson et al, 1942). Because rotaviral diarrhea is less common than TGE in piglets younger than 7 days of age, hypoglycemia may not be as

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Pathophysiology

Rotavirus infection in pigs causes desquamation of the villous epithelial cells resulting in loss of membrane-bound enzymes such as lactose, sucrose, and sodium-potassium ATPase (Davidson et al, 1977; Graham et al, 1984). Loss of microvillus-associated enzymes is accompanied by reduced transport of 3-0-methyglucose and decreased absorption of sodium and water (Graham et al, 1984).

The segments of intestine in which altered enzyme activities are detected may, in part, be influenced by the strain of rotavirus used to infect the piglets. For example, Davidson et al (1977) found that 8- to 10-day-old piglets infected with a human rotavirus strain had decreased sodium-potassium ATPase activity in the upper jejunum. In contrast, when Graham et al (1984) infected piglets with a porcine rotavirus (OSU strain), sodium-potassium ATPase activities were depressed in the ileum only.

Loss of membrane-associated digestive enzymes leads to nutrient malabsorption. Miniature piglets infected with porcine rotavirus (OSU strain) had an increase in stool osmolarity from 248 ± 20 mOsm/liter to 348 ± 20 mOsm/liter at 75 hours after inoculation. The majority of the osmotic gap in the feces could be accounted for by lactose in the stools. Sodium concentration in the stool

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increased, but potassium stayed the same (Graham et al, 1984). Malabsorption also has been measured by a reduced uptake of D-xylose in the small intestine of rotavirus-infected children and calves (Mavromichaelis et al, 1977; Woode et al, 1978).

These studies indicate the following sequence of events in the pathophysiology of rotaviral diarrhea: (a) rotavirus destroys columnar villous epithelial cells, (b) mucosal surface area and important digestive enzymes are reduced, and (c) osmotic diarrhea occurs due to nutrient malabsorption.

Lesions and Pathogenesis

Gross Lesions

At necropsy, lesions in piglets infected with rotavirus are confined to the intestinal tract. The stomachs of experimentally-infected piglets are usually partially filled with milk regardless of differences in time between the last feeding and necropsy (Torres-Medina and Underdahl, 1980). The color of the fecal material reflects the diet and may be white, yellow, or grey. The small intestinal wall is reduced in thickness, and the cecum and colon may be distended with fluid. Visible fat in lacteals in gnotobiotic piglets infected with rotavirus is absent between 16 and 72 hours after inoculation (Theil et al, 1978). Evidence of villous atrophy in the small intestine, especially in the jejunum, can be seen by subgross examination (Pearson and

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Histopathologic Lesions

The histopathologic lesions of rotavirus infection are similar in many mammalian and avian species (Mebus et al, 1971b; Theil et al, 1978; Conner et al, 1980; Johnson et al, 1983). The lesions are characterized by shortening of small intestinal villi, desquamation of epithelial cells lining the distal portion of the villus, and exposure of the lamina propria. The latter is quickly relined by squamous to cuboidal epithelial cells with no microvillous border (Woode et al, 1976b) and with biochemical characteristics of crypt cells (Middelton, 1978).

Diarrhea in piglets precedes the development of small intestinal lesions. If small intestine is obtained within 1-3 hours after the onset of diarrhea, little villous atrophy is evident, but if greater than 12 hours after the onset of diarrhea, villous atrophy is distinct (Bohl et al, 1978). Villous atrophy is most severe 24-72 hours after infection; villi may be essentially absent in some cases (Pearson and McNulty, 1977; Theil et al, 1978; Crouch and Woode, 1978). Villous atrophy persists for 48 to 168 hours after infection (Pearson and McNulty, 1977).

There is disagreement about the stage of infection at which fusion of villi occurs. McAdaragh et al (1980) and Theil et al (1978) reported fusion of villi in the early stages of infection whereas Pearson and McNulty

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(1977) and Torres-Medina and Underdahl (1980) noticed fusion of villi only after several days.

The reported distribution of small intestinal lesions in piglets infected with porcine rotavirus is variable. Pearson and McNulty (1977) found villous atrophy at all levels of the intestine (duodenum, jejunum, and ileum). McAdaragh et al (1980) also noticed villous atrophy and fusion of villi at all levels of the small intestine. In fact, the duodenum of 2 piglets killed 36 or 72 hours after infection, respectively, had severe villous atrophy and fusion of villi. Other investigators have found lesions primarily in the middle and distal small intestine and "less strikingly" in the duodenum (Woode et al, 1976; Crouch and Woode, 1978; Torres-Medina and Underdahl, 1980; Graham et al, 1984). In one of the earliest reports of "white scours" in piglets, the distribution of lesions varied. The duodenal mucosa was most severely altered in one-third of the pigs (Mouwen et al, 1971). One-half of the pigs had lesions of greatest severity in the distal small intestine. When a bovine rotavirus strain was given to gnotobiotic piglets, lesions were most severe in the middle small intestine, but milder lesions were detected in the upper small intestine (Hall et al, 1976). Differences in age of the piglets, virus strains, and methods of sampling the intestine might explain these differences in lesion distribution.

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Glandular crypts respond to the loss of villous epithelial cells caused by rotavirus infections by increased proliferation. Crypt hyperplasia in gnotobiotic piglets occurs by 48-60 hours after infection and is maximal by 96 hours (Theil et al, 1978; McAdaragh et al, 1980). Piglets infected with the OSU strain of porcine rotavirus had crypt hyperplasia in the jejunum and mid-small intestine but not in the ileum (Graham et al, 1984). However, crypt hyperplasia in the duodenum and ileum of gnotobiotic piglets infected with a field strain of porcine rotavirus has been reported by others (McAdaragh et al, 1980). Interestingly, Johnson et al (1983) noted that crypt hyperplasia in the duodenum of gnotobiotic pups infected with canine rotavirus was not preceded by villous atrophy.

The severity and distribution of intestinal lesions is influenced by the type of infecting rotavirus. Theil et al (1985) studied the pathogeneis of a rotavirus-like virus (group B) in gnotobiotic piglets and found variable and mild histologic changes. Sloughing of villous epithelial cells occurred only at the tips of villi and villous atrophy was evident only occasionally. McNulty et al (1983) compared the clinical and virologic findings associated with infection by a group A avian rotavirus (ch 1) and a non-group A avian rotavirus (ch 132) in specific-pathogen-free chickens. There was a distinct difference between the two rotavirus isolates in viral

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antigen distribution. Rotavirus strain ch 1 (group A) had a predilection for the duodenal mucosa, whereas rotavirus strain ch 132 (non-group A) replicated best in the mid-small intestine. This was the first report of differences in intestinal tropism for different isolates of rotavirus.

Eosinophilic cytoplasmic inclusions have been observed histologically in the intestinal epithelium of mice infected with murine rotavirus (Adams and Kraft, 1967), rats infected with an atypical rotavirus (Vonderfecht et al, 1984), and in cell culture (Carpio et al, 1981a). Inclusions, in most instances, were evident only with the use of special stains. The nature of the inclusions is unclear. Adams and Kraft (1967) could not correlate inclusions seen by light microscopy with structures seen by electron microscopy.

The pathogenesis of rotavirus infections tends to be uncomplicated by lesions in other organs. Lesions in the gastric mucosa have been reported (Woode 1975b; Pearson and McNulty, 1977) but have not been associated with viral antigen (McNulty et al, 1976a; Davidson et al, 1977; Theil et al, 1978). Rodriguez et al (1980) described cecal and colonic vacuolation of superficial epithelial cells in mice infected with a murine rotavirus. Murine rotavirus has been detected in the cecum and colon of mice by immunofluorescent and electron microscopic techniques (Adams and Kraft, 1967; Narita et

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Scanning Electron Microscopy

Scanning electron microscopy (SEM) of intestine offers the advantages of tridimensional view, larger specimens, and observation of details not readily visualized by histologic examination. Despite these advantages, porcine rotavirus infections have been studied by SEM infrequently (McAdaragh et al, 1980; Torres-Medina and Underdahl, 1980). Torres-Medina and Underdahl (1980) described the sequential SEM changes in the intestinal mucosa of 6-day-old gnotobiotic piglets given a rotavirus strain (B-317) isolated from a field case of porcine rotaviral diarrhea. Lesions were first visualized at 12 hours after infection in the middle and lower small intestine. Enterocytes were swollen and occluded transverse furrows and goblet cell openings. By 36 hours after inoculation, villi were one-third the length of control villi and detachment of epithelial cells exposed lamina propria. Microvilli on degenerate epithelial cells were sparse and short. Regeneration of epithelial cells was evident by 156 hours and villi were nearly normal in dimension by 16 days after infection. There is disagreement about the time at which villous fusion occurs. McAdaragh et al (1980) observed villous fusion as early as 12 hours post-inoculation (PI), and fused villi were numerous by 72 hours. Torres-Medina and Underdahl (1980) did not observe fused villi until 4.8

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Transmission Electron Microscopy

Rotavirus particles can be seen by transmission electron microscopy (TEM) in the jejunum of gnotobiotic piglets by 12 hours after infection (Narita et al, 1982). Virions at this time are on and between microvilli and within vesicles in the apical cytoplasm and terminal web. The earliest cytopathologic alterations, detectable between 18-24 hours after infection, are cytoplasmic swelling, reduction in the number and size of microvilli, cytoplasmic lipid droplets, rough endoplasmic reticulum distended with vacuoles, and virus particles in the rough endoplasmic reticulum. By 48 hours, immature cuboidal cells with an uneven, incomplete microvillous border can be seen. No virus particles are evident in newly synthesized cells. Virus particles may or may not be visible in goblet cells (Pearson and McNulty, 1979; Narita et al, 1982).

Immunofluorescence and Location of Viral Antigens

The location and number of antigen-positive cells observed by immunofluorecence (IF) is a function of time after rotavirus infection. Maximal fluorescence in piglets is observed in the enterocytes near villous tips in the middle small intestine 24 hours after infection (Pearson and McNulty, 1977; Crouch and Woode, 1978; Bohl et al, 1978). McAdaragh et al (1980) first observed fluorescence in the duodenum and upper jejunum of

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gnotobiotic piglets 12 hours after infection. The fluorescence progressively descended through the upper ileum by 36 hours. These findings are in disagreement with findings by other investigators (Chasey and Lucas, 1977; Bohl et al, 1978; Theil et al, 1978; Torres-Medina and Underdahl, 1980). The latter investigators found infected villous epithelial cells throughout the small intestine, but most consistently in the jejunum and ileum. Duodenal fluorescence was sparse and variable or not observed. Enterocytes infected by group A rotaviruses desquamate so IF is only detected in piglets for 96 hours (McAdaragh et al, 1980) to 168 hours (Theil et al, 1978) after infection. Gnotobiotic piglets infected with a group B rotavirus had few IF cells later than 24 hours after infection (Theil et al, 1985).

Viral antigen has been detected by IF in sites other than small intestine epithelium. Immunofluorescence was seen in the lamina propria of the small intestine of piglets (Theil et al, 1978), dogs (Johnson et al, 1983), and calves (Mebus et al, 1971b), and the mesenteric lymph nodes of piglets and calves (Theil et al, 1978; Mebus et al, 1971b). Fluorescence observed in the small intestinal lamina propria of calves was not regarded as specific by Mebus et al (1971b) because many eosinophils were present. But Stair et al (1983) substantiated the presence of rotavirus in macrophages in the lamina propria by TEM. A few infected enterocytes have been

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detected in the cecum of rotavirus-infected piglets (Narita et al, 1982), the colon and rectum of mice (Banfield et al, 1968), colon of chickens (McNulty et al, 1983), and cecum and colon of calves (Reynolds et al, 1985).

Viral Shedding

Compared to IF, rotavirus can be detected in negatively stained fecal material for a longer period of time. Electron microscopic examination of intestinal content from piglets just beginning to show clinical signs contain few rotaviral particles. Viral particles are numerous by 24 hours after infection, and the greatest number of particles are evident in specimens from pigs 48 to 72 hours after infection (Lecce et al, 1976; Bohl et al, 1978). The number of rotavirus particles is reduced or they cannot be detected between 5-8 days after infection (Tzipori and Williams, 1978; Torres-Medina and Underdahl, 1980).

Immunology

Humoral Immunity

Resistance to rotavirus diarrhea is mediated by neutralizing antibodies present within the intestinal tract (Pearson and McNulty, 1977; Snodgrass and Wells, 1978; Offit and Clark, 1985). These antibodies may be secreted into the intestinal lumen following intestinal infection (Corthier and Vannier, 1983) or be obtained passively via colostrum and milk (Bridger and Brown,

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1981; Saif et al, 1984). The presence of serum rotavirus antibody does not correlate with resistance to rotavirus infection (Snodgrass and Wells, 1978; Offit and Clark, 1985).

Passive immunity against enteric pathogens occurs in suckling pigs as a result of frequent ingestion of an adequate amount of specific antibodies. Antibodies to rotavirus in swine occur in the immunoglobulin classes IgG, IgA, and IgM in mammary secretions (Hess and Bachmann, 1981). The IgG class of immunoglobulin predominates in colostrum, but the highest and longest persisting rotavirus antibody is associated with the IgA class which constitutes the predominant immunoglobulin class in the milk of pigs (Vairman et al, 1970).

The antirotaviral antibody in sows' milk protects pigs less than 7-days-old from rotavirus infection unless there are inadequate antibody titers in the milk, infrequent suckling, or dilution of antibodies by creep feed (Bohl et al, 1978). Protective IgA antirotaviral antibody is found in sows' milk until weaning at 6 to 8 weeks after parturition (panel report on the Colloquium on Selected Diarrheal Diseases of the Young, 1978). Thus an increased incidence of rotavirus diarrhea is observed at this time.

Rotavirus infection induces local production of intestinal antibody. Immune complexes appeared in the feces by 4 days after experimental inoculation of newborn

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piglets with rotavirus. Free IgA and IgM coproantibodies were demonstrated by 7 days after inoculation (Corthier and Vannier, 1983). Maximal excretion of IgA in the feces of human infants occurred 7 days after the onset of clinical signs and corresponded with clinical improvement (Stals et al, 1984). In human infants, there also is evidence of an immunologic memory because reinfection with rotavirus is associated with an intestinal IgA anamnestic response (Yamaguchi et al, 1985). The production of intestinal antibody in calves may be short lived (42-70 days), thereby allowing reinfection with the same rotavirus strain with which they were originally infected (Bachmann et al, 1983).

Cell-Mediated Immunity

The exact role of the different components of the host response to rotavirus infection is not clearly understood. Attention has been given only recently to some aspects of the cellular immune response to rotavirus infection (Riepenhoff-Talty et al, 1982b; Little et al, 1983). In Balb-c mice, virus-specific cell mediated immunity appeared in the splenic lymphocytes as early as 2 days after rotavirus infection and peaked around 10 days after infection (Riepenhoff-Talty et al, 1982). Because cell-mediated immunity (CMI) increased simultaneously with cessation of viral replication, these investigators concluded that CMI may be important in recovery from the disease.

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Little et al (1983) studied the role of T and B lymphocytes in recovery from rotaviral infections. Infant nude mice were transfused with equal numbers of B and T lymphocytes from syngenic adult mice. Animals receiving spleen cells from immunologically mature animals resolved their infections more quickly than those that did not, which suggested that T and B cells were important in the resolution of the rotavirus infection.

Rotavirus infection in athymic nude mice was studied by Eiden et al (1986). Neonatal T-cell-deficient mice experienced a self-limited gastrointestinal infection which was identical to that observed in age-matched immunocompetent mice. Also, adult T-cell-deficient seronegative mice and age-matched normal mice showed a similar extent of resistance to rotavirus infection. In both the neonatal and adult mice, the infection was resolved without the generation of antirotavirus antibody. These investigators suggested that the host defense against murine rotavirus requires neither functional T-lymphocytes nor specific antirotaviral antibody.

Cell-mediated immunity to rotavirus can be passively transferred to neonatal calves by giving colostral leukocytes from immunized heifers (Duhamel and Osburn, 1985). Although significant cell-mediated immunity is passively acquired by the calves, it does not confer protection when calves are orally challenged with live

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Immunization

The high morbidity and mortality caused by rotavirus gastroenteritis in many mammalian and avian species underscores the need for an efficacious and safe vaccine. Commercial vaccines containing bovine or porcine rotavirus strains are available, but results have been disappointing (Hoblet et al, 1984; Van Zaame et al, 1986).

Two approaches have been used to try to prevent rotavirus infections in calves. The first involves stimulation of active immunity in the intestinal tract of newborn calves by oral inoculation with attenuated modified live rotavirus vaccines. These vaccines afforded protection to gnotobiotic calves as early as 48 hours after inoculation (Mebus et al, 1973). Furthermore, use of this vaccine was reported to decrease neonatal calf morbidity and mortality in field trials (Thurber et al, 1977). However, other double-blind trials failed to substantiate these findings (Acres and Radostits, 1976).

It has been suggested that colostral rotavirus antibodies may interfere with active immunization of the newborn calf. This has been documented recently (Van Zaane et al, 1986). Oral vaccination, with modified live rotavirus, of calves fed colostrum with rotavirus antibodies did not induce a protective intestinal immune

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response. Oral vaccination did induce protective immunity in calves which were not fed colostral rotavirus antibodies, for practical purposes, it is not sensible to delay or omit colostrum feeding.

A second approach used to protect calves from rotavirus infection is immunization of the pregnant dam with subsequent passive transfer of antibodies to the calf via colostrum and milk. Parenteral immunization of cows with an adjuvated modified live rotavirus vaccine increased virus-neutralizing antibody titers in colostrum and milk, which were protective when fed to calves as a 1% supplement (Saif et al, 1983). In the same experiment, a commercially available vaccine did not increase virus-neutralizing antibody titers. Because multiple serotypes of bovine rotavirus exist (Murakami et al, 1983) and do not show cross protection, it was initially believed that for parenteral immunization of cows to be effective, a vaccine must contain all of the bovine rotavirus serotypes. However, Snodgrass et al (1984) have shown that after monovalent vaccination, cows will respond heterotypically to all rotavirus serotypes with which they were exposed. Therefore, in some instances, single serotype vaccination may be sufficient.

A modified-live TGE virus and serotype 1 porcine rotavirus vaccine to be administered to pregnant swine or to nursing piglets prior to weaning is federally licensed. There are no published controlled studies on

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the efficacy of this vaccine, but preliminary studies have shown no differences in the incidence or frequency of diarrhea, rotavirus shedding, and post-weaning weight gains between vaccinates and controls (Hoblet et al, 1984). The rotavirus strain in a commercially-available calf vaccine has been used to vaccinate swine. However, this has been shown to be ineffective because the virus does not replicate in newborn colostrum-free piglets nor does it protect them from disease (Lecce and King, 1979).

Rotaviruses are now considered one of the major causes of gastroenteritis in children younger than two years of age (Flewett and Woode, 1978). Thus, an urgent need for an effective and safe vaccine for use in people has been recognized. A number of approaches to vaccine development are being studied. Use of a bovine rotavirus strain as a potential vaccine candidate for people is under active investigation (Zissis et al, 1983, Wyatt et al, 1979). Vesikari et al (1983) have shown that oral administration of a bovine rotavirus (NCDV strain) induces resistance in infants and young children against moderate to severe diarrheal illness caused by human rotaviruses.

Another approach to vaccine development takes advantage of the propensity of rotaviruses to undergo genetic reassortment (Greenberg et al, 1981). Co-infection of cell cultures with animal rotaviruses and non-cultivatable human rotaviruses has yielded

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reassortants which contain the gene segment coding for the major neutralization protein from the human rotavirus and the ability to replicate in vitro from the animal rotavirus (Midthun et al, 1985). Reassortants of this type represent potential live vaccine strains.

Diagnosis

Enteric disease caused by rotavirus and several other enteropathogens is common in nursing and weanling pigs. Although history, clinical, and necropsy findings assist in obtaining an accurate diagnosis, they alone are insufficient to distinguish among these diseases. A definitive diagnosis of rotavirus infection depends on the demonstration of rotaviral antigen in specimens from diarrheic animals and correlation of these findings with the presence of histopathologic lesions.

Many useful techniques for rotavirus detection have been described, and the details of the relative sensitivities, specificities, and advantages of each test often have been provided (Yolken et al, 1978a; Yolken and Stopa, 1979; Benfield et al, 1984; Reynolds et al, 1984). The laboratory tests that have been most widely compared are enzyme-linked immunosorbent assays (ELISA), electron microscopy (EM), and immunofluorescence (IF).

Enzyme-linked Immunosorbent Assays

Enzyme-linked immunosorbent assays are systems that are similar in design to radioimmunoassays, but which utilize an enzyme rather than radioactive isotope as the

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immunoglobulin marker (Engvall and Perlmann, 1972). This enzyme, when bound to the "solid phase" in a series of antigen-antibody reactions, interacts with added substrate to produce a product. The product, usually a color, can be detected visually or with the aid of a colorimeter. The sensitivity of these assays is determined by the lowest concentration of visible color that can be detected. Yolken and Stopa (1979) described a modification of a rotavirus ELISA in which a substrate was used that yielded a fluorescent product. This modification improved the sensitivity of human rotavirus detection by greater than 100 times compared to standard ELISA or radioimmunoassay techniques.

The sensitivity of ELISA has been compared to other rotavirus detection methods. In most instances, ELISA were more sensitive than EM (Rubenstein and Mille, 1982; Reynolds et al, 1984). For example, comparison of several commercially-available ELISA indicated that one of the ELISA was more sensitive (98%) than immunoelectron microscopy (93%), IF (86%), and EM (84%) (Mounet et al, 1984).

Commercially-available ELISA correlate well with EM for the detection of bovine rotavirus (Benfield et al, 1984; Reynolds et al, 1984). Reynolds et al (1984) compared EM to enzyme-linked immunosorbent assay for the detection of rotavirus and coronavirus in bovine feces. Results showed excellent correlation with the detection

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of rotavirus by EM in feces from experimentally infected calves as well as from diagnostic laboratory specimens. Benfield et al (1984) found a commercial enzyme-linked immunosorbent assay as sensitive as EM but more sensitive than IF or viral isolation for detecting bovine rotavirus. However, EM was more sensitive than IF, enzyme-linked immunosorbent assay, or viral isolation for detecting porcine rotavirus. The commercially-available enzyme-linked immunosorbent assay used by Benfield et al is not sensitive for the detection of porcine rotaviruses, so it should be used in combination with other laboratory procedures.

An advantage of ELISA when compared to EM is that viral antigen need not be assembled into viral particles for detection. This is useful because Mathan et al (1977) have shown that much of the rotavirus antigen in feces is not assembled into viral particles. Disadvantages of some ELISA are non-specificity (Chrystie et al, 1983) and in some instances, inability to detect viral antigen-antibody complexes which commonly occur in feces of naturally infected animals (Crouch and Acres, 1984).

Electron Microscopy

Because many rotaviruses associated with diarrhea in animals have been difficult to adapt to cell culture, EM remains the primary method for their detection. Flewett (1978b) reported that, with skill and perseverance, EM

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will detect 10^5 viral particles/ml, 10^6 particles/ml should not be missed, and 10^8 particles/ml will be rapidly diagnosed. These sensitivities can be greatly improved, however, by the aggregation of rotavirus particles with antisera prior to EM (Saif et al, 1977). This technique is termed immunoelectron microscopy (IEM). The sensitivity of EM also can be greatly enhanced by the use of solid-phase immunoelectron microscopy. This technique utilizes protein coated grids treated with antirotavirus antibody. Solid phase immunoelectron microscopy has been reported 30 times more sensitive than direct EM and 10 times more sensitive than ELISA (Yolken et al, 1979).

Immunofluorescence

All group A rotaviruses possess a common antigen that can be detected by IF, IEM, ELISA, gel diffusion, and complement fixation (Woode et al, 1976a). Thus, group specific antigen and antisera prepared from any of the group A rotaviruses can be used for the diagnosis of group A rotavirus infections in any species. Detection of non-group A rotaviruses requires antisera specific for the common antigen of each group.

Immunofluorescence techniques which utilize antisera against the common antigen of group A porcine rotaviruses have been described (Theil et al, 1978). In these reports, it has been demonstrated that rotaviral antigen in intestinal smears or frozen sections can

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usually only be detected for 72-96 hours after infection. Thus, in field cases of rotaviral diarrhea, specimens must be selected from piglets in the early stages of infection if IF tests are to be successful. Additional disadvantages of IF procedures are subjective interpretation, interference by non-specific background fluorescence, and interference by post mortem decomposition.

Other Diagnostic Techniques

Other techniques for rotavirus detection have been described (Middleton et al, 1974; Middleton et al,1976; Woode and Bohl, 1981). One of these, the inoculation of gnotobiotic piglets, has been utilized to demonstrate the presence of rotavirus when other techniques have proved negative. Inoculation of the gnotobiotic piglet may be the most sensitive means of demonstrating the presence of rotavirus (Woode and Bohl, 1981).

Treatment and Control

At present, there are no practical methods for preventing rotavirus infection; commercially available vaccines have not proved efficacious (Hoblet et al, 1984; Van Zaane et al, 1986). Management factors, therefore, have assumed an important role in moderating the severity of these infections.

Thorough cleaning and disinfection of the farrowing and nursery units and the use of "all-in-all-out" management systems will reduce the amount of virus in the

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piglets' environment. Reduction of the quantity of virus to which piglets are exposed may reduce the severity and delay the onset of rotaviral diarrhea (Lecce and King, 1980). Rotaviruses are extremely resistant to environmental conditions and disinfectants, so they cannot be completely eliminated from the farm environment (Snodgrass et al, 1977; Sattar et al, 1983).

Under natural and experimental conditions, a drop in ambient temperature has been shown to increase mortality associated with rotaviral infection in piglets (Crouch and Woode, 1978). Meeting ambient temperature needs of piglets, especially preventing chilling of neonates and piglets with diarrhea, assists in controlling losses caused by rotavirus.

Resistance to rotaviral diarrhea is mediated by neutralizing antibodies present within the intestinal tract (Snodgrass and Wells, 1978; Offit and Clark, 1985). Most sows are positive for group A rotavirus serum antibodies and will transfer variable amounts of passive immunity to their piglets via colostrum and milk (Hess and Bachmann, 1981). Passive protection obtained in this way is important because a low infectious dose of rotavirus in the presence of protective milk-secreted antibody may result in immunity without disease (Woode and Bohl, 1981). If piglets receive rotavirus antibodies at birth, the onset of infection will be delayed, and the severity of diarrhea and mortality caused by rotavirus

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will be reduced. This is important because rotavirus infections are most severe in piglets less than 2-weeks-old (Lecce et al, 1976).

In general, rotaviral diarrhea of pigs can best be controlled by avoiding environments that are heavily contaminated with rotavirus and by ensuring that piglets receive an adequate amount of colostrum and are kept warm, dry, and well fed.

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MATERIALS AND METHODS

Gnotobiotic Pigs

Sixty-three pigs were obtained from 7 sows by closed hysterotomy and maintained in stainless steel isolators as described previously (Miniats and Joel, 1978). Control pigs were kept in isolators separate from inoculated pigs. Isolators containing these gnotobiotic pigs were kept in a room with an ambient temperature of 25-30°C. The pigs were fed 50 ml of SPF-lac (Borden, New York, NY) three times (morning, afternoon, and evening) on the first day. The volume of milk replacer was increased by 13.5 ml/feeding daily as recommended by Miniats and Joel (1978). Pigs were fasted 3 to 6 hours before necropsy.

Immediately prior to inoculation, fecal and oral swabs from 1 pig in each isolator and a composite swab of residual milk in feed pans were collected. Ileum from each pig was collected at necropsy. Swabs and ilea were cultured for bacteria by use of sheep blood, tergitol-seven, and brilliant-green agars incubated in aerobic and anaerobic atmospheres. Evidence of bacterial contamination also was obtained by histopathologic examination of intestine.

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Microorganisms were not detected in any isolators before inoculation of the pigs. Bacillus sp. was cultured from the ileum of 1 pig 3 days after inoculation with the SDSU strain of porcine rotavirus. A few enteric organisms were cultured from 1 pig 7 days after inoculation with the OSU strain of porcine rotavirus. Bacteria were seen by histologic examination in the large intestine of 2 pigs necropsied 1 day after inoculation with rotavirus (1 pig--OSU; 1 pig--SDSU) and 2 pigs necropsied 3 days after inoculation with the OSU strain. Viability of the observed bacteria was not demonstrated by aerobic and anaerobic culture.

Virus

Porcine rotavirus designated as the Ohio State University (OSU) isolate was obtained from Dr. Linda Saif (Ohio Agricultural Research and Development Center (OARDC), Wooster, OH). The OSU strain of porcine rotavirus has serotype 1 and subgroup I specificities (Bohl et al, 1984; Hoshino et al, 1984). Additional morphologic and antigenic properties of this rotavirus strain have been described (Saif et al, 1977; Theil et al, 1978). The other porcine rotavirus was from an intestinal homogenate of a pig with diarrhea. This porcine rotavirus was designated as the South Dakota State University (SDSU) isolate and was obtained from Dr. David Benfield (South Dakota State University, Brookings, SD). The SDSU strain of porcine rotavirus has not been

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isolated in cell culture. Antisera against the SDSU strain neutralized a serotype 2 porcine rotavirus (Gottfried strain) at a titer of greater than 1:1024 (personal communication: Dr. Linda Saif, OARDC, Wooster, OH), so this strain was considered a serotype 2 porcine rotavirus. Each strain of virus was serially passaged 4 times in gnotobiotic pigs; diarrhea was observed in inoculated pigs at each passage. At the fourth gnotobiotic pig passage, the intestinal tract and contents from 2 pigs given the OSU or SDSU isolates, respectively, were aseptically collected 48 hours after inoculation. To avoid cross-contamination, intestine was collected and processed on different days and in different locations. A 50% suspension of intestinal tract and contents in Hanks' balanced salt solution was prepared by homogenization in a blender (Waring Blender, New York, NY). The homogenate was frozen and thawed twice to rupture infected epithelial cells, thereby increasing the quantity of released virus. The intestinal homogenate was centrifuged 20 minutes at 10,000 x g. Aliquots (10 ml) of supernatant were pipetted into sterile plastic tubes and refrigerated at -70°C. As needed, supernatant was thawed and sterilized by filtration through a series of Millipore filters (Millipore Corp., Bedford, MA) with average pore diameter (APD) of 0.65 µm, 0.45 µm, and 0.22 µm. Sterility of the filtrate was confirmed by bacterial culture as described

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above. Negatively stained specimens of inocula were examined by EM. Filtrates of the SDSU and OSU isolates contained +4 (see Electron Microscopic Examination of Feces) rotavirus particles. No contaminating organisms were observed.

Virus Infectivity Titrations

The SDSU strain of porcine rotavirus has not been adapted to cell culture, so an in vitro method was not available to determine accurately the number of infective virus particles in the viral pools. Therefore, the SDSU and OSU virus pools were titrated for infectivity in gnotobiotic pigs.

Two litters, one containing 11 and the other 12 pigs, were used for the titration of the OSU and SDSU virus pools, respectively. Various dilutions (10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}) of the SDSU and OSU virus pools were prepared in Hanks' balanced salt solution. Groups of pigs inoculated with different dilutions and different viruses were housed in separate isolators. Groups of 3 pigs (except the group given the 10^{-5} dilution of the OSU rotavirus, which contained 2 pigs) were inoculated orally with 1 ml of either the diluted SDSU or OSU rotavirus pools. Pigs were examined three times daily for clinical signs of diarrhea. Feces were collected for electron microscopic examination 48 hours after inoculation. Piglets that became diarrheic and had rotavirus particles in their feces at 48 hours after inoculation were

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considered infected. The dilution of the virus pool that caused diarrhea in 50% of the piglets was defined as one pig-infectious-dose fifty (PID_{50}). The calculated PID_{50} for the SDSU and OSU rotavirus pools were $10^{-7.2}$ and $10^{-5.8}$, respectively.

Experimental Design

Forty gnotobiotic pigs were assigned to treatment groups by use of a random number table. At 3 days of age, 28 pigs were each inoculated orally with 1 ml containing 10^5 PID_{50} of either the OSU strain of porcine rotavirus (14 pigs) or the SDSU strain of porcine rotavirus (14 pigs). Immediately after inoculation, each pig was fed SPF-lac to which an additional 1 ml of the appropriate viral inoculum had been added. Controls (12 pigs) were inoculated with filtered Hanks' balanced salt solution in the same manner as the principals. Five pigs in each of the virus-inoculated groups and four control pigs were killed at 24 and 168 hours after inoculation. Four pigs in each of the three experimental groups (OSU, SDSU, and control) were killed at 72 hours after inoculation.

Clinical Signs and Body Weight

Control and infected pigs were observed for clinical signs 3 times daily at the time of feeding. Body weight was measured prior to inoculation and at 24 hour intervals thereafter. Body weights were measured by suspending pigs in a sling attached to a spring scale

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(Ohaus, Florham Park, NJ) with accuracy to ±20 gms. Pigs were fed after body weights were recorded.

Clinical Pathology

Blood collected at necropsy was dispensed into vacutainer tubes (Becton-Dikinson, Rutherford, NJ) which for the collection of serum contained no anti-coagulant and for the collection of whole blood contained ethylenediamine-tetracetic acid (EDTA).
Packed-cell-volume of unclotted blood was measured by the microhematocrit method (Benjamin, 1978). Serum chemistry profiles were measured by the Laboratory of Clinical Medicine (Sioux Falls, SD). Technicon SRA 2000 and reagents for the "basic panel" from Technicon (Technicon Industrial Systems, Tarrytown, NY) were used.

Necropsy

Pigs were bled by cardiac puncture and then killed by an intracardiac injection of a solution containing barbiturates. Segments (3 cm in length) from 6 sites in the small intestine were removed immediately for light microscopy (LM), fluorescent antibody testing (FAT), and scanning electron microscopy (SEM). The first segment was removed at the duodenal-jejunal flexure (DU) and the last portion was removed from the lower ileum (LI) approximately 5 cm proximal to the ileocecal junction. The remaining segments were removed at approximately 1/6, 1/3, 1/2, and 2/3 the distance from the duodenal-jejunal flexure to the ileocecal junction and were designated as

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the upper jejunum (UJ), mid-portion of the small intestine (MI), lower jejunum (LJ), and upper ileum (UI), respectively. The lumen of each section of intestine for LM and SEM was rinsed with 10% neutral buffered formalin. A small portion (0.5 cm^2) of each segment of small intestine, cecum, and colon was removed and immersed in Karnovsky's fixative at pH 7.4 (Karovsky, 1965) for SEM and refrigerated at 4°C. The remaining small intestine was incised longitudinally along the mesenteric attachments, pinned to rigid sections of cardboard for straight flat fixation, and immersed in neutral buffered formalin. Sections of unfixed stomach, cecum, colon, rectum, and mesenteric lymph node were collected for FAT. In addition to these tissues, lung, liver, kidney, brain, heart, thymus, pancreas, and spleen were immersed in neutral buffered formalin for LM.

Preparation of Fluorescein-Conjugated Gamma Globulin

Gnotobiotic pig hyperimmune serum to the SDSU strain of porcine rotavirus (serotype 1) and rabbit hyperimmune serum to the OSU strain of porcine rotavirus (serotype 2) were kindly supplied by Dr. David Benfield (South Dakota State University, Brookings, SD). Hyperimmune serum was stored at -70°C until used. Fluorescein-conjugated gamma-globulins were prepared from hyperimmune serum by a modification of procedures developed by Dr. I. C. Pan (Plum Island Research Laboratories, Plum Island, NY). Unless noted, all procedures were done at room

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temperature. Serum was dispensed into an Erlenmyer flask. The gamma-globulin was precipitated by the addition of an equal volume of saturated ammonium sulfate solution dropwise while stirring with a mechanical magnetic stirrer. After all the ammonium sulfate had been added, the mixture was stirred for 20 minutes. Precipitated gamma-globulin was then centrifuged at 1,900 x g for 30 minutes at 4°C. After the supernatant was decanted, the pellet was resuspended in one-half the original volume of ammonium sulfate and centrifuged as before. This step was repeated (usually 3 times) until the last pellet was completely white. The pellet was resuspended in the smallest amount possible of 0.01 M Tris-hydrochloric acid buffer (HCl) (pH 9.0). The dissolved pellet was centrifuged at 1,100 x g for 30 minutes at 4°C. To remove residual ammonium sulfate, the supernatant was dispensed into Spectrapor membrane tubing (Spectan Medical Industries, Inc., Los Angeles, CA) and dialyzed overnight at 4°C against a large volume of 0.01 M Tris-HCl in a beaker with a magnetic stirrer. The total protein of the concentrated gamma-globulin was determined by the biuret method (Sigma Diagnostics, St. Louis, MO). Sufficient isomer I of fluorescein isothiocyanate (FITC) (Sigma Chemical Co., St. Louis, MO) was added to the gamma globulin in a 50 ml beaker to give a fluorochrome-to-protein ratio by weight of 1:100. The mixture was slowly (to prevent denaturation of the

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globulin) stirred overnight by a mechanical stirrer in a refrigerator at 4°C. Unconjugated FITC was removed from the mixture by gel filtration through a glass column (60 cm in height x 5 cm in diameter) packed with Sephadex-25 (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) equilibrated with 0.1 M phosphate buffered saline (PBS) (pH 7.3).

Because of non-specific fluorescence, the conjugate was adsorbed with liver powder (Sigma Chemical Co., St. Louis, MO). The conjugate was adjusted with 0.1 M PBS (pH 7.3) to a concentration of 3.3 mg of protein/ml. Ten mg of liver powder per mg of conjugate protein were mixed and allowed to stand 1 hour with occasional stirring. Tissue powder was removed by centrifugation for 30 minutes at 20,000 x g. This process was repeated one time and the conjugate was then filtered through a 0.45 µm APD Millipore filter. Small aliquots of conjugate in plastic vials were stored at -20°C until used. Various dilutions of conjugate in 0.1 M PBS were tested in staining trials to determine the appropriate conjugate dilution for FAT.

Examination by Immunofluorescence

Specimens for fluorescent antibody testing were placed in embedding medium (Miles Laboratories, Inc., Naperville, IL), and quick frozen at -70°C. Frozen tissues were cut 8-10 µm thick with a freezing microtome (International Equipment Company, Needham Heights, MA)

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and then two sections were placed on one glass slide. After fixation in acetone for 10 minutes, one of these tissue sections was stained with fluorescein-conjugated anti-rotavirus (SDSU strain) globulin and the other section was stained with fluorescein-conjugated anti-rotavirus (OSU strain) globulin. After the stains were applied, the slides were placed in a humid chamber for 30 minutes at 37°C. Then slides were washed for 5 minutes in each of 2 rinses of 0.1 M PBS and then for 1 minute in distilled water. Slides were air-dried and mounting medium pH 9 (Difco Laboratories, Detroit, MI) and coverslips were applied. Stained preparations were examined by immunofluorescence microscopy using a Zeiss immunofluorescent microscope with epifluorescent illumination and a Xenon light source (Carl Zeiss, West Germany). At least 4 sections of small intestine per site were evaluated. Specimens with intense fine to coarsely granular cytoplasmic fluorescence when compared to control tissues were considered positive (+) for rotavirus antigen. Tissues from uninfected pigs served as the controls.

Light Microscopy

Formalin-fixed tissues were embedded in paraffin, sectioned 6 μm thick, and stained with hematoxylin and eosin using standard histologic techniques (Thompson and Hunt, 1966). Histopathologic changes in tissues were evaluated with the use of a light microscope. In

addition, small intestine was examined using a calibrated ocular micrometer to determine crypt depth and villous height (Moon et al, 1975). Five, well-oriented villi and crypts were measured from each segment of small intestine. Because submucosal lymphoid nodules affect mucosal architecture (Mouwen et al, 1971), no attempt was made to measure villi or crypts in these areas. Additional changes in intestinal segments were subjectively graded on a negative (-) to plus three (+3) scale where (-) represented no change from the control and a (+3) represented the greatest change. Squamous, cuboidal, and low columnar epithelial cells were considered immature. Cytoplasmic vacuolation was scored by the number of vacuolated epithelial cells in each segment of intestine. Vacuolation ranged from a negative (-) which represented no vacuolation to a plus four (+4) which equaled vacuolation of approximately 100% of the villous epithelial cells.

Scanning Electron Microscopy

Tissues for SEM were refrigerated at 4°C until processed for SEM (usually within 24-48 hours). All additional processing was done at room temperature unless otherwise specified. Duodenum, UJ, and LI from 3 principal pigs and 1 control pig per treatment group at each time period were rinsed in each of 3 changes of 0.2 M phosphate buffer and washed for 8 hours in each of 3 changes of double-distilled water. Tissue was post-fixed

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in 1% osmium tetroxide for 30 minutes. The above rinse, wash, and post-fixation steps were repeated one additional time.

Tissue staining with thiocarbohydrazide (Polysciences, Inc., Warrington, PA) and osmium tetroxide were done using procedures described previously (Malick and Wilson, 1975) with the following modifications: the thiocarbohydrazide was a saturated solution; each treatment with osmium tetroxide was for 30 minutes; and tissue was washed in 3 changes of double-distilled water over 30 minutes.

Tissue dehydration was done by reported methods (Maser and Trimble, 1977) except that only 2 ml of acidified dimethoxypropane (DMP) (1 drop of concentrated HCl/50.0 ml of DMP) was applied to each tissue section for 15 minutes. The DMP was removed and each of 3 changes of 100% ethanol was applied for 15 minutes. The dehydrated ethanol treated specimens were placed in 100% ethanol in a critical point drying apparatus (Denton Vacuum, Inc., Cherry Hill, NJ) and were dried in liquid carbon dioxide. Dried specimens were attached with silver conducting paint (Acme Chemicals and Insulation Co., New Haven, CT) to aluminum SEM stubs and were examined using a Super IIIA scanning electron microscope (International Scientific Instruments, Korea) at an accelerating voltage of 15kv and at various magnifications and working distances. Images were

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Electron Microscopic Examination of Feces

Fecal specimens were collected from each pig prior to inoculation and from the remaining pigs in each isolator 24, 72, and 168 hours after inoculation. Sterile plastic tubes (12 x 75 mm) were used to collect fecal samples, or if pigs were not scouring, sterile swabs were used. Cecal contents were collected from each control and infected pig at necropsy. Specimens were stored in a freezer (Revco, Inc., Deerfield, MI), at -70°C until processed.

Each specimen was thawed at room temperature, suspended in Hanks' balanced salt solution, and clarified by centrifugation at 3,000 x g for 15 minutes. The pellet was discarded and the supernatant was centrifuged at 40,000 x g for 30 minutes. The supernatant was discarded and the pellet was suspended in 0.5 ml of distilled water.

A negative-staining procedure as described by Ritchie and Fernelius (1968) was used. Briefly, 20 drops of distilled water, 2 drops of 4% phosphotungstic acid (PTA) pH 6.2-6.8, 1 drop of 0.1% bovine serum albumin (BSA), and 1 drop of the fecal suspension were mixed in a depression dish with a Pasteur pipette. The mixture was applied with an all-glass nebulizer (Ted Pella, Inc., Tustin, CA) to nitrocellulose coated 200 mesh grids.

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Specimens were examined immediately with an Hitachi HU-12A transmission electron microscope (Hitachi, Tokyo, Japan) at an accelerating voltage of 75kv. If needed, the PTA, BSA, or sample concentrations were adjusted to optimize spreading and contrast.

The density of viral particles was estimated by criteria described by Benfield et al (1984).

Statistical Analysis

Body weights, villous heights, and crypt depths were analyzed statistically by using the one-way analysis of variance. Differences between group means were analyzed by using the Waller-Duncan K-ratio T-test. Differences from control values were considered significant at the level of $p<0.05$. (The statistical analysis was done by Dr. William Tucker, Agricultural Experiment Station, South Dakota State University, Brookings, SD).

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RESULTS

Clinical Signs

All pigs in the control group and pigs inoculated with the OSU or SDSU strains survived. Diarrhea was observed in 10/14 pigs 19-36 hours PI with the OSU strain. Four pigs inoculated with the OSU strain did not develop diarrhea until 44-48 hours PI. Pigs inoculated with the SDSU strain had incubation periods of varying lengths before the onset of diarrhea. Diarrhea was observed in 11/14 pigs 24 to 54 hours PI with the SDSU strain. Three pigs inoculated with the SDSU strain did not have diarrhea when killed at 24 hours PI.

The appearance of feces from pigs inoculated with the SDSU or OSU strains were similar. Initially, pigs with diarrhea had watery yellow feces. Curds of undigested milk in the feces were first observed 48-96 hours PI. Diarrhea with curds of undigested milk persisted until pigs were killed 168 hours PI. Pigs inoculated with the OSU strain in most instances became reluctant to drink milk shortly before the onset of diarrhea, and milk remained in the feeding bowls at the time of the next feeding. The pigs infected with the OSU strain remained anorectic until 48-96 hours PI. Vomiting

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was observed in 5 of 14 (36%) of the pigs infected with the OSU strain, whereas pigs inoculated with the SDSU strain did not vomit.

Control pigs remained active and alert throughout the experiment and rapidly consumed their entire ration after each feeding. Control pigs had pasty golden feces characteristic of milk-fed gnotobiotic pigs.

Body Weight

Pigs inoculated with the OSU or SDSU strains of rotavirus had lower body weights and reduced weight gains compared to the controls (Table 1). At 168 hours PI, pigs infected with the OSU or SDSU strains and control pigs had weight gains of 16, 22, and 46%, respectively. The rotavirus-infected pigs had highly variable weight gains irrespective of the strain of rotavirus used as the inoculum. These differences in weight gain are reflected by the high standard deviations (Table 1). For example, by 144 hours PI, a pig inoculated with the OSU strain had a weight loss of 25% whereas another pig inoculated with the same virus had a weight gain of 21%. Similarly, a pig inoculated with the SDSU strain had a weight loss of 15% whereas another pig inoculated with the same pool of inoculum and kept in the same isolator had a weight gain of 27%. Because of this extreme pig to pig variation, body weights of virus infected pigs were not statistically different ($p<0.05$) from controls.

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Table 1. Mean body weights (g ± SD) for gnotobiotic pigs infected with the OSU or SDSU strains of porcine rotavirus

Treatment group	Hours after inoculation							
	0	24	48	72	96	120	144	168
Control	1254[a] ±142	1295 ±142	1456 ±158	1520 ±143	1620 ±127	1652 ±153	1741 ±160	1832 ±138
OSU	1320 ±222	1323 ±255	1386 ±177	1363 ±232	1362 ±248	1386 ±265	1432 ±292	1526 ±311
SDSU	1257 ±224	1316 ±227	1309 ±220	1346 ±228	1378 ±88	1412 ±99	1464 ±112	1534 ±126

[a] Body weights of control, OSU, and SDSU treatment groups were not significantly different ($p<0.05$).

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Clinical Pathology

Hematocrit values varied among pigs because some pigs had excessive umbilical cord bleeding after delivery. Therefore, hematocrit measurements were not useful as an index of hemoconcentration. Pigs inoculated with the OSU or SDSU strains had similar changes in serum chemistry values (Tables 2, 3, and 4). The total serum protein and BUN values were greater in rotavirus inoculated pigs than in controls. The elevations in total protein and BUN were greatest at 168 hours PI. At 24, 72, and 168 hours PI, serum triglycerides were reduced in virus infected pigs when compared to controls. However, results of serum triglyceride measurements at 168 hours PI were not meaningful because sera from 3 controls, 3 pigs inoculated with the OSU strain, and 2 pigs inoculated with the SDSU strain were lipemic at this time. Lipemia, which indicates insufficient fasting before the collection of serum, may have interfered with serum chemistry determinations. Sodium, potassium, chloride, cholesterol, glucose, and creatinine values for control and rotavirus infected pigs were similar.

Gross Lesions

The external appearance of virus-inoculated pigs varied and was independent of the strain of rotavirus used as the inoculum. Some pigs were thin and dehydrated (as judged by decreased skin pliability and prominence of bony processes) and had rough hair coats whereas others

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Table 2. Serum chemistry and hematocrit values for gnotobiotic pigs 24 hours after inoculation with the OSU or SDSU strains of porcine rotavirus

Parameter	Units	Treatment group		
		Control	OSU	SDSU
Hematocrit	%	29.0 ± 1.2	29.8 ± 0.2	26.4 ± 0.5
Total protein	gm/dl	2.5 ± 0.3	2.6 ± 0.1	2.6 ± 0.2
Glucose	mg/dl	97 ± 14	110 ± 5	83 ± 0
BUN	mg/dl	7.8 ± 0.3	9.4 ± 4.9	18.6 ± 14.1
Creatinine	mg/dl	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Sodium	meq/l	149 ± 0.6	148 ± 3.4	151 ± 4.3
Potassium	meq/l	5.4 ± 0.2	5.8 ± 0.6	5.6 ± 0.7
Chloride	meq/l	108 ± 2.3	108 ± 1.1	108 ± 4.0
Cholesterol	mg/dl	149 ± 33	136 ± 14	180 ± 8
Triglyceride	mg/dl	90 ± 2	34 ± 17	51 ± 18

Data are expressed as the mean ± SD.

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Table 3. Serum chemistry and hematocrit values for gnotobiotic pigs 72 hours after inoculation with the OSU or SDSU strains of porcine rotavirus

Parameter	Units	Treatment group		
		Control	OSU	SDSU
Hematocrit	%	27.0 ± 2.3	28.3 ± 0.4	25.3 ± 2.0
Total protein	gm/dl	2.8 ± 0.3	3.0 ± 0.5	2.9 ± 0.2
Glucose	mg/dl	116 ± 26	103 ± 4	110 ± 17
BUN	mg/dl	7.5 ± 0.6	27.0 ± 18.5	26.3 ± 28.6
Creatinine	mg/dl	1.1 ± 0.1	0.9 ± 0.2	1.1 ± 0.3
Sodium	meq/l	154 ± 9.2	153 ± 10.7	154 ± 2.7
Potassium	meq/l	6.1 ± 0.8	6.5 ± 0.7	5.7 ± 0.1
Chloride	meq/l	118 ± 10.7	116 ± 8.4	116 ± 5.8
Cholesterol	mg/dl	157 ± 15	137 ± 21	113 ± 12
Triglyceride	mg/dl	89 ± 17	46 ± 34	48 ± 19

Data are expressed as the mean ± SD.

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Table 4. Serum chemistry and hematocrit values for gnotobiotic pigs 168 hours after inoculation with the OSU or SDSU strains of porcine rotavirus

Parameter	Units	Treatment group		
		Control	OSU	SDSU
Hematocrit	%	32.0 ± 4.6	28.4 ± 2.9	27.0 ± 0.0
Total protein	gm/dl	2.7 ± 0.3	3.3 ± 0.4	3.3 ± 0.0
Glucose	mg/dl	151 ± 8	119 ± 8	105 ± 24
BUN	mg/dl	5.0 ± 0.0	37.2 ± 30.4	31.6 ± 22.8
Creatinine	mg/dl	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.0
Sodium	meq/l	148 ± 2.3	154 ± 12.1	150 ± 9.6
Potassium	meq/l	5.8 ± 0.0	5.2 ± 0.2	5.1 ± 0.3
Chloride	meq/l	111 ± 4.0	119 ± 12.8	114 ± 10.1
Cholesterol	mg/dl	143 ± 25	157 ± 26	128 ± 51
Triglyceride	mg/dl	203 ± 13	120 ± 92	62 ± 25

Data are expressed as the mean ± SD.

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appeared healthy except for the presence of diarrhea and fecal staining on the perineum.

Internally, gross changes were similar in pigs infected with either the OSU or SDSU strain of rotavirus. The stomachs of virus-infected pigs contained various amounts of clotted milk. Ceca and colons were distended with watery yellow contents which contained small clots of incompletely digested milk in pigs killed at 72 and 168 hours PI. The small intestine in pigs with diarrhea was pale, flaccid, and thin-walled at 24, 72, and 168 hours PI. The intestines of two pigs inoculated with the SDSU strain which did not have diarrhea at 24 hours PI were normal grossly.

The majority of virus-infected pigs did not have chyle in mesenteric lymphatics 24 hours after inoculation. Small amounts of chyle were seen in the proximal one-third of the small intestine at 72 and 168 hours PI. Other organs of infected pigs were normal grossly.

Gross lesions were not seen in control pigs. Lymphatics in the small intestine of control pigs contained various amounts of chyle in the proximal small intestine 24, 72, and 168 hours PI.

Light and Scanning Electron Microscopic Changes

Villi in each of the small intestinal sites sampled from pigs inoculated with the OSU or SDSU strains of rotavirus were shorter than control villi at 24 hours PI (Table 5). The DU was less severely affected by

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Table 5. Villous length (μm) in the small intestines of gnotobiotic pigs infected with the OSU or SDSU strains of porcine rotavirus

Hours after inoculation	Treatment group	Intestinal site DU	UJ	LJ	MI	UI	LI
24	Control	855 ±4	906 ±8	813 ±12	825 ±6	781 ±48	815 ±18
	OSU	645 ±5	342[a] ±44	501 ±12	541 ±27	594 ±11	546 ±11
	SDSU	675 ±31	526[a] ±40	544 ±30	537 ±58	513 ±0	422 ±24
72	Control	827 ±7	943 ±7	833 ±28	839 ±9	897 ±63	952 ±12
	OSU	614[a,b] ±82	227[a] ±37	124[a,b] ±19	82[a] ±7	194[a] ±11	249[a] ±4
	SDSU	354[a,b] ±13	311[a] ±23	196[a,b] ±2	155[a] ±0	247[a] ±3	366[a] ±7
168	Control	845 ±19	810 ±27	823 ±27	939 ±9	1016 ±44	1002 ±64
	OSU	874 ±42	419[a] ±10	364[a] ±9	321[a,b] ±21	376[a] ±10	686[a] ±5
	SDSU	439[a,b] ±1	322[a] ±26	289[a] ±4	245[a,b] ±9	293[a] ±7	640[a] ±22

Data are expressed as the mean ± SE (N = 20 except for the OSU and SDSU groups at 24 and 168 hours after inoculation in which N = 25).

[a] Values significantly different from control values, $p<0.05$.

[b] Significant difference ($p<0.05$) in villous length between the OSU and SDSU inoculated pigs.

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villous atrophy than the other sites. The reduction in villous length in the UJ (Figure 1) was significantly different ($p<0.05$) when each of the rotavirus inoculated groups of pigs were compared to the control (Table 5). At 24 hours PI, mean villous lengths for pigs inoculated with the OSU or SDSU strains were not significantly different. Villous atrophy in the small intestines of pigs infected with the OSU or SDSU strains was maximal at 72 hours PI (Figure 2). At this time, villous lengths in virus infected pigs were significantly ($p<0.05$) shorter than controls (Table 5). Villous atrophy was most severe in the UJ, LJ, MI, and UI of pigs infected with the OSU or SDSU strains of rotavirus at 72 and 168 hours PI. The mean villous lengths in the DU of pigs infected with the SDSU strain were significantly shorter ($p<0.05$) at 72 and 168 hours PI when compared to pigs infected with the OSU strain or control pigs (Figures 3, 4, 5, 6, and 7). Villi in the duodenum of pigs infected with the OSU strain 168 hours PI were the same length as the control villi. Villi in the LJ of pigs infected with the OSU strain 72 hours PI were significantly shorter ($p<0.05$) than villi in the LJ of pigs infected with the SDSU strain and control pigs. Villi in the small intestine of pigs infected with the OSU or SDSU strains were longer at 168 hours than at 72 hours PI, but were still significantly shorter, except for the DU of pigs infected with the OSU strain, than control villi at each site.

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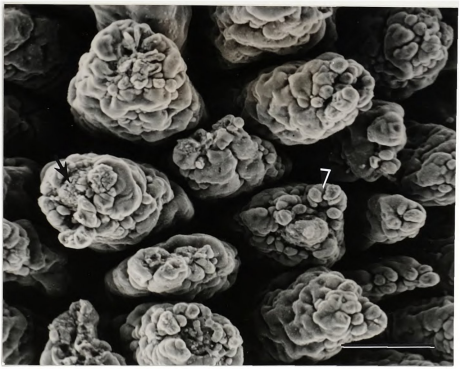

Figure 1. Scanning electron micrograph of the UJ of a pig 24 hours after inoculation with the OSU strain of porcine rotavirus. Notice the rounded absorptive cells (arrowhead) and the exposed lamina propria (arrow) on the tips of atrophic villi. Bar = 100 μm.

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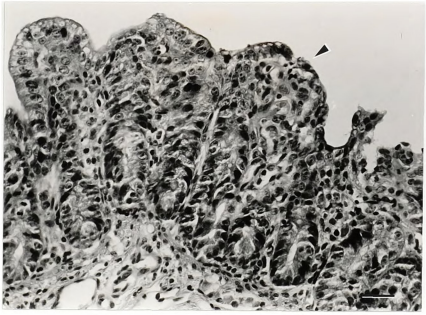
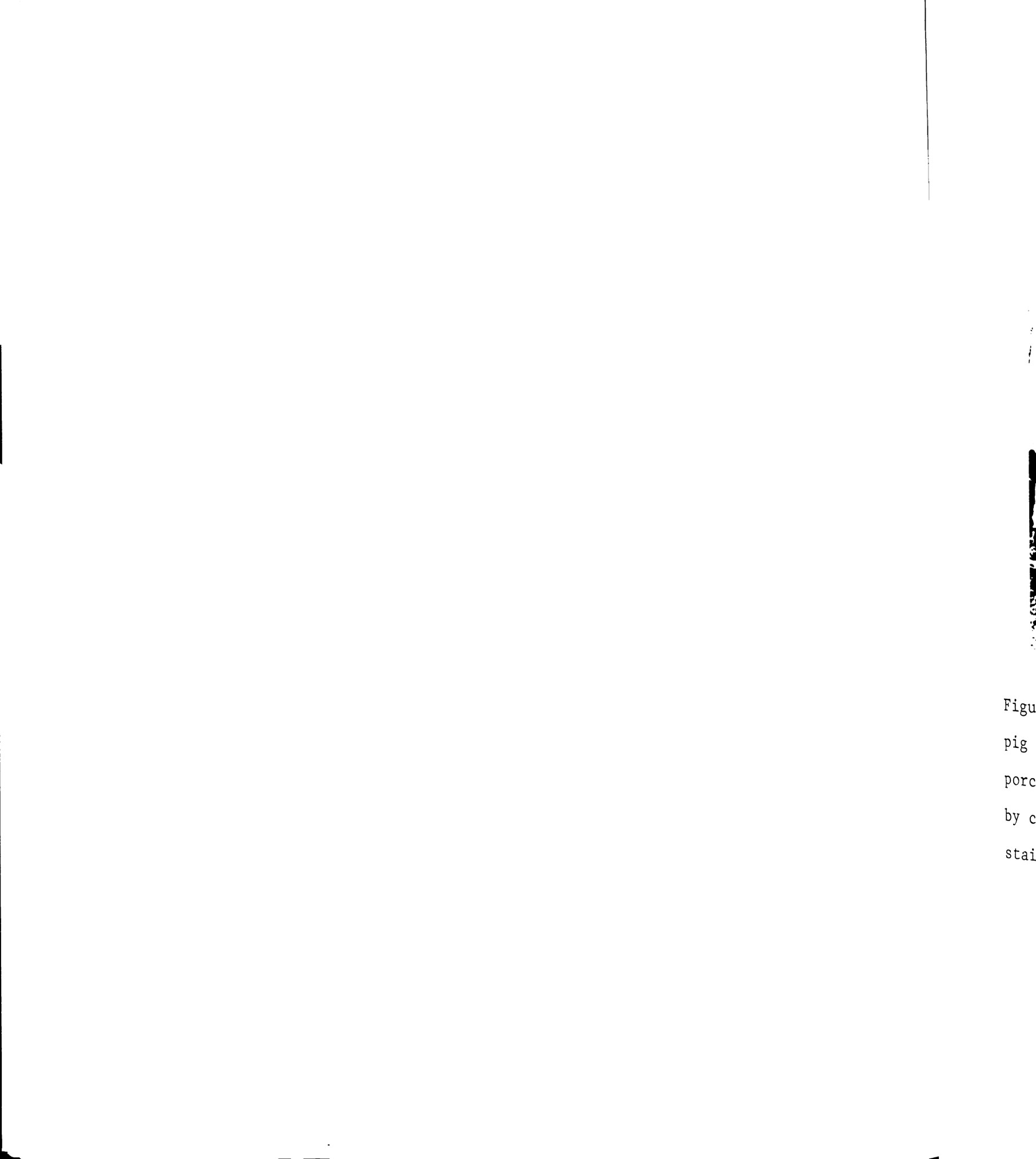


Figure 2. Section of small intestine from the MI of a pig 72 hours after inoculation with the OSU strain of porcine rotavirus. Villi are short, fused, and focally denuded (arrow). Hematoxylin and eosin stain; bar = 10 µm.



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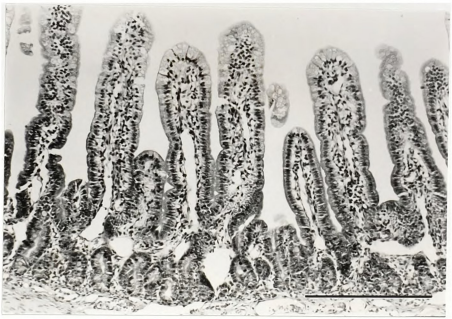

Figure 3. Section of small intestine from the DU of a pig 72 hours after inoculation with the SDSU strain of porcine rotavirus. Moderately shortened villi are lined by columnar absorptive cells. Hematoxylin and eosin stain; bar = 100 μm.

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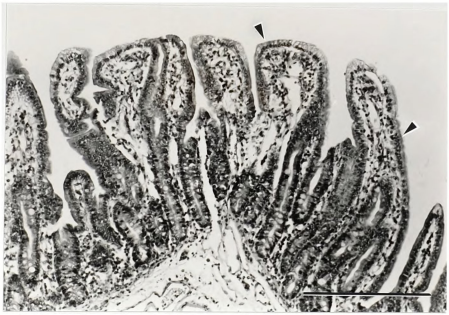

Figure 4. Section of small intestine from the DU of a pig 168 hours after inoculation with the SDSU strain of porcine rotavirus. Villi are short and fused (arrows) and lined by low to tall columnar absorptive cells. Hematoxylin and eosin stain; bar = 100 μm.

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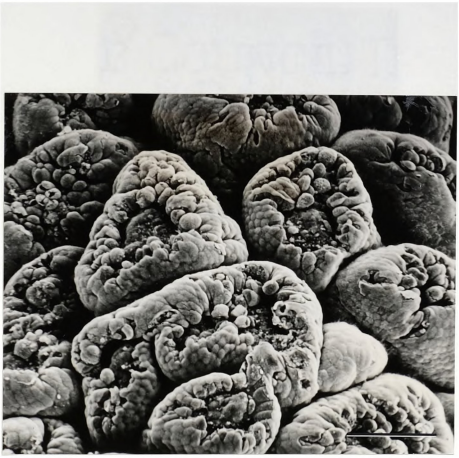

Figure 5. Scanning electron micrograph of the DU of a pig 168 hours after inoculation with the SDSU strain of porcine rotavirus. The tips of severely shortened and fused villi have areas of exposed lamina propria. Bar = 100 μm.

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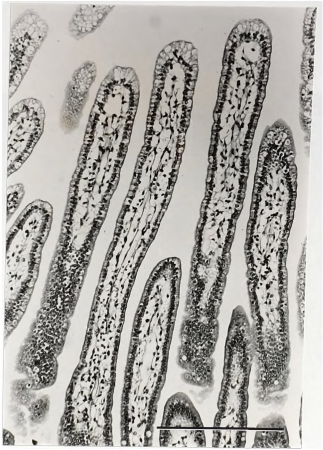

Figure 6. Section of small intestine from the DU of a pig 168 hours after inoculation with the OSU strain of porcine rotavirus. Notice the histologically normal villous structure. Hematoxylin and eosin stain; bar = 100 μm.

Figure 7

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Figure 7. Scanning electron micrograph of the DU of a pig 168 hours after inoculation with the OSU strain of porcine rotavirus. Villous structure is similar to control villi. Epithelial cell extrusion zones can be seen (arrows). Bar = 100 μm.

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Small intestinal crypt depths in control and principal pigs were not significantly different at 24 hours PI, except for the crypt depths in the UI of pigs infected with the SDSU strain (Table 6). At 72 hours PI, crypts in pigs infected with the OSU or SDSU strains were deeper in the UJ-LI than those of controls and by 168 hours PI this difference in crypt depths was significant at all sites. Crypts at each site in the small intestine of pigs infected with the SDSU strain 168 hours PI were significantly deeper, except in the LI, when compared to crypt depths in pigs infected with the OSU strain.

Fusion of villi in the small intestine of rotavirus inoculated pigs was observed 24, 72, and 168 hours PI (Tables 7, 8, and 9). All of the segments of small intestine were affected, but fused villi were most numerous in the UJ-UI (Figures 8, 9, and 10). Fused villi were observed more frequently in the DU of pigs inoculated with the SDSU strain (9 of 9 pigs) than in pigs inoculated with the OSU strain (2 of 9 pigs) at 72 and 168 hours PI. The small intestine of control pigs had a few areas of villous fusion 24 and 72 hours PI.

Control pigs had vacuolated absorptive epithelial cells in the UJ-LI and less commonly in the DU (Figure 11). The amount of epithelial cell vacuolation increased in the distal small intestine and decreased as the pigs aged. Cytoplasmic vacuolation in the small intestine of rotavirus infected pigs, particularly at 168 hours PI, was

Table 6.
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Table 6. Crypt depths (μm) in the small intestines of gnotobiotic pigs infected with the OSU or SDSU strains of porcine rotavirus

Hours after inoculation	Treatment group	Intestinal site					
		DU	UJ	LJ	MI	UI	LI
24	Control	97 ±6.8	98 ±0.8	90 ±2.6	77 ±11.6	91 ±0.5	85 ±0.9
	OSU	94 ±3.4	89 ±1.4	92 ±6.2	75 ±0.6	74 ±10.0	80 ±0.5
	SDSU	85 ±1.6	82 ±1.1	71 ±1.4	70 ±0.5	66[a] ±2.4	70 ±1.8
72	Control	120 ±1.5	105 ±1.7	93 ±0.0	93 ±0.1	89 ±3.9	101 ±0.6
	OSU	122 ±4.4	122 ±5.1	135[a] ±5.6	143[a] ±1.5	128[a] ±2.1	121 ±3.0
	SDSU	125 ±0.0	130 ±2.1	121[a] ±0.6	118 ±9.4	124[a] ±3.7	109 ±6.0
168	Control	113 ±4.7	107 ±6.9	101 ±4.2	102 ±1.4	89 ±2.1	98 ±8.3
	OSU	151[a,b] ±2.9	165[a,b] ±2.0	149[a,b] ±4.3	153[a,b] ±9.8	139[a,b] ±4.3	136[a] ±3.7
	SDSU	181[a,b] ±4.3	189[a,b] ±2.4	193[a,b] ±6.9	179[a,b] ±0.8	160[a,b] ±3.1	140[a] ±1.1

Data are expressed as the means ± SE (N = 20 except for the OSU and SDSU groups 24 and 168 hours after inoculation in which N = 25).

[a] Values significantly different from control values $p<0.05$.

[b] Significant difference ($p<0.05$) in crypt depth between the OSU and SDSU inoculated pigs.

Table 7. Light microscopic findings in the small intestine of gnotobiotic pigs 24 hours after inoculation with the OSU or SDSU strain of porcine rotavirus

Table 7. Light microscopic findings in the small intestine of gnotobiotic pigs 24 hours after inoculation with the OSU or SDSU strain of porcine rotavirus

Treatment group	Intestinal site	Absorptive cell vacuolation[a]	Absorptive cell detachment[b]	Villous fusion[b]	Immature villous epithelium[b]
Control	DU	-, -, -, 1	-, -, -, -	-, -, -, -	-, -, -, -
	UJ	1, 1, 1, 2	-, -, -, -	-, -, -, -	-, -, -, -
	LJ	2, 1, 1, 3	-, -, -, -	-, 1, 1, -	-, -, -, -
	MI	3, 2, 2, 3	-, -, -, -	-, 1, 1, -	-, -, -, -
	UI	4, 3, 3, 4	-, -, -, -	1, 1, 1, -	-, -, -, -
	LI	4, 4, 4, 4	-, -, -, -	1, -, -, -	-, -, -, -
OSU	DU	-, -, -, -, 2	2, 1, -, 2, -	1, -, -, -, -	3, 2, -, 2, -
	UJ	-, -, 2, 2, 1	3, 3, -, -, 3	3, 3, -, 2, -	3, 2, -, -, 2
	LJ	-, -, 1, 3, 3	3, 1, -, -, -	3, 3, -, 1, -	3, 3, -, 1, -
	MI	-, 1, 4, 3, 4	3, 1, -, -, -	3, 2, 1, 1, -	3, 1, -, -, -
	UI	-, -, 4, 3, 4	3, 1, -, -, -	3, 2, -, -, -	3, 2, -, -, -
	LI	-, 1, 4, 4, 4	3, 1, -, -, -	3, 3, -, -, -	3, 3, -, -, -
SDSU	DU	-, -, -, -, -	-, -, -, -, -	1, -, -, -, -	-, 1, -, -, -
	UJ	-, -, 1, 1, 1	-, 1, -, -, 1	1, 3, -, -, 1	-, 3, -, -, 3
	LJ	1, -, 2, 1, 1	-, 1, -, -, -	-, 3, -, 1, -	-, 3, -, -, -
	MI	1, -, 3, 2, 1	-, 1, -, -, 1	-, 3, -, 1, 1	-, 3, -, -, 1
	UI	1, -, 4, 3, 3	-, -, -, -, -	-, 3, -, 1, 1	-, 3, -, -, 1
	LI	1, 1, 4, 4, 3	2, -, -, -, -	1, 3, -, -, -	2, 3, -, -, -

[a] Cytoplasmic vacuolation was scored on the degree of vacuolation, ranging from (-) = no vacuolation to (4) = vacuolation of approximately 100% of the villous epithelial cells; a comma separates results from each pig.

[b] Detachment, fusion, and immaturity were scored on the basis of -, 1, 2, 3; (-) = no change from control and a 3 representing the greatest change.

Table 8. Light microscopic findings in the small intestine of gnotobiotic pigs 72 hours after inoculation with the OSU or SDSU strains of porcine rotavirus

Table 8. Light microscopic findings in the small intestine of gnotobiotic pigs 72 hours after inoculation with the OSU or SDSU strains of porcine rotavirus

Treatment group	Intestinal site	Absorptive cell[a] vacuolation	Absorptive cell[b] detachment	Villous[b] fusion	Immature[b] villous epithelium
Control	DU	-, -, -, -	-, -, -, -	-, -, -, -	-, -, -, -
	UJ	-, 1, 1, 1	-, -, -, -	-, -, -, -	-, -, -, -
	LJ	1, 2, 3, 1	-, -, -, -	-, -, -, -	-, -, -, -
	MI	2, 3, 3, 3	-, -, -, -	-, -, -, 1	-, -, -, -
	UI	2, 4, 4, 3	-, -, -, -	-, -, -, -	-, -, -, -
	LI	3, 4, 4, 4	-, -, -, -	-, -, -, -	-, -, -, -
OSU	DU	2, 1, -, 1	-, -, -, -	-, -, -, 1	-, -, -, 1
	UJ	2, 2, -, 1	-, 1, 1, 1	2, 3, -, 3	-, 3, -, 2
	LJ	2, 2, 1, 1	1, 3, 2, 1	3, 3, 2, 3	-, 3, 2, 3
	MI	1, 1, 1, 1	2, 3, 2, 3	3, 3, 3, 3	1, 3, 2, 3
	UI	1, 1, -, 1	-, -, 2, 1	1, 3, 3, 3	1, 1, 3, 3
	LI	2, 2, -, 1	-, -, 1, -	2, 2, 2, 2	-, -, -, -
SDSU	DU	1, 2, 1, 1	2, 1, -, -	3, 1, 1, 1	1, -, -, -
	UJ	-, 1, 3, 1	1, 1, -, -	3, 1, 2, 1	1, 1, -, -
	LJ	1, -, 2, 1	1, 1, -, 1	3, 3, 3, 2	2, -, -, 1
	MI	1, -, 2, 1	3, 1, 1, -	3, 2, 3, 2	3, 2, 2, -
	UI	1, 1, 4, 2	3, 1, -, 1	3, 1, 1, 1	3, -, -, -
	LI	-, 1, 4, 2	-, -, -, -	2, 2, 1, 1	-, -, -, -

[a] Cytoplasmic vacuolation was scored on the degree of vacuolation, ranging from (-) = no vacuolation to (4) = vacuolation of approximately 100% of the villous epithelial cells; a comma separates results from each pig.

[b] Detachment, fusion, and immaturity were scored on the basis of -, 1, 2, 3; (-) = no change from control and a 3 representing the greatest change.

Table 9. Light microscopic findings in the small intestine of gnotobiotic pigs 168 hours after inoculation with the OSU or SDSU strains of porcine rotavirus

Table 9. Light microscopic findings in the small intestine of gnotobiotic pigs 168 hours after inoculation with the OSU or SDSU strains of porcine rotavirus

Treatment group	Intestinal site	Absorptive cell[a] vacuolation	Absorptive cell[b] detachment	Villous[b] fusion	Immature[b] villous epithelium
Control	DU	-, 1, -, -	-, -, -, -	-, -, -, -	-, -, -, -
	UJ	-, 1, -, -	-, -, -, -	-, -, -, -	-, -, -, -
	LJ	1, 1, -, 1	-, -, -, -	-, -, -, -	-, -, -, -
	MI	2, 2, 1, 2	-, -, -, -	-, -, -, -	-, -, -, -
	UI	3, 3, 2, 3	-, -, -, -	-, -, -, -	-, -, -, -
	LI	3, 3, 2, 4	-, -, -, -	-, -, -, -	-, -, -, -
OSU	DU	-, -, -, 1, 1	-, -, -, -, -	-, -, -, 2, -	1, -, -, -, -
	UJ	-, -, -, 1, 1	-, -, -, -, -	2, -, 2, 3, 3	1, -, -, -, -
	LJ	-, -, 1, 1, 1	-, -, -, -, -	2, 2, 3, 2, 2	-, -, -, -, -
	MI	1, -, 1, -, 1	-, -, -, -, -	2, 1, 2, 1, 3	-, -, -, -, -
	UI	1, 1, -, 1, 1	-, -, -, -, -	2, -, 2, 2, 1	-, -, -, -, -
	LI	1, -, 1, 1, 2	-, -, -, -, -	1, 2, -, 1, 1	-, -, -, -, -
SDSU	DU	-, 1, -, 1, -	-, -, 1, 1, -	1, 3, 2, 2, 2	-, -, 3, 2, 1
	UJ	-, -, -, 1, -	1, -, 1, -, -	2, 2, 3, 2, 3	2, -, -, -, 1
	LJ	-, -, -, 1, -	-, -, -, -, -	3, 2, 2, 3, 3	3, -, -, -, -
	MI	-, -, -, 1, 1	-, -, -, -, -	3, 1, 3, 2, 3	3, -, -, -, 1
	UI	-, -, -, 1, -	-, -, -, -, -	3, 1, 2, 2, 3	1, -, 1, -, 1
	LI	-, -, -, -, -	-, -, -, -, -	1, -, -, 1, 1	1, -, -, -, -

[a] Cytoplasmic vacuolation was scored on the degree of vacuolation, ranging from (-) = no vacuolation to (4) = vacuolation of approximately 100% of the villous epithelial cells; a comma separates results from each pig.

[b] Detachment, fusion, and immaturity were scored on the basis of -, 1, 2, 3; (-) = no change from control and a 3 representing the greatest change.

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Figure 8. Villous fusion in a section of small intestine from the LJ of a pig 72 hours after inoculation with the SDSU strain of porcine rotavirus. Hematoxylin and eosin stain; bar = 10 µm.

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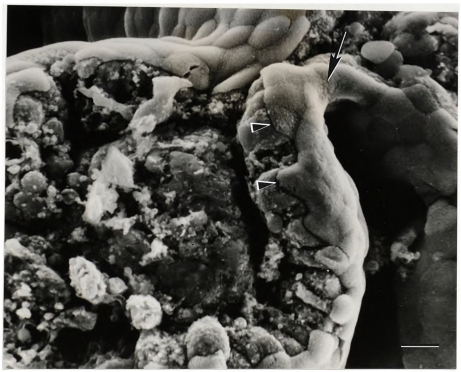

Figure 9. Scanning electron micrograph of small intestine from the LJ of a pig 168 hours after inoculation with the SDSU strain of porcine rotavirus. Loss of absorptive cells creates a sharp line of demarcation (arrowheads) bordering an area of exposed lamina propria. An area of villous fusion (arrow) can be seen. Bar = 10 µm.

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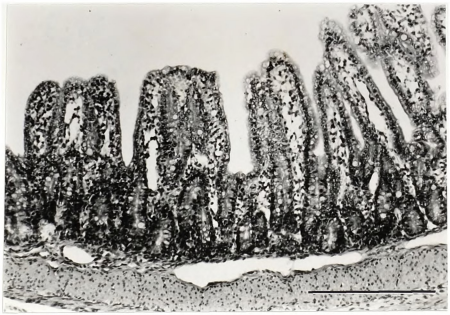

Figure 10. Villous fusion in a section of UJ from a pig 168 hours after inoculation with the OSU strain of porcine rotavirus. Hematoxylin and eosin stain; bar = 100 µm.

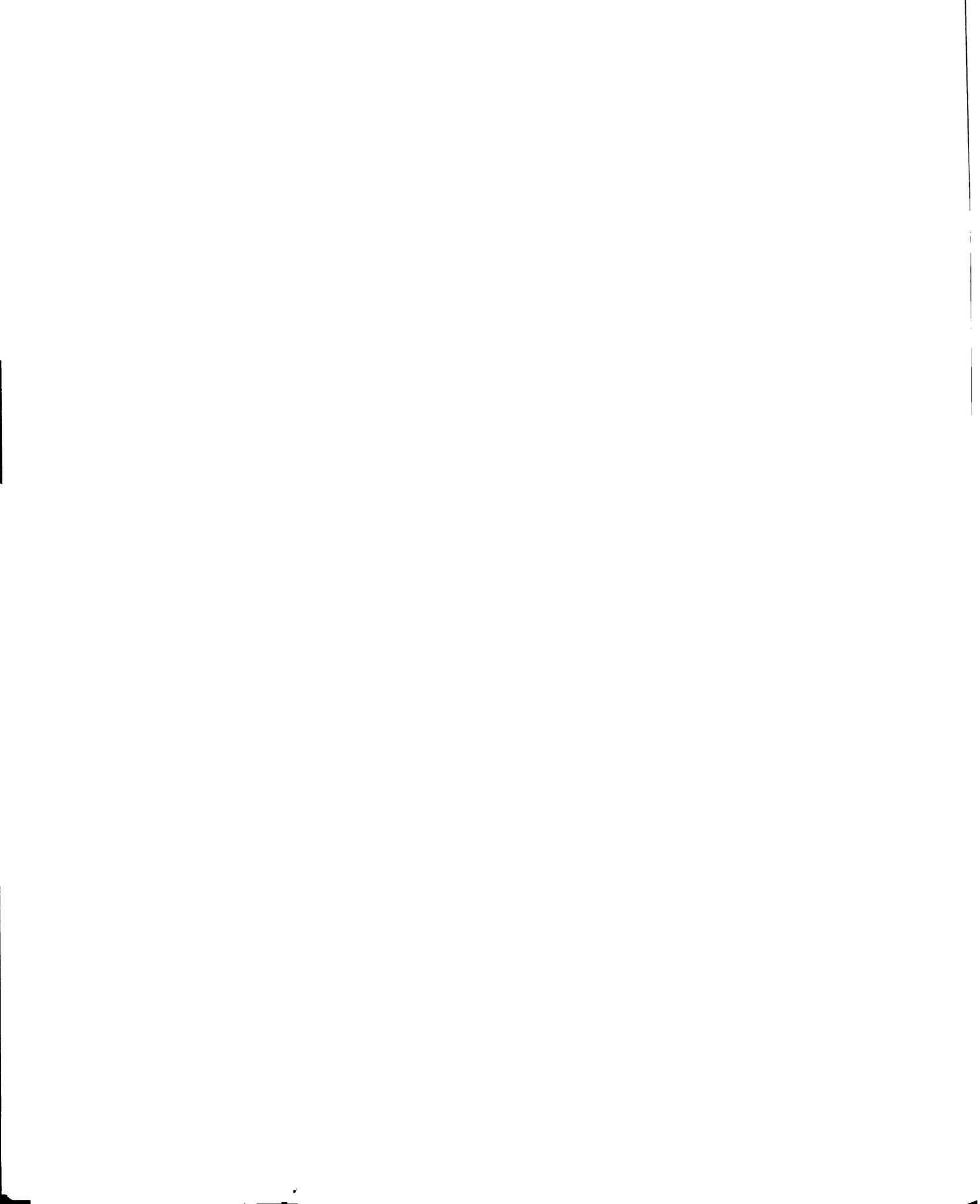

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Figure 11. Most of the absorptive cells lining villi in the LI of a control pig 24 hours after inoculation with media are vacuolated (+4). Hematoxylin and eosin stain; bar = 10 μm.

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much less conspicuous (Figure 12) than in controls (Tables 7, 8, and 9).

Rounding and detachment of absorptive epithelial cells on the tips and sometimes the sides of villi were observed in the small intestine of rotavirus infected pigs at 24 hours PI (Table 7). At this time, there was much pig-to-pig variation in the amount of cell detachment. Rounding and detachment of absorptive cells with exposure of the underlying lamina propria were most numerous in the UJ-UI of pigs infected with the OSU or SDSU strains 72 hours PI (Table 8). Microvilli in areas adjacent to rounded or detached absorptive cells were disorganized or reduced in number. Areas devoid of microvilli were often sharply demarcated from normal microvilli on the same absorptive cell. Absorptive cell detachment was observed in the duodenum of pigs infected with the OSU strain 24 hours PI, but not at 72 or 168 hours PI (Tables 7, 8, and 9). In contrast, pigs infected with the SDSU strain had cell detachment in the duodenum at 72 and 168 hours PI, but not at 24 hours. Pigs inoculated with the OSU strain did not have epithelial cell detachment 168 hours PI, and cell detachment was uncommon in pigs infected with the SDSU strain at this time. Control pigs did not have areas of cell detachment or exposed lamina propria (Figures 13, 14, and 15).

Immature villous epithelium lining the small intestinal villi of rotavirus infected pigs was observed

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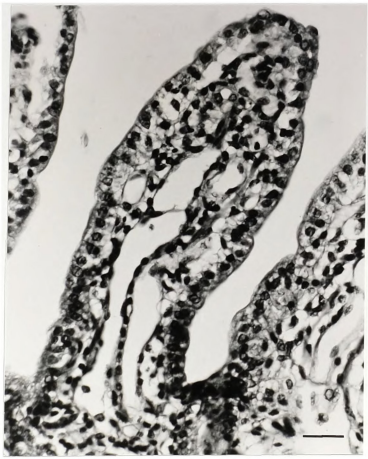

Figure 12. Immature cuboidal to low columnar absorptive cells line an atrophic villus in the UJ of a pig 24 hours after inoculation with the OSU strain of porcine rotavirus. Hematoxylin and eosin stain; bar = 10 μm.

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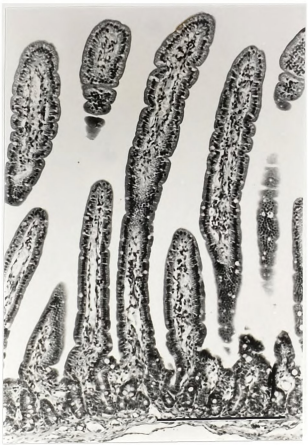

Figure 13. Duodenum of a control pig killed at post-inoculation hour 24. Hematoxylin and eosin stain; bar = 100 μm.

Figure 14. Scanning electron micrograph of the duodenum of a control pig at post-inoculation hour 24. Long slender villi have prominent transverse fissures (arrows). Bar = 100 μm.

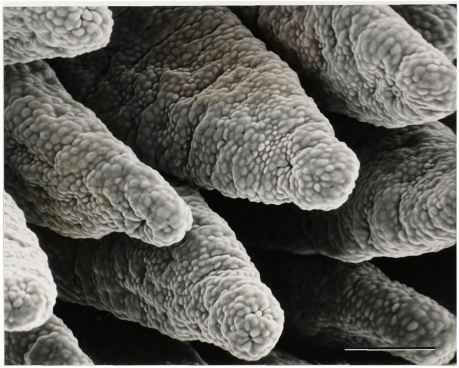

Figure 15. Scanning electron micrograph of the lower ileum of a control pig at post-inoculation hour 24. Villi are broader in the lower ileum when compared to villi in the duodenum (Figure 14). Enterocytes bulge into the intestinal lumen giving a cobblestone appearance to the mucosal surface. Bar = 100 μm.

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at 24 and 72 hours PI (Figure 12). By 168 hours, the majority of villi were lined by low to tall columnar epithelial cells (Tables 7, 8, and 9).

Aside from the occasional fused villi, small intestine of control pigs was histologically normal (Figure 13). Villi were long and slender with prominent transverse fissures (Figure 14). Villi in the lower ileum of controls were broader and had prominent enterocytes (Figure 15) and numerous goblet cells. Histopathologic changes were not observed in the cecum, colon, rectum, lung, liver, heart, pancreas, spleen, brain, thymus, tonsil, and mesenteric lymph node of control or rotavirus infected pigs.

Immunofluorescence

Pigs inoculated with either the OSU or SDSU strains of rotavirus had finely granular cytoplasmic fluorescence in enterocytes lining the sides and tips of villi in the small intestine (Tables 10 and 11). Immunofluorescent enterocytes were most consistently detected in the UJ, MI, LJ, UI, and LI of pigs 24 and 72 hours after inoculation with either rotavirus strain. Viral antigen was detected in the DU of pigs 24 hours (5 of 5 pigs) and 72 hours PI (2 of 4 pigs) after inoculation with the OSU strain. Immunofluorescent cells were seldom observed in the DU of pigs 24 hours (1 of 5 pigs) or 72 hours (0 of 4 pigs) after inoculation with the SDSU strain. By 168 hours, immunofluorescence was rarely observed in the

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UJ
LJ
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LI

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Table 10. Immunofluorescence in the small intestine of gnotobiotic piglets infected with the SDSU strain of porcine rotavirus

Intestinal site	Hours after inoculation 24	72	168
DU	-, -, -, +, -*	-, -, -, -	-, -, -, -, -
UJ	-, +, +, +, -	+, -, +, -	-, -, -, -, -
LJ	-, +, +, +, -	+, +, +, +	-, -, -, -, -
MI	-, -, +, +, -	+, +, +, +	-, -, -, -, +
UI	+, -, -, +, -	+, +, +, -	-, -, -, -, -
LI	+, -, +, +, -	+, -, -, -	-, -, -, -, -

* - = No specific fluorescence for rotavirus; + = specific immunofluorescence for rotavirus; a comma separates results obtained from each pig.

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Intesti site
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UJ
LJ
MI
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Table 11. Immunofluorescence in the small intestine of gnotobiotic piglets infected with the OSU strain of porcine rotavirus

Intestinal site	Hours after inoculation 24	72	168
DU	+, +, +, +, +*	-, -, +, +	-, -, -, -, -
UJ	+, -, -, +, +	+, +, +, +	-, -, -, -, -
LJ	+, +, -, +, +	+, +, +, +	-, -, -, -, -
MI	+, -, +, +, +	+, -, +, +	-, -, -, -, -
UI	+, -, +, +, +	+, -, +, +	-, -, -, -, -
LI	+, -, +, +, +	+, -, +, +	-, -, +, -, -

* - = No specific fluorescence for rotavirus; + = specific immunofluorescence for rotavirus; a comma separates results from each pig.

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small intestine of pigs infected with either virus strain.

A few surface enterocytes with cytoplasmic fluorescence were observed in the cecum from one pig 24 hours after inoculation with the OSU strain. Two additional sections of cecum from this pig did not contain immunofluorescent cells. Specific immunofluorescence was not seen in the cecal mucosa from pigs inoculated with the SDSU strain, but a few detached immunofluorescent cells were observed in the cecal lumen of one pig 24 hours after inoculation.

Specific immunofluorescence was not observed in frozen sections of stomach, tonsil, spleen, mesenteric lymph node, colon, or rectum. Frozen tissue sections from control pigs did not have specific immunofluorescence when stained for porcine rotaviral antigen.

Electron Microscopic Examination of Feces

Feces collected from control and principal pigs before inoculation did not contain viral particles. At 24 hours PI, most virus-inoculated pigs had +2 to +3 rotavirus particles in their feces (Table 12). Two pigs, one inoculated with the OSU strain and another with the SDSU, did not have clinical signs of diarrhea at 24 hours PI, but rotavirus particles were identified in cecal contents collected at necropsy. Cecum and colon in these pigs were dilated with fluid yellow contents indicating

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Table 12. Results of direct electron microscopic examination of feces or cecal content from pigs infected with the SDSU or OSU strain of porcine rotavirus

	Number of specimens[a]					
	24 hrs[b]		72 hrs		168 hrs	
EM score	OSU[c]	SDSU	OSU	SDSU	OSU	SDSU
Neg	6	6	0	1	2	1
+1	0	1	1	1	0	2
+2	3	4	1	3	3	2
+3	5	3	7	4	0	0
+4	0	0	0	0	0	0
Total no. of specimens	14	14	9	9	5	5

[a] Viral particles were not observed in feces obtained before rotavirus inoculation.

[b] Hours after inoculation.

[c] The number of rotavirus particles per grid square was estimated and rated as +1 (0 to 5 virus particles/grid square), +2 (6 to 20), +3 (21 to 100), or +4 (>100).

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that diarrhea would have occurred had these pigs been allowed to survive. Virus particles were not detected at 24 hours after inoculation in the feces from the remainder of the non-diarrheic pigs.

Virus particles had been seen in the feces of all virus infected pigs by 72 hours after inoculation. The feces of one diarrheic pig inoculated with the SDSU strain contained virus particles at 24 hours, but not at 72 hours PI. The feces from this pig were becoming less fluid so it may have been recovering from the rotavirus infection.

The number of rotavirus particles in the feces began to decrease by 168 hours PI. Virus particles were not seen in the feces of 3 pigs (2 OSU, 1 SDSU) at 168 hours PI. Feces or cecal content collected at 24, 72, or 168 hours PI contained approximately equal numbers of complete and incomplete virus particles. Rotaviral particles were not observed in the fecal samples collected from control pigs 24, 72, or 168 hours after inoculation. In general, the virus excretion patterns were similar for both rotavirus strains.

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DISCUSSION

Findings in this study indicate that in 3-day-old gnotobiotic pigs serotype 1 (OSU) and serotype 2 (SDSU) strains of porcine rotavirus cause different clinical signs and histopathologic lesions. The SDSU strain caused more severe villous atrophy and fusion at the duodenal-jejunal flexure when compared to pigs inoculated with the OSU strain. Pigs infected with the OSU strain frequently vomited whereas pigs infected with the SDSU strain did not vomit.

Vomiting by gnotobiotic pigs infected with the OSU strain but not the SDSU strain is consistent with the findings of other investigators (Theil et al, 1978; McAdaragh et al, 1980; Graham et al, 1984). Vomiting after infection by some strains of rotavirus has been reported in conventional (Bohl et al, 1978), colostrum-deprived (Lecce et al, 1976; Pearson and McNulty, 1977), and gnotobiotic pigs (Theil et al, 1978; Graham et al, 1984). In other instances, no vomiting was reported (Tzipori et al, 1978; McAdaragh et al, 1980; Narita et al, 1982). In fact, a strain of porcine rotavirus (B-317) studied by Torres-Medina and Underdahl (1980), has never been observed by these investigators to

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cause vomiting in gnotobiotic pigs. Therefore, findings in this and other studies suggest that differences in the occurrence of vomiting are related to rotavirus strain differences. Epidemiologic studies of human rotavirus infections also indicate differences in clinical signs caused by different strains of rotavirus. Vomiting occurred more frequently following infection by serotype 2 than serotype 1 human rotavirus (Yolken et al, 1978b). More recently, Uhnoo and Svenson (1986), reported that in people, subgroup II rotavirus strains caused diarrhea and vomiting of greater severity when compared to subgroup I. This is in contrast, however, to the findings of White et al (1984), who could find no differences in clinical signs caused by different subgroups of human rotavirus. The OSU strain of porcine rotavirus and the DS-1 strain of human rotavirus are classified as subgroup I, whereas all other human rotavirus strains and the Gottfried strain of porcine rotavirus are subgroup II (Hoshino et al, 1984). The subgroup specificity of the SDSU strain used in the present study has not yet been identified.

Vomiting may be caused by irritation of the upper gastrointestinal tract or by stimulation of chemoreceptors in the medulla (Ganong, 1983). In the present study, mucosal histopathologic lesions were most severe in the upper small intestine of pigs infected with the SDSU strain, yet none of these pigs vomited. Futhermore, rotaviral antigen was not detected by IF in

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the stomachs of pigs infected with either rotaviral strain nor has it been detected in the stomachs of pigs in other studies (McNulty et al, 1976a; Davidson et al, 1977; Theil et al, 1978). This suggests that vomiting associated with rotavirus infection may be initiated by mechanisms other than irritation of the gastrointestinal tract.

In the present study, attempts were made to minimize the effects of environmental and host factors on the course of the rotaviral infections. The OSU and SDSU strains were studied while keeping diet, environmental temperature, and housing constant. Experimental animals were randomly assigned to treatment groups to account for pig-to-pig variation in susceptiblity to rotavirus infection. Furthermore, the OSU strain of rotavirus underwent four gnotobiotic pig passages prior to inoculation of experimental animals. This was done to prevent attenuation, i.e. loss of virulence which may occur after passage in cell culture (Bohl et al, 1984).

Under these experimental conditions, all of the pigs infected with the OSU or SDSU strains survived until necropsied. This lack of mortality caused by the OSU and SDSU virus strains is in agreement with the findings of other investigators (Theil et al, 1978; McAdaragh et al, 1980). Mortality associated with porcine rotavirus infection has been reported in conventional (Bohl et al, 1978), colostrum-deprived (Lecce et al, 1976; McNulty et

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al, 1976), and gnotobiotic pigs (Tzipori et al, 1978; Narita et al, 1982). However, in these studies experimental conditions differed so it is possible that host or environmental factors and not rotavirus strain differences influenced the observed mortality. Age, environmental temperature, and intercurrent bacterial infection have been shown to influence the severity of rotavirus infections (Woode et al, 1976b; Crouch and Woode, 1978; Tzipori et al, 1980b; Torres-Medina, 1984; Newsome and Coney, 1985). When a strain of porcine rotavirus which caused high mortality in 1-day-old colostrum-deprived conventionally-derived pigs was given to 1-day-old gnotobiotic pigs kept under the conditions used in this experiment, no mortality was observed (personal data). Therefore, caution must be exercised when suggesting that field strains of rotavirus differ in virulence, particularly because environmental and host factors may influence the severity of the disease.

Differences in virulence between rotavirus strains are evident when rotaviruses are inoculated into a heterologous host (Bridger and Brown, 1984; Offit et al, 1986). For instance, the SA-11 strain (primate origin) of rotavirus was more virulent than the NCDV strain (bovine origin) when inoculated orally into newborn mice. However, evidence indicating that field strains of rotavirus are more virulent than others when inoculated into a homologous host such as in the present study is

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scant (Woode, 1979) and often based on clinical reports (Castrucci et al, 1983) or personal communications (Bridger, J. C. in Reynolds et al, 1985). A report by Carpio et al (1981b) is frequently cited as evidence indicating that field isolates with different virulence characteristics exist. In their report, four field isolates of bovine rotavirus consistently caused different amounts of villous atrophy when inoculated into ligated intestinal loops in colostrum-deprived calves. The authors concluded that the difference in the severity of villous atrophy corresponded to differences in virulence among the rotavirus isolates tested. However, it may be incorrect to assume that villous atrophy is equated with virulence. Reynolds et al (1985) observed villous atrophy in clinically normal (non-diarrheic) calves infected with rotavirus. The calves used in the experiment by Carpio et al (1981b) were maintained on intravenous chloramphenicol throughout the experiment. Chloramphenicol administered to healthy calves has been shown to induce diarrhea (Huffman et al, 1981). Chloramphenicol or the rotavirus strains, or both, may have influenced the amount of villous atrophy observed.

The incubation periods after inoculating pigs with the OSU or SDSU strains, were longer and more variable than those previously reported (Crouch and Wood, 1978; Theil et al, 1978; McAdaragh et al, 1980). The prolonged and variable incubation periods were probably due to the

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lower dosage of rotavirus used in the present study. Previous investigations on the pathogenesis of rotaviral diarrhea caused by the OSU or SDSU strains used 10% suspensions of intestinal or fecal filtrate (Theil et al, 1978; McAdaragh et al, 1980). Decreasing dosages of rotavirus have been shown to increase the length of the incubation period before the onset of clinical signs but do not affect the severity of the clinical disease (Woode et al, 1976b).

Variation in incubation periods may have been responsible for the differences in the amount of villous atrophy observed in the UJ-UI of pigs infected with the OSU and SDSU strains. Villi in the UJ-UI of pigs infected with the OSU strain were shorter at 72 hours PI but longer at 168 hours PI when compared to those of pigs infected with the SDSU strain. Glandular crypts were significantly deeper in pigs infected with the SDSU strain than in pigs infected with the OSU strain 168 hours PI. Pigs infected with the OSU or SDSU strain, because of different incubation periods, may have been at different stages of the infection when studied at these times.

Rotavirus-infected pigs had reduced body weights when compared to controls. Weight gains were highly variable even when pigs were kept in the same isolator and inoculated with the same pool of virus. Pig-to-pig variation in body weight gain also was reported by Woode

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Reasons for the pig-to-pig variation are not known, but may be related to host physiological factors. For instance, the availability of trypsin in the small intestine might affect the expression of virulence by rotaviruses. It is now clear that a trypsin-sensitive protein plays a major role in gastrointestinal virulence (Offit et al, 1986). It has been suggested that qualitative or quantitative differences in intestinal enzymes (trypsin) may be responsible for the resistance of the human neonate to rotaviral diarrhea (Holmes et al, 1976; McClean and Holmes, 1981). Gastric pH might also affect the clinical expression of rotavirus infection. A recent report indicated that three bovine and several primate-origin rotaviruses are rapidly inactivated at pH 2.0, but inactivated at a much slower rate at pH 3.0; inactivation at pH 4.0 was minimal (Weiss and Clark, 1985). The effect of host factors such as gastric pH, intestinal trypsin content, and the availability of enterocyte receptors on the expression of virulence by rotaviruses deserves further study.

Serum biochemical changes that occur in rotavirus infected pigs seldom have been reported (Mouwen et al, 1972). In the present study, elevations in BUN which suggested dehydration were detected in pigs inoculated with the OSU or SDSU strains. However, as evidenced by the large standard deviation of the mean, elevations in

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BUN were detected inconsistently. Another enteric pathogen of pigs, transmissible gastroenteritis virus, has been reported to cause elevations in BUN and total protein (Cornelius et al, 1968; Drolet et al, 1984). Serum triglycerides were decreased in rotavirus infected pigs when compared to controls. Higher levels of fat are present in the feces of piglets with "white scours", a condition presumably caused by rotavirus, when compared to normal pigs (Mouwen et al, 1972). Decreased serum triglycerides may be related to the malabsorptive state of the damaged small intestine.

Serum electrolyte values of rotavirus infected pigs in the present study did not differ from the controls regardless of the strain of rotavirus used as inoculum. This finding, coupled with the elevations in BUN and total protein suggests that, when dehydration occurs, it is isotonic. Isotonic dehydration also has been reported more frequently than hypertonic or hypotonic dehydration in children hospitalized with rotavirus infection (Tallette et al, 1977; Rodriguez et al, 1977).

An important finding in this study was that the SDSU strain of rotavirus produced more severe villous atrophy in the upper small intestine. Villi were significantly shorter in the DU of pigs inoculated with the SDSU isolate at 72 and 168 hours PI when compared to pigs given the OSU strain. Fusion of villi also was more frequently seen in the duodenum of pigs infected with the

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SDSU strain than pigs infected with the OSU strain. This is the first time that different serotypes of the same mammalian rotavirus have been shown to cause histopathologic lesions of different severity in different sites in the small intestine.

Previous investigators disagreed about the extent of villous atrophy in the upper small intestine. For instance, McAdaragh et al (1980) observed atrophy and fusion of villi in the duodenum as early as 24 hours PI whereas Torres-Medina and Underdahl (1980) found no villous atrophy in the duodenum. In the experiment by McAdaragh et al (1980), the SDSU strain of rotavirus was used whereas Torres-Medina and Underdahl used a field strain (serotype unknown) of porcine rotavirus designated B-317. Differences in the severity of villous atrophy and fusion in the upper small intestine might be explained by rotavirus strain differences as suggested by the findings in the present study.

Viral antigen was consistently detected in the DU of pigs infected with the OSU but not SDSU strain 24 and 72 hours PI. This was unexpected since histologic lesions were most severe in the DU of pigs infected with the SDSU strain. Three of the pigs killed 24 hours PI with the SDSU strain did not have diarrhea. Viral antigen was not detected in the small intestine from 1 of these pigs and the other two only had immunofluorescence in a few intestinal segments. It is possible that duodenal

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enterocytes in the pigs infected with the SDSU strain became infected between 24 and 72 hours PI, sloughed, and could no longer be detected at 72 hours PI.

McNulty et al (1983) were the first to report different intestinal tropisms for different strains of rotavirus. Avian rotavirus strain ch 1 (group A) had a predilection for the duodenal mucosa of specific pathogen-free chickens, whereas avian rotavirus strain ch 132 (non-group A) replicated best in the mid-small intestine.

Based on the differences in intestinal tropism, McNulty et al (1983) suggested that mixed infections by some strains of avian rotavirus may cause more severe clinical disease than infection by either virus alone. The Gottfried strain of porcine rotavirus was originally isolated from a mixture of rotaviruses (Bohl et al, 1984) suggesting that mixed infections occur naturally in pigs. Mixed infections by different electropherotypes of human rotavirus also have been reported (Spencer et al, 1983; Rodriguez et al, 1983). It is possible that mixed infections in pigs by two or more strains of rotavirus may cause more severe clinical disease than infection by either strain alone. The findings in the present study suggest that mixed infections by serotypes 1 and 2 of porcine rotavirus might result in more severe villous atrophy with the serotype 2 rotavirus producing the upper small intestinal lesion. This hypothesis deserves to be

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Histopathologic and scanning electron microscopic findings were similar to those reported previously (Pearson and McNulty, 1977; Theil et al, 1978; Crouch and Woode, 1978; McAdaragh et al, 1980; Torres-Medina and Underdahl, 1980; Narita et al, 1982). However, previous reports disagree about the time at which villous fusion first appears after inoculating gnotobiotic pigs with rotavirus. Theil et al (1978), using the OSU strain, and McAdaragh et al (1980), using the SDSU strain, observed villous fusion in gnotobiotic pigs by 24-48 hours PI. In contrast, villous fusion was first observed 4.5 days after inoculating pigs with a field strain (B-317) of porcine rotavirus (Torres-Medina and Underdahl, 1980). In the present study, villous fusion was seen at 24 hours PI, so results are in agreement with findings by Theil et al (1978) and McAdaragh et al (1980).

Gnotobiotic pigs in the present study did not have lesions or specific immunofluorescence in organs other than the small intestine. Immunofluorescence in the lamina propria and mesenteric lymph node of gnotobiotic pigs was not observed in the present study, but has been reported by others (Theil et al, 1978).

Immunofluorescence was observed in the cecal mucosa and cecal lumen of one pig infected with the OSU strain and one pig infected with the SDSU strain at 24 hours PI and has been observed in the cecum of gnotobiotic pigs by

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other investigators (McAdaragh et al, 1980; Narita et al, 1982). In general, the absence of histologic lesions and immunofluorescence in organs other than the small intestine is consistent with previous reports (Pearson and McNulty, 1977; Theil et al, 1978; McAdaragh et al, 1980).

The virus-excretion patterns for pigs infected with the OSU and SDSU strains in the present study were similar and in agreement with previous reports (Lecce et al, 1976; Bohl et al, 1978; Tzipori and Williams, 1978; Torres-Medina and Underdahl, 1980). The greatest number of rotavirus particles as detected by electron microscopy were shed by gnotobiotic pigs at 72 hours PI. The number of rotavirus particles was reduced, or virus particles could not be detected 7 days after infection.

As has been shown by other investigations (Torres-Medina and Underdahl, 1980), virus could be detected by electron microscopic examination of feces for a longer period of time when compared to immunofluorescence of the small intestine. Enterocytes infected by rotaviruses desquamate so immunofluorescent cells can only be detected up to 96 to 168 hours PI (Theil et al, 1978; McAdaragh et al, 1980). Immunofluorescent cells may be detected for even shorter periods of time after infection by some porcine rotavirus strains (Theil et al, 1985).

SUMMARY

The purpose of the present study was to compare serotypes 1 (Ohio State University strain) and 2 (South Dakota State University strain) of porcine rotavirus to determine if specific clinical signs, mortality rates, virus shedding patterns, virus antigen distribution, or lesion distribution and severity were related to the serotype of rotavirus with which pigs were infected.

Forty hysterotomy derived gnotobiotic pigs were inoculated orally at 3 days of age with 2 ml of homogenate containing 10^5 pig-infectious $dose_{50}$ of either the Ohio State University (OSU) strain or the South Dakota State University (SDSU) strain of porcine rotavirus. Controls were inoculated with media only. Mortality, clinical signs, and body weights were monitored daily. Five pigs in each of the virus inoculated groups and 4 control pigs were killed at 24 and 168 hours after inoculation. Four pigs in each of the 3 experimental groups (OSU, SDSU, and control) were killed 72 hours after inoculation. Specimens were collected at necropsy for serum chemistry, histologic, immunofluorescent, and scanning electron microscopic examinations.

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All of the pigs inoculated with the OSU or SDSU strains survived. Control pigs remained healthy throughout the study. Pigs inoculated with the OSU or SDSU strains developed diarrhea 19-48 hours and 24-54 hours after inoculation, respectively. Vomiting was observed in 5 of 14 (36%) of the pigs infected with the OSU strain whereas pigs inoculated with the SDSU strain did not vomit. Pigs inoculated with the OSU or SDSU strains had reduced weight gain compared to control pigs, but there was no difference in weight gain between pigs given the OSU or SDSU strains. Results of immunofluorescent examinations were similar for pigs given either rotavirus strain except that at 24 hours after inoculation, viral antigen was detected at the duodenal-jejunal flexure in 5/5 pigs given the OSU strain and in 1/5 pigs given the SDSU strain. Villous atrophy and fusion were more severe at the duodenal-jejunal flexure of pigs inoculated with the SDSU strain when compared to pigs inoculated with the OSU strain. Although the OSU and SDSU strains did show differences in the occurrence of vomiting and distribution of villous atrophy, these strains were equally virulent for 3-day-old gnotobiotic pigs. Therefore, prevention and control practices must be directed against both serotypes of porcine rotavirus.

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