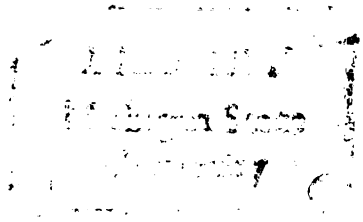

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EFFECT OF SERIAL PASSAGE OF TRANSMISSIBLE GASTROENTERITIS VACCINE VIRUS THROUGH GNOTOBIOTIC PIGS

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EFFECT OF SERIAL PASSAGE OF TRANSMISSIBLE GASTROENTERITIS VACCINE VIRUS THROUGH GNOTOBIOTIC PIGS

By

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ABSTRACT

EFFECT OF SERIAL PASSAGE OF TRANSMISSIBLE GASTROENTERITIS VACCINE VIRUS THROUGH GNOTOBIOTIC PIGS

By

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The effects of serial passage of transmissible gastroenteritis (TGE) vaccine virus were determined by inoculating gnotobiotic piglets from 3 litters with vaccine virus or saline. Control piglets remained normal while piglets infected with the vaccine virus developed mild clinical signs including diarrhea, dehydration and rough hair coat. There was no evidence of increase or decrease in severity of clinical signs with passage of the virus.

Measurement of histologic sections of small intestine revealed a consistent decrease in length of villi and a consistent increase in depth of glands of virus infected piglets when compared to control piglets. The decrease in villus length and increase in gland depth between passages 1 and 2 were statistically significant ($p<0.01$), but the changes between passages 2 and 3 were not significant ($p>0.01$).

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INTRODUCTION

Transmissible gastroenteritis is a coronavirus infection that has caused severe losses in very young pigs. The disease is characterized by vomiting, diarrhea, and dehydration. These are more pronounced in the young animal. The mortality rate in piglets over 3 weeks of age is diminished, and there is almost no mortality in adult swine.

Treatment of infected young pigs is not practical, and researchers have, therefore, devoted their efforts to developing clinically useful methods of prophylaxis.

Up to this date there has been no efficient and safe method of vaccinating the neonatal pigs soon after birth. For this reason, attention has been focused on vaccinating the pregnant sow so that she in turn can provide passive immunity to her pigs through the colostrum and milk. In order to maintain an effective protection for the piglet, antibodies must be supplied locally to the intestinal tract on a continuous basis. Not all the vaccines tested have provided this type of immunity.

One vaccine, which has been commercially available for a number of years, contains a live, attenuated strain of the virus. It is recommended that the pregnant sow be given 2 parenteral injections of this vaccine with the last injection being 7 to 30 days before parturition. However, one study has shown that the oral administration of this vaccine to young pigs resulted in clinical signs and lesions similar to TGE. Serial passage of this attenuated virus in conventional pigs apparently

caused an increased virulence of the virus.

Since it is possible that other infectious agents may have played a role in the development of the clinical signs and lesions reported in conventional pigs, it seemed wise to conduct a similar study in gnotobiotic pigs. This would help to further assess the disease-producing capability of the vaccine virus.

The objectives of this study were to:

1) Pass the TGE vaccine virus through a series of gnotobiotic pigs.
2) Assess the disease-producing capabilities of the virus at various passages by studying incubation period, clinical signs, and gross, histologic and fluorescence microscopic lesions.

LITERATURE REVIEW

History of Transmissible Gastroenteritis

The first report of an enteric disease in baby pigs characterized by vomiting, diarrhea, and high morbidity and mortality was recorded by Doyle and Hutchings (1946). This was the first accurate description of TGE in the United States. Whitehair et al. (1948) found a similar disease in young suckling pigs. This disease resulted in almost 100% morbidity and 70-90% mortality. These authors were unable to isolate and demonstrate any pathogenic organisms, and the primary lesions were confined to the small intestine. Another early suggestion of TGE was reported by Young and Underdahl (1955). Smith (1972) found a similar disease in Minnesota. Feenstra et al. (1948) studied TGE in baby pigs and compared it with a gastrointestinal disease in human infants. Wood (1969) described TGE in other countries.

Etiology

Doyle and Hutchings (1946) suggested a viral etiology when they described the filterable nature of the infectious agent. These authors produced clinical TGE by inoculating ground intestinal contents, or bacteria-free filtrates, from infected to susceptible pigs. Wilner (1969) stated that the etiological agent of TGE is an RNA-enveloped virus. It is very similar to the avian infectious bronchitis virus (IBV). However, Ritchie and Norman (1968) isolated 2 morphologically distinct agents from stock cultures of TGE virus. There was some controversy

concerning the etiological agent of TGE (McClurkin and Norman, 1966). The virus of TGE is now classified as having physical, chemical, and biological properties common to the genus coronavirus (Tajima, 1970). The chemical, physical, and biological properties of TGE virus have been studied almost exclusively with cell cultured-adapted strains. The genome consists of RNA as indicated by the inability of deoxyuridine derivatives to inhibit replication (McClurkin and Norman, 1967).

The fact that the virus of TGE is resistant to trypsin (Cartwright et al., 1965; Sheffy, 1965) aids its successful infection of the small intestine. The virus is sensitive to lipid solvents (Pensaert et al., 1970) and thus might be expected to be inactivated by bile in the duodenum. Harada et al. (1968), however, demonstrated the TGE virus to be relatively stable in 2% and 5% pig bile, but sensitive to a 50% suspension. This relative resistance of the virus to low concentration of bile should not be surprising since the virus is able to resist the concentration of bile in the duodenum to reach the remainder of the small intestine.

The virus of TGE appears to be relatively stable at acidic pH. McClurkin and Norman (1966) found the virus to be stable at a pH range of 4 to 8. Haelterman (1972) reported a two-log loss in infectivity of TGE virus after being held at pH 2 for 30 minutes. Since the most effective route of infection is by ingestion, the virus of TGE must reach the target organ, the small intestinal mucosa, via the stomach because the 18- to 24-hour incubation period seems too short to involve a hematogenous route of spread (Haelterman, 1972). Thus, TGE virus must be sufficiently resistant to the gastric pH (range 1.5 to 3.2) in order to reach the small intestine.

Antigenic diversity between isolants of TGE virus has not been demonstrated (Cartwright, 1966; Harada et al., 1968). Kemeny (1976) did not detect differences among 10 TGE isolants using a reciprocal plaque reduction neutralization test. Haelterman (1973) has emphasized that this should facilitate serological identification of virus in a control program.

Epizootiology

Haelterman (1962) stated that clinical disease had not been produced in any species other than swine, but that dogs and foxes had been shown to be susceptible to infection with TGE virus. Starlings fed infectious TGE virus have also been reported to shed virus in their feces for short periods (Pilchard, 1965). Antibodies of TGE have been detected in dogs (McClurkin et al., 1970; Norman et al., 1970). Cartwright and Lucas (1972) stated that a virus serologically related or identical to TGE of pigs is capable of producing a disease in dogs similar to that in pigs.

Larson et al. (1979) recently indicated that the dog was capable of having a role in the epizootiology of TGE. Fourteen neonatal dogs, ages 4 through 11 days, were given various dosages of TGE virus orally. Clinical signs of enteric disease did not develop, and jejunal epithelium from dogs euthanatized at 12, 24, 48, and 96 hours and at 10 days after exposure did not have morphologic alterations detectable by light microscopy. Electron microscopic examination, however, indicated that TGE virus was in the jejunal tissue of dogs as early as 12 hours and as late as 10 days after exposure to the virus.

Pathogenesis

The pathogenesis of TGE has been studied almost exclusively in baby pigs, and the main features have been outlined (Hooper and Haelterman, 1966; Haelterman, 1972). The virus of TGE is ingested and infects the columnar epithelial cells of the small intestine. Replication of the virus takes place in 4 to 5 hours. The absorptive cells of the villous epithelium are destroyed causing villi to shorten and broaden, and immature cuboidal epithelium replaces the columnar absorptive cells on the villi. This lesion is referred to as "villous atrophy". As a result of the destruction or alteration in function of these cells, an acute malabsorption syndrome occurs (Hooper and Haelterman, 1969). As a result of the malabsorption, diarrhea is seen as a clinical sign. Diarrhea occurs because both the surface areas and the enzymes are deficient in the flattened mucosa (Cross and Bohl, 1969; Thake, 1968; Maronpot and Whitehair, 1967). The diarrhea of TGE is largely osmotic in character (Haelterman, 1972). In pigs surviving the infection, villous atrophy is reversible because the cells in the crypts of Lieberkühn, which do not support the growth of TGE virus, regenerate new functional epithelial cells most of which are resistant to or are protected from reinfection with TGE virus (Haelterman, 1972). The atrophic villi elongate as the immature crypt cells migrate to the tips of the villi and mature from cuboidal to columnar cells. In pigs which survive TGE, the small intestinal mucosa usually returns to normal within 6 to 10 days following infection (Hooper and Haelterman, 1969; Pensaert et al., 1970).

Swine of all ages are susceptible to infection with TGE virus. However, the severity of the clinical signs and the duration of the disease decrease with age, while the mortality is inversely related to

the age of the pig. Vomiting is considered to be the first clinical sign of TGE in baby pigs. It usually occurs 20-24 hours after the infection, but it may be seen as early as 18 hours. Diarrhea follows vomition by a few hours, and the stools are profuse, watery, and yellow to green in color with flecks of undigested milk curds being seen. If the pigs survive, the diarrhea may last for 8-9 days but usually stops between the 4th and 6th days, and weight gains return to normal (Haelterman, 1963; Hooper and Haelterman, 1966). The clinical signs of infection in sows and feeder swine are inappetence, temporary weight loss, and profuse diarrhea. On the other hand, the diarrhea may be transient or the infection may even be subclinical. Some lactating sows with affected litters may become clinically ill with elevated temperatures, agalactia, vomiting, inappetence and diarrhea (Goodwin and Jennings, 1959), whereas sows having no contact with infected litters of pigs usually have mild or subclinical signs of disease (Bohl, 1975). The signs of TGE in feeder swine vary from a profuse, watery diarrhea with anorexia and soft stools to a complete absence of any signs (Morin et al., 1973; Olson, 1971; Morin and Morehouse, 1974). These clinically inapparent infections are important epidemiologically because the infected pigs are unlikely to be detected unless baby pigs are present on the premises. Pigs with clinically inapparent infections establish a new focus of TGE when they are introduced into clean herds.

Pigs are most rapidly infected by the oral route, and pig-to-pig transmission in the field occurs through ingestion of feces from sick or recently recovered pigs (Haelterman, 1972). Parenteral inoculation of TGE virus has been described, but much higher doses are required to reproduce the disease (Young et al., 1955; Haelterman, 1965). Kemeny

(1976) described mild clinical signs of TGE infection in 2 or 3 day old pigs given a single dose of relatively attenuated virus intravenously.

During the last few years, evidence has been accumulating indicating that fetuses from a variety of mammalian species are able to respond to an antigenic stimulus (Redman et al., 1974). The virus of TGE has not been incriminated in transplacental infections in swine. However, clinical evidence suggests that infections of sows with this virus during the breeding period, or shortly thereafter, may influence the embryonic survival rate (Dunne, 1975). Two fetuses in each of 3 sows were inoculated (intramuscularly) with a live attenuated Purdue strain of TGE on the 95th, 77th, or 74th days of gestation. All pigs inoculated in utero became serologically positive for TGE (Redman et al., 1978).

The disease has also been produced by intranasal inoculation (Young et al., 1955). These workers estimated that approximately 90% of their intranasal inoculum was probably swallowed. Underdahl et al. (1975) reported finding the virus in pulmonary and intestinal tissues of pigs at 104 days after they were inoculated. Isolation of virus from the nasal secretion of sows during the first few days of illness has been reported (Kemeny et al., 1975). Two of 10 sows used in this study had virus in their milk on the third or fourth day of illness. The involvement of sows' milk in spreading TGE infection has important epizootiologic implications. Kemeny and Woods (1977) have tested the role of sows in spreading TGE. Eleven sows were given virulent virus intravenously, intranasally, or intramammarily within 5 days of farrowing. All sows became clinically ill with signs of anorexia, depression, and fever. The disease can be transmitted by aerosol (Reber, 1956). The initial site of infection with virus must be the intestine, and the virus must reach the intestine through

the stomach since the incubation period seems to be too short to involve a hematogenous route (Haelterman, 1972).

When TGE virus is inoculated orally and/or intranasally, it is swallowed and is able to withstand intragastric pH and lipolytic activity of bile in the duodenum to reach the small intestine in the infective form. However, bile may prevent infection of cells in the anterior part of the duodenum (Haelterman, 1972). Hooper and Haelterman (1966) stated that TGE virus replicated rapidly and to a high titer in the jejunum and duodenum and to a lesser extent in the ileum but that it did not replicate in the stomach or colon.

Waxler (1972) and Olsen et al. (1973) used scanning electron microscopy to study the lesions of TGE in pigs infected under conventional and gnotobiotic conditions. They described marked shortening of villi in the jejunum and ileum. Less severe changes were described in the duodenum. Frederick et al. (1976) reported that 1 attenuated, tissue-culture-adapted strain of TGE virus caused atrophy of villi in only the posterior half or 2/3 of the small intestine of either gnotobiotic or conventionally-reared pigs. This is in contrast to lesions throughout the small intestine when the virulent virus was used and may explain the milder clinical signs produced by the attenuated virus. Spotts (1974) studied the regeneration of small intestinal villi in gnotobiotic pigs infected with TGE virus. Scanning electron microscopic studies indicated that incomplete regeneration of villi was present at 4 days after exposure in the cranial jejunum, but appeared much later in the ileum. Even at 12 and 15 days, villi in the ileum did not appear normal.

Moon et al. (1975) studied the degree of villous atrophy and apparent rates of regeneration of intestinal villi in newborn, 3-week-old, and

adult swine for 1 week after they were exposed to TGE virus. There was a maximal villous atrophy and comparatively slow regeneration in the newborn group. In the other 2 groups, villous atrophy was less severe and regeneration was more rapid.

Gross Lesions

The gross lesions of TGE in pigs have been described by Bay et al. (1949) and Bay (1952). Later, Hooper and Haelterman (1966a) described the gross lesions of TGE in more detail. They consist of dehydration and loss of body weight, engorgement of gastric and mesenteric blood vessels, distention of stomach with milk curd, and absence of chyle in the subserosal and mesenteric lymph vessels and nodes. Distention of the intestine with white to yellow-green fluid and urate deposits in the renal pelvis have also been described. Hooper and Haelterman (1966b) described the most characteristic lesion of TGE which is "villous atrophy". The functional significance of villous atrophy was interpreted as a response to the rapid loss of mucosal epithelium (Hooper and Haelterman, 1966a; Thake, 1968). Gross lesions as observed in gnotobiotic pigs by Olsen et al. (1973) were excessive gas and liquid-ration curd in the stomach, hyperemia of gastric and mesenteric blood vessels, and distention of the small intestine with gas and straw-colored fluid.

Hooper and Haelterman (1969) stated that villous atrophy is associated with a failure of cells, migrating upward from the crypts of Lieberkühn, to undergo normal differentiation and maturation into the columnar epithelium. Moon et al. (1970) and Moon (1978) stated that villous atrophy with TGE is a primary factor in the pathogenesis of diarrhea. Trapp et al. (1966) described the lesion in gnotobiotic pigs

exposed to TGE virus. The villous atrophy was marked in the jejunum and ileum but not in the duodenum. Olsen et al. (1973) studied the lesions of TGE in gnotobiotic pigs. There was uniform and consistent atrophy of all villi in jejunum and ileum by 18 hours after exposure to the TGE virus. Hooper and Haelterman (1969) indicated that, within 1 day after exposure, most of the villi were atrophied to a uniform and maximum degree.

Microscopic Lesions

Cellular degeneration in the small intestine with TGE has been described (Bay et al., 1951; Hooper and Haelterman, 1966; Trapp et al., 1966). The microscopic lesions mainly involved the jejunum and ileum (Hooper and Haelterman, 1966). Atrophy of small intestinal villi has been reported as a major lesion of TGE (Thake, 1968; Trapp et al., 1966; Waxler, 1972). Epithelial cells covering the villi in the jejunum and ileum were lost rapidly and in large numbers in TGE. The cells covering the shortened villi were undifferentiated and appeared as low cuboidal or squamous type cells (Hooper and Haelterman, 1969). Although there was a rapid and severe loss of intestinal epithelium, the mucosa was not as severely denuded as might be expected. Thake (1968), however, noticed that the tips of some villi were partially denuded. Denudation of epithelium involving the upper 1/3 to 1/2 of the infected villi has been described (Bay et al., 1951).

Hooper and Haelterman (1969) described vacuolation of epithelial cells in pigs with TGE. Alexander et al. (1969) and Olson et al. (1973) described the vacuolation of epithelial cells of jejunum and ileum as a finding in normal gnotobiotic pigs. Christie (1967) and Dress and Waxler (1970) also noted vacuole formation in gnotobiotic pigs experimentally

infected with Escherichia coli 0138:K81:NM. Vacuolated epithelium did not occur in pigs 2-3 weeks old after exposure of the jejunum to E. coli enterotoxin (Moon et al., 1971). Moon (1972) hypothesized that differentiation of porcine ileal absorptive cells to vacuolated, pinocytic cells is a function of cell age and occurs about 3 to 4 days after DNA synthesis.

Moon has the following evidence supporting his hypothesis: (1) Cells remained on the villi of day-old pigs for 7-10 days after synthesis of DNA and only for 2-4 days in 21-day-old pigs (Moon, 1971). Thus, the disappearance of ileal vacuoles when pigs were about 3 weeks old coincides with disappearance of cells that survive more than 4 days after synthesis of DNA. (2) Vacuolated ileal epithelium also disappeared in rats at about 3 weeks of age. The long-lived epithelial cells disappeared in this species as well (Koldovsky et al., 1966). (3) The percentage of a villus covered by vacuolated cells tended to decreased from 70% or more in newborn pigs, to 50% or less during the second week of life, to zero in most 21-day-old pigs. (4) The short, blunt villi over Peyer's patches were not vacuolated. If cells were replaced at the same rate (number of cells per unit time) on these villi as on the long villi with vacuolated epithelium, then the cells would be sloughed from these short villi before they reach the age of vacuolation. Moon et al. (1973) stated that the differences between germ-free and conventional pigs in mucosal dimensions, cell migration rates, and vacuolation were similar to those between 1 and 21-day old suckling pigs (Moon, 1971, 1972). Fat globules were found in the cytoplasm of epithelial cells from the small intestine infected with TGE virus (Thake, 1968).

The microvillous border of intestinal epithelial cells of pigs with TGE was often reduced in height, indistinct, or absent (Maronpot and

Whitehair, 1967; Thake, 1968; Trapp et al., 1966). Trapp et al. (1966) and Hooper and Haelterman (1969) reported a normal ratio of villous-height/crypt-depth (V/C) of 7:1 in the small intestine. In TGE, the V/C ratio was reduced to 1:1. Moon (1971), however, reported a similar situation in the normal animal and stated that a decreased epithelial cell replacement time in the intestine with age is associated with an increase in crypt depth, a decrease in villous length, and a resultant 3-fold decrease in V/C ratio.

Moon et al. (1970) reported that TGE was not the only common enteric disease of pigs which causes villous atrophy. They stated that atrophy of small intestinal villi has also been demonstrated in piglets in the terminal stages of E. coli diarrhea. Arbuckle (1975) described the macroscopic and microscopic appearance of villous atrophy in weaned pigs orally infected with Salmonella choleraesuis.

Rotavirus has been incriminated as a cause of diarrhea in young pigs (Rodger et al., 1975; Saif et al., 1977; Bohl et al., 1978; Bohl, 1979), especially in 2- to 4-week-old suckling pigs. Bohl (1979) described villous atrophy in rotaviral infection. The author stated that the pathogenesis of rotaviral infection appears similar to that which occurs in TGE.

The disease due to rotavirus in suckling pigs appeared similar to the syndrome commonly referred to as milk scours, white scours, or 3-week scours (Bohl, 1978).

Immunity to TGE

During the past 4 decades, there has been considerable research effort toward the development of an effective method of immunizing swine

against TGE. The practical use of immunity in the control of TGE is complicated by the fact that the animals most in need of protection are pigs in the first few days of life (Saif et al., 1972; Bohl et al., 1975; Saif and Bohl, 1977). Even if such baby pigs were exposed at once after birth, the time required for the immune response to reach an effective level would appear to preclude active immunization of newborn pigs. This is coupled with the fact that neither antiviral drugs nor chemotherapeutic measures have been found to be effective against TGE.

Although 2- to 3-week-old pigs may survive infection with TGE (Abou-Youssef and Ristic, 1975; Moon et al., 1975), neonatal pigs 1 to 10 days after farrowing are extremely susceptible to infection with this virus (Ferris, 1973). Thus, a considerable amount of attention now has been devoted to means for providing passive immunity to suckling pigs. Bay et al. (1953) stated that exposing pregnant swine to TGE virus at least 3 weeks before farrowing will provide protection to the pigs nursing the dam after farrowing. Haelterman (1965) stated that this immunity is of a passive nature and results from antibody levels in the colostrum and milk. Evidence for this concept has been reviewed (Bohl et al., 1975). It is important to remember that transplacental transfer of maternal antibodies does not occur in swine and that the newborn pig gets all its circulating passive antibody through the colostrum. Evidence shows that the offspring of sows exposed to virulent TGE virus are protected against TGE if the suckling pigs ingest colostrum and milk during the first 2 weeks of life (Abou-Youssef and Ristic, 1975; Bohl et al., 1975; Stone et al., 1974). Circulating antibodies in the newborn pigs, whether derived from the colostrum or by injection of antiserum, are not protective because the humoral antibodies do not reach the major site of viral infection --

the epithelial cells of the small intestine (Morin et al., 1973; Thake et al., 1973).

Because neonatal pigs are virtually agammaglobulinemia (Abou-Youssef, 1975; Bourne, 1974), they must continually receive colostrum and milk containing a high concentration of TGE immune globulins during the first 10 days to protect the intestinal mucosa against infection by TGE virus. Suckling pigs are protected as a result of the frequent ingestion of colostrum and milk which contains TGE-neutralizing antibodies. As long as a continuous supply of TGE antibodies is present in the lumen of the small intestine of susceptible pigs, it will neutralize ingested TGE virus and thus protect against the disease. Haelterman (1965) and Hooper and Haelterman (1966) have referred to this immunologic mechanism as lactogenic immunity. Because colostrum and early milk contain the basic spectrum of immunoglobulins which include immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM), (Abou-Youssef and Ristic, 1975; Stone et al., 1974), there is controversy as to which of the three is most efficiently effective. Some investigators have reported that IgA offers greatest protection (Abou-Youssef et al., 1975; Bohl et al., 1975) although antibodies as measured by the plaque-reduction test may be found in all immunoglobulin classes.

Abou-Youssef and Ristic (1975) and Stone et al. (1977) studied the efficiency of isolated colostral IgA, IgG and IgM that protect neonatal pigs against the coronavirus of TGE. The data showed that all 3 immunoglobulin classes in immune colostrum were protective for neonatal pigs against exposure to virulent TGE virus.

Gel filtration studies of colostrum and milk fractions from naturally exposed sows showed early postpartum colostral antibodies, primarily of

IgG class, which were probably derived from serum. This was followed by high IgA to IgG ratios in the later milk (Svendsen and Brown, 1973). The anti-TGE activity in the milk was associated with secretory IgA throughout lactation (Bohl et al., 1972b; Saif and Bohl, 1977). It was only after stimulation of the intestinal tract of the sow, following oral or natural infection with virulent TGE virus, that IgA TGE antibodies were produced in the milk (Bohl et al., 1972a,b).

Oral inoculation with a live, high-cell-culture-passaged strain of TGE virus produced IgG antibodies in the milk and only small amounts of IgA (Bohl and Saif, 1975; Morilla et al., 1976). Saif and Bohl (1979) have reviewed the passive immunity in TGE and stated:

> "Six pregnant sows and 4 gnotobiotic piglets were inoculated orally and intranasally with the Miller strain of transmissible gastroenteritis (TGE) virus passaged 14 times in porcine kidney cell culture (vaccinal virus). The 4 gnotobiotic piglets developed severe clinical illness after viral inoculation, and mortality was high. Of the 6 sows, 4 seroconverted and 2 remained serologically negative for TGE. Milk titres from the single sow (seroconverted) which was clinically ill remained high, and TGE antibodies were associated primarily with the IgA class. Challenge of this sow's litter with virulent TGE virus resulted in low mortality (11%). In contrast, the 3 sows (seroconverted) which remained well after inoculation had low TGE antibody titres in their serum, and milk titres declined rapidly after the sows had farrowed. Gel filtration studies indicated that milk antibodies in these 3 sows were almost exclusively of the IgG class. Protection of their pigs after oral challenge with virulent TGE virus was poor (50% to 71% mortality) and all 3 sows were clinically affected. These sows were not experimentally challenged, but were exposed to TGE by contact with their infected litters. It was calculated that protective concentrations of IgA antibodies were produced in the milk only after an adequate stimulation of the intestinal tract of the sow with TGE virus."

If lactogenic immunity is to succeed, TGE antibodies must exist in the lumen of the gut in sufficient concentration and at the right time

to neutralize or modify the variable quantity of virus presented in nature to the susceptible pig. Sows recovered from natural or planned infections are capable of transmitting effective passive immunity to their suckling pigs (Bay et al., 1953; Bohl et al., 1975). Planned infections have been accomplished by feeding intestines of infected pigs to gestating sows in infected herds, and it has been effective where inoculated sows farrowed at least 2 weeks or more after exposure. Planned infection is dangerous from an epidemiological standpoint. This is because TGE virus may spread to other herds, some of which may contain suckling pigs. Also, the preparation used for infection may contain pathogens other than TGE virus. This procedure of planned infection could be more efficient and safe if virulent TGE were used as an immunizing agent during the summer months when the chance of the disease spreading to other herds would be lessened (Bohl, 1975).

The basic mechanism of active immunity against TGE is not known. One possible explanation is the elaboration of TGE antibodies of the secretory IgA class by plasma cells of the lamina propria of the intestinal tract and their secretion onto the mucosal surface, thus providing protection to the mucous membrane of the small intestine against infection (Bohl et al., 1972b; Sprino et al., 1976). Another mechanism that might be responsible for active immunity is local cell-mediated-immunity (CMI) in the small intestine. Frederick and Bohl (1976) found that the production of a macrophage-migration-inhibition factor by lymphocytes obtained from the lamina propria of the small intestine was greater than that by splenic lymphocytes in pigs that had been exposed orally to TGE virus and that the reverse was true for pigs exposed subcutaneously to the virus. The authors, however, stated that the duration of CMI was not known

because the number of pigs used in their experiment was not great enough.

Woods (1977), using the direct leukocyte-migration-inhibition (LMI) test, demonstrated CMI in pigs exposed orally to TGE virus. Sprino et al. (1976) have suggested that the resistance to reinfection in TGE might be established by the combined action of local CMI and intestinal secretory IgA. Recently Woods (1979) described his results using LMI procedures to compare the CMI response of swine exposed to virulent or attenuated TGE virus and uninfected cell fluids. Woods (1979) stated:

> "Swine exposed to attenuated transmissible gastroenteritis virus had higher virus-neutralizing antibody titres than did swine exposed to virulent virus. The cellular response, measured by the direct leukocyte-migration-inhibition (LMI) procedure, was greater in swine exposed to virulent virus than in swine exposed to the attenuated virus. Leukocytes from exposed swine were inhibited more in LMI procedure in the presence of the homologous sensitizing antigen than in the presence of the heterologous viral antigen. The humoral response measured by virus neutralization reached a peak 21 days after exposure, and the cellular response measured by LMI reached a peak 28 days after exposure."

Vaccine

Several attempts have been made to develop an effective and safe vaccine for pregnant sows which could provide passive immunity to their piglets. This is a goal of considerable importance. An inactivated vaccine (Welter, 1965; Fuller and Welter, 1966; Fuller, 1967) became commercially available in the United States in October, 1965. Directions called for intramuscular injection of the vaccine at 60 and 30 days prior to farrowing to provide protection to the suckling pigs. However, the Veterinary Biologics Division of the USDA did not renew the license of this product because of questionable or inadequate efficiency, and the vaccine has not been commercially available since October, 1967 (Bohl, 1975).

Another commercial TGE vaccine was licensed in 1970 and is currently available (Fuller, 1971). It is composed of a live, attenuated TGE virus and is to be administered intramuscularly. Two doses of vaccine should be given with a 30-day interval between vaccinations. The second dose is recommended to be given to sows one month after the first vaccination and at 7 to 30 days before farrowing. The vaccine stimulates the production of high levels of circulating antibodies, but does not protect the intestine of the sow nor does it stimulate production of specific IgA in the milk of sows (Bohl et al., 1975). Baby pigs suckling these immunized sows appear to resist challenge to virulent virus during the first few days of life due to the ingestion of high levels of IgG concentrated in the colostrum (Bohl et al., 1972b; Abou-Youssef and Ristic, 1975). This protection, however, wanes after this time due to an increase in milk production and absence of specific IgA (Bohl et al., 1975; Bohl et al., 1972b; Saif et al., 1972). This vaccine is restricted to use in pregnant sows and not in growing and finishing pigs which remain susceptible to infection although they are present in a vaccinated herd.

Safety of a modified live virus TGE vaccine has been demonstrated by feeding it to 3-day-old pigs (Tamoglia, 1972). He reported a mortality of 38% in pigs nursing sows that had been vaccinated with the commercial vaccine, 71% in pigs nursing nonvaccinated control sows and 100% in pigs nursing sows that had been experimentally infected with virulent virus during gestation. The morbidity rate was 100% for pigs nursing either the vaccinated or the nonvaccinated sows and 14% for pigs nursing the experimentally infected sows. Tamoglia (1972) also suggested that this vaccine is of limited value in preventing infection and clinical signs of TGE in baby pigs, but tends to reduce the high mortality associated

with the occurrence of this disease in neonatal pigs.

Morehouse (1975) indicated that, in limited trials, clinical signs and lesions typical of TGE were seen in pigs exposed orally at 2 days of age to the commercially available vaccine and that further passage of the virus decreased survival time. Mild TGE in pigs suckling sows vaccinated with the commercial TGE vaccine has been reported (Larson et al., 1980). The authors stated:

> "A strain of transmissible gastroenteritis (TGE) virus of low virulence was isolated from 14-day-old pigs suckling sows vaccinated with an attenuated TGE vaccine. Diarrhea developed in suckling pigs approximately 14 days after farrowing in 4 farrowings; however, none of these pigs died from diarrhea. Diarrhea ceased after the fourth farrowing, when vaccination of sows was discontinued. Experimentally, both the field isolate and the vaccine strain were ineffective and in some instances lethal for 2-day-old pigs exposed orally. However, neither strain was as virulent as the Purdue strain."

Recently a new oral vaccine for protection of pigs against TGE has been developed (Welter, 1980). He stated:

> "The vaccine can safely be fed to pregnant sows, as well as baby pigs (thereby inducing both active and passive local immunity). Results of experimental and clinical trials showed this vaccine to be more effective than a commercially available vaccine (administered intramuscularly)."

MATERIALS AND METHODS

Animals

Gnotobiotic pigs were obtained by hysterotomy from 3 crossbred sows. The technique used was described by Waxler et al. (1966). Epidural anesthesia was obtained by injection of 25 ml of 2.5% procaine hydrochloride[a] at the lumbosacral articulation (Getty, 1963). Following the administration of the epidural anesthetic, 5 ml of tranquilizer[b] was given intramuscularly. The sow was restrained in lateral recumbency with the left side uppermost. After surgical preparation of the left flank region, the skin was sprayed with a sterile surgical adhesive[c], and the bottom of the isolator was placed over this area. Working through the rubber gloves of the isolator, the operators cut through the vinyl floor of the isolator and skin of the sow with an electrical cautery unit. The rest of the abdominal wall was incised with scissors. Each pig was removed through a separate incision in the wall of the uterus.

When the pigs were removed from the uterus, self-locking clamps[d] were placed on the umbilical stump 3 cm from the abdominal wall to prevent hemorrhage, then the umbilical cord was severed. The surviving pigs were aseptically transferred into plastic rearing isolators.

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[a]Epidural, Haver-Lockhart, Kansas City, MO.

[b]Sparine, Wyeth Laboratories, Philadelphia, PA.

[c]Vi-Drape, Parke-Davis, Detroit, MI

[d]"Double-Grip" disposable cord-clamp, Hollister, Inc., Chicago, IL.

The 3 litters consisted of 11, 12, and 10 pigs, respectively, at the time of delivery. Each litter comprised one experimental group. Three pigs from each litter were maintained in a separate isolator and acted as unexposed controls. The rest of the pigs were divided into 3 groups with 2 or 3 pigs being put in each isolator, depending on the litter size (Table 1). Each isolator contained a maximum of 3 pigs in individual cages along with equipment for feeding, inoculation, and sample collection for microbiologic determination. An adequate supply of a commercial sterilized liquid diet[e] was aseptically transferred into each isolator, and a measured quantity was placed in pans at each feeding.

Table 1. Number and Distribution of Pigs from 3 Litters.

Litter No.	Isolator No.			
	I	II	III	IV
1	3	3	2	3
2	3	3	3	3
3	2	2	3	3

Two pigs died before exposure (1 at 2 days of age, and 1 at 5 days of age) and were not included as part of the experimental groups. The remaining 31 pigs were used in this study. The pigs were maintained in individual cages in sterile plastic rearing isolators at 32 C. The

[e]SPF-Lac, Borden, Inc., Norfolk, VA.

temperature was gradually reduced to 29 C after 72 hours. Each pig was fed 120 ml of the liquid ration 3 times per day.

Bacteriological Status

Swabs were taken from each isolator prior to exposure of the pigs. Specimens consisted of rectal swabs and waste material from the cages. Material was streaked on tryptose blood agar[f] plates and inoculated into thiglycollate broth[f]. The media were incubated both aerobically and anaerobically at room temperature, 37 C and 55 C. Material was also inoculated into PPLO broth[f] and incubated aerobically at 37 C for 5 days. A blind passage was then made from the broth onto a PPLO plate and into another broth and incubated in the same manner for 5 days. The same blind passage and incubation were repeated 2 more times. All cultures were observed for 3 weeks.

TGE Vaccine Virus

The TGE vaccine virus[g] was prepared according to the manufacturer's instructions. The vaccine was diluted to 50 ml and filtered through an 0.45 µm membrane filter[h]. The filtrate was then transferred into 5 ml sterile glass ampules and heat sealed.

Control Inoculum

Ampules containing sterile Hank's balanced salt solutions (HBSS) were prepared (Kalter, 1963). The HBBS was filtered through an 0.45 µm

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[f]Difco Laboratories, Detroit, MI.

[g]TGE-VAC, Diamond Laboratories, Des Moines, IA.

[h]Nalge, Sybron Corporation, Rochester, NY.

membrane filter, and 1 ml was transferred into a sterile ampule which was heat sealed.

Ampules containing the vaccine or the control inoculum were checked for leaks by placing them upside down in a beaker of dilute methylene blue[i]. The beaker was then put into a dessicator which was attached to a vacuum pump. A vacuum equivalent to 27 inches of mercury was obtained and held for 2 to 3 minutes. The vacuum was then released. If a leak was present in the ampule seal, stain was pulled into the ampule. Any leaking ampules were then discarded.

Serial Passage of Infectious Agent

The ampules containing the TGE vaccine virus were aseptically transferred into the first isolator, and each pig to be exposed was given 1.0 ml of the material orally at 2 days of age. The liquid was aspirated into a 5 ml disposable plastic syringe and expelled onto the base of the pig's tongue. One pig in the fourth (control) isolator was given 1.0 ml of HBSS.

Three days following exposure, the pigs in the exposed isolator were euthanatized within the isolator. Euthanasia was accomplished by injection of 1.5 ml sodium pentobarbital[j] intravenously into the anterior vena cava. After the peritoneal cavity was opened, the contents of the cecum and spiral colon from each of the pigs were aspirated into a sterile 10 ml plastic syringe. The syringes were passed out of the isolator, and the contents were pooled and placed into sterile 5 ml glass ampules. An

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[i]Sigma Chemical Co., St. Louis, MO.

[j]Haver-Lockhart Laboratories, Kansas City, MO.

ampule containing the pooled intestinal contents was aseptically transferred into the second isolator, and each pig was given 1 ml of this pooled intestinal matter. In the same manner, the pig in the control isolator was euthanatized, and 1 ml of pooled intestinal contents was given orally to the second control pig in the same isolator. The ampules containing the rest of the pooled intestinal contents from the exposed and control pigs were frozen.

Using the same techniques, pigs in the third isolator were exposed to intestinal contents from pigs in the second isolator, and in the control isolator, contents were passed from the second to the third pig. The distribution of exposed and control pigs in the three passages is shown in Table 2.

Determination of Clinical Signs

Throughout the experiment, pigs were observed carefully for clinical signs including inappetence, weakness, diarrhea and vomiting.

Necropsy Procedures

The pigs were killed at 72 hours after exposure by giving 1.5 ml of sodium pentobarbital[j] intravenously into the anterior vena cava. They were then placed in dorsal recumbency, and the sternum and ventral abdominal wall were incised and reflected caudally exposing the abdominal viscera. The small intestine was removed by severing it adjacent to the pylorus and to the ileocecal valve and incising along the mesenteric attachment. Sections approximately 2 cm in length were removed from each of 7 locations. The first (Level 1) was taken from the duodenum 5 cm from the pylorus, and the last (Level 7) was taken from the ileum 5 cm

Table 2. Numbers of Pigs Exposed to TGE Vaccine Virus and Control Pigs in the Three Passages.

Litter No.	1st Passage		2nd Passage		3rd Passage		Total
	Exposed	Control	Exposed	Control	Exposed	Control	
1	3	1	3	1	2	1	11
2	3	1	3	1	2	1	11
3	2	1	2	1	2	1	9

anterior to the ileocecal valve. The other sections (2,3,4,5,6, respectively) were taken at equal distances between Levels 1 and 7. These sections of intestine, along with pieces of kidney, liver and spleen, were fixed in 10% buffered neutral formalin solution, dehydrated, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin according to established procedures (Luna, 1968). These sections were used for light microscopic studies.

Histologic Examination

Length of villi and depth of glands were measured at 7 levels in the small intestine by use of an ocular micrometer. At each level, the lengths of the villi were measured from the tip of the villi to the base of the villi. The depths of glands were measured from the base of villi to the deepest part of the glands. Ten measurements were taken from each level. Data were analyzed by analysis of variance. Differences were compared by students' t test (Goulden, 1952).

Immunofluorescence

Sections approximately 7 cm in length were taken adjacent to levels 1,3,5 and 7 as described above, for fluorescent-antibody determinations. Each section was placed on a cork disc 2.2 cm in diameter and 0.3 cm thick. Enough embedding medium[k] was added to cover and support these cross sections. This disc was placed in a beaker which was put into a jar containing a dry ice and acetone slurry. When the tissue was frozen,

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[k]Tissue-Tek II O.C.T. Compound, Lab-Tek Products Division, Miles Laboratories, Inc., Naperville, IL.

it was placed into a small labeled plastic bag and sealed. The sections were stored at -70 C until processed. They were mounted onto cryostat stubs and quickly frozen at -25 C in a cryostat[1]. Four- to five-micron thick cross sections were cut, mounted on glass slides, and dried at 37 C overnight. The sections were then fixed in acetone for 10 minutes at room temperature. They were then overlaid with hyperimmune anti-TGE globulin which had been conjugated with fluorescein isothiocyanate[m] (Black, 1971) and incubated for 30 minutes at 37 C in a moist chamber. Sections were then washed twice for 15 minutes, each time in phosphate buffered saline (PBS) at pH 7.5. The slides were then coverslipped with a mixture of PBS and glycerol (1:9).

The mounted specimens were examined on a Zeiss photomicroscope III[n] equipped for immunofluorescence.

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[1]International Equipment Co., Needham Haight, MA.

[m]TGE Conjugate, N.A.D.C., Ames, IA.

[n]Carl Zeiss, Oberkochen, West Germany.

RESULTS

Clinical Signs

One pig from litter 2 died 5 days after birth and 1 pig from litter 3 died 2 days after birth. These pigs died before being exposed to TGE vaccine. They had little or no liquid diet in the digestive tracts with the stomachs and intestines containing only a small amount of mucus.

The 9 control pigs did not have evidence of any clinical illness throughout the experiment. The feces were light brown and had a creamy consistency.

Most of the 22 exposed pigs had evidence of diarrhea (Table 3). Other clinical signs noted were dehydration and rough hair coat, but the exposed pigs maintained a more or less normal appetite and remained active throughout the experiment. None of the exposed pigs died before euthanasia.

Gross Lesions

There was no evidence of gross lesions in any of the control pigs. The stomach and intestinal contents appeared to be normal and there were no gross lesions.

Most exposed pigs in all the 3 litters had evidence of gross lesions except 3 pigs in the first litter in which the gross lesions were absent.

In addition to the dehydration noted under clinical signs, gross lesions were confined to the gastrointestinal tract. These included

Table 3. Presence of Diarrhea at the Time of Euthanasia in Pigs Exposed to TGE Vaccine Virus or Intestinal Contents 3 Days Previously.

Litter No.	Pig No.	Passage No.	Diarrhea
1	1	I 1	-
	2	I 1	+
	3	I 1	-
	4	C 1	-
	5	I 2	+
	6	I 2	++
	7	I 2	++
	8	C 2	-
	9	I 3	++
	10	I 3	+++
	11	C 3	-
2	12	I 1	+
	13	I 1	++
	14	I 1	+
	15	C 1	-
	16	I 2	++
	17	I 2	++
	18	I 2	+
	19	C 2	-
	20	I 3	++
	21	I 3	++
	22	C 3	-
3	23	I 1	++
	24	I 1	+
	25	C 1	-
	26	I 2	++
	27	I 2	+
	28	C 2	-
	29	I 3	++
	30	I 3	-
	31	C 3	-

I = Infected
C = Control
1 = Passage number 1
2 = Passage number 2
3 = Passage number 3
- = No visible diarrhea
+ = Slight diarrhea
++ = Moderate diarrhea
+++ = Marked diarrhea

distention of the stomach with a mixture of gas and semisolid, undigested liquid diet and engorgement of gastric and mesenteric blood vessels. The wall of the small intestine was thin. Distention of the small intestine was variable with some exposed pigs having marked distention but other only moderate distention. The fluid in the small intestine was either white or sometimes brown in color. Mucus was observed in the small intestine. The contents of the colon and cecum were generally creamy in consistency and yellow to tan. In some exposed pigs the contents were fluid and brown in color. One exposed pig in litter 3 had perirenal edema.

Microscopic Lesions

Control Pigs: Examination of the small intestine by light microscopy revealed variation in the length and configuration of villi. Most were long and finger-like (Figure 1). The surface of the villi was often folded inward along the sides of the villi, and many goblet cells were seen (Figure 2). The crypts of Lieberkühn had a normal histological pattern. The surface of the villi in the lower jejunum and ileum had the same microscopic appearance, but vacuolation of the epithelial cells was increased (Figure 3). The glands in most pigs were 85-100 microns in depth, while most of the villi were 550-700 microns in height. The ratio of villus height to gland depth was about 7:1 in the small intestine (Tables 4,5,6). There were some variations from these figures.

Exposed Pigs: In the pigs exposed to the TGE vaccine virus, the microscopic lesions were confined mainly to the posterior one-half or two-thirds of the small intestine. In Levels 5, 6 and 7, which represent the lower jejunum and ileum, the microscopic changes were marked as compared to Levels 1, 2 and 3 (Figure 4). In Levels 5, 6 and 7, there was

Figure 1. Photomicrograph of Level 1 of the intestine of a pig euthanatized 3 days after exposure to control inoculum. The villi have normal configurations with most being long and finger-like. The surface of the villi is covered by simple columnar epithelium. H & E stain, 48X.

Figure 2. Photomicrograph of Level 1 of the intestine of a pig euthanatized 3 days after exposure to control inoculum. The villi have normal configurations. Many goblet cells are seen (arrow). H & E stain, 120X.

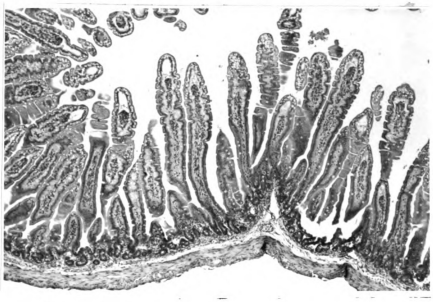


Figure 1

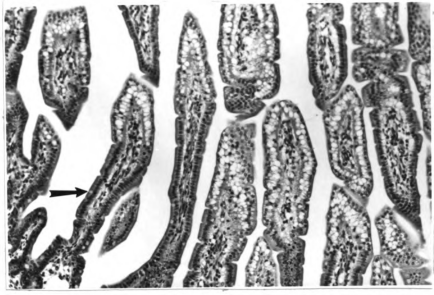


Figure 2

Figure 3. Photomicrograph of Level 5 from the intestine of a pig euthanatized 3 days after exposure to control inoculum. The villi have a normal appearance, but epithelial cell vacuolation is increased as compared to the duodenum. H & E stain, 48X.

Figure 4. Photomicrograph of Level 5 of the intestine of a pig euthanatized 3 days after exposure to the TGE vaccine virus. Marked atrophy of nearly all villi. H & E stain, 48X.

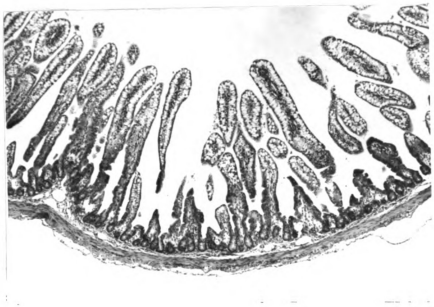


Figure 3

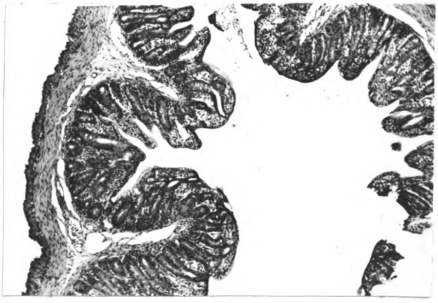


Figure 4

Table 4. Length of Intestinal Villi and Depth of Glands in Small Intestine of Gnotobiotic Pigs Exposed to TGE Vaccine Virus. Litter 1.

Passage No.	Level of Intestine	Vaccine Virus			Control	
		Villi	Glands		Villi	Glands
1	1*	(3)** 487***	118	(1)	628	92
	2	355	106		617	88
	3	371	105		512	85
	4	424	103		604	87
	5	475	104		446	78
	6	387	102		701	80
	7	390	105		320	90
2	1	(3) 448	132	(1)	561	122
	2	386	118		864	87
	3	184	119		612	99
	4	164	142		616	98
	5	152	140		726	87
	6	148	145		515	90
	7	176	136		577	99
3	1	(2) 674	100	(1)	600	110
	2	392	117		542	88
	3	267	131		557	91
	4	150	131		594	85
	5	145	141		479	98
	6	126	147		458	87
	7	178	154		503	95

*Level 1 = 5 cm from pylorus; Level 7 = 5 cm from ileocecal junction; Levels 2 through 6 equally spaced between Levels 1 and 7.
**Number of pigs in each group.
***Values expressed in microns, represent the means of 10 measurements made on each level of intestine of each pig.

Table 5. Length of Intestinal Villi and Depth of Glands in Small Intestine of Gnotobiotic Pigs Exposed to TGE Vaccine Virus. Litter 2.

Passage No.	Level of Intestine	Vaccine Virus			Control	
		Villi	Glands		Villi	Glands
1	1*	(3)** 543***	92	(1)	554	88
	2	635	91		522	94
	3	634	94		665	78
	4	617	93		831	86
	5	540	103		603	77
	6	506	98		855	81
	7	421	121		739	84
2	1	(3) 317	114	(1)	759	84
	2	296	106		545	81
	3	270	144		721	85
	4	137	145		362	87
	5	73	157		417	88
	6	67	168		484	86
	7	143	177		464	86
3	1	(2) 536	110	(1)	641	97
	2	476	121		602	90
	3	404	138		643	96
	4	444	140		718	107
	5	372	138		848	97
	6	389	136		825	98
	7	462	131		901	103

*Level 1 = 5 cm from pylorus; Level 7 = 5 cm from ileocecal junction; Levels 2 through 6 equally spaced between Levels 1 and 7.
**Number of pigs in each group.
***Values, expressed in microns, represent the means of 10 measurements made on each level of intestine of each pig.

Table 6. Length of Intestinal Villi and Depth of Glands in Small Intestine of Gnotobiotic Pigs Exposed to TGE Vaccine Virus. Litter 3.

Passage No.	Level of Intestine	Vaccine Virus		Control	
		Villi	Glands	Villi	Glands
1	1*	(2)** 478***	168	(1) 594	112
	2	376	116	617	100
	3	202	128	751	124
	4	268	121	804	96
	5	186	116	718	97
	6	268	110	773	99
	7	192	133	632	89
2	1	(2) 363	161	(1) 632	167
	2	237	146	614	183
	3	218	143	632	158
	4	239	114	858	115
	5	148	121	804	96
	6	129	141	889	107
	7	186	148	614	132
3	1	(2) 425	142	(1) 717	162
	2	365	147	551	115
	3	290	145	546	108
	4	195	143	615	99
	5	156	157	637	95
	6	157	157	657	103
	7	217	164	683	117

*Level 1 = 5 cm from pylorus; Level 7 = 5 cm from ileocecal junction; Levels 2 through 6 equally spaced between Levels 1 and 7.
**Number of pigs in each group.
***Values, expressed in microns, represent the means of 10 measurements made on each level of intestine of each pig.

uniform and consistent atrophy of most of the villi. Villi were absent or very short and difficult to identify in some areas. Many villi were fused with adjacent ones so that individual villi were indistinct. The crypts of Lieberkühn were increased in depth. Numerous cells in the glands were undergoing mitosis, and the epithelial cells were crowded and sometimes appeared pseudostratified. The epithelium covering most of the shortened villi had undergone marked change to become low cuboidal or sometimes squamous in nature. Extensive bulging and sloughing of groups of epithelial cells from the tips and sides of villi were observed (Figure 5).

In Levels 1 and 2 (duodenum) of exposed pigs, the changes were slight to nonexistant (Figure 6). When histologic sections of the intestine from second and third passage pigs were examined, the lesions noted were similar to those seen in tissues from pigs receiving the vaccine virus directly.

Measurement of Villi and Glands

Analysis of variance indicated that exposure to the TGE vaccine virus resulted in a statistically significant ($p<0.0005$) reduction in the length of intestinal villi and increase in the depth of intestinal glands as compared to unexposed pigs. Analysis did not reveal a statistically significant difference in the shortening of the villi at different levels of the intestine for exposed and unexposed pigs combined. However, when the average length of the villi was expressed graphically (Figure 7), there was a decided trend, in exposed pigs, toward more severe shortening in the posterior portion of the small intestine. The significance of this result is supported by the strong interaction of treatment and level in the

Figure 5. Photomicrograph of Level 5 of the intestine of a pig euthanatized 3 days after exposure to the TGE vaccine virus. The epithelium covering most of the shortened villi has undergone marked changes to become low cuboidal or sometimes squamous in nature. Epithelium is missing in some areas (arrow). H & E stain, 120X.

Figure 6. Photomicrograph of Level 1 of the intestine of a pig euthanatized 3 days after exposure to the TGE vaccine virus. Changes are slight or nonexistent when compared to lower jejunum (Figure 4). H & E stain, 48X.

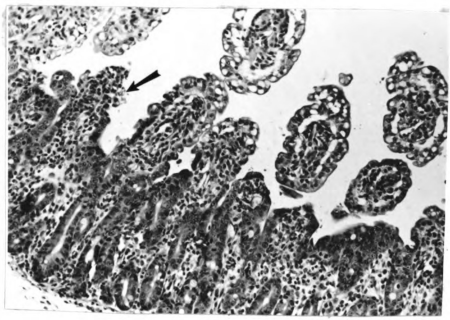


Figure 5

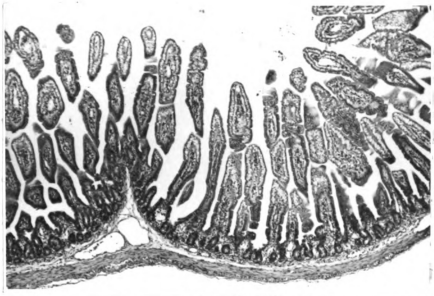
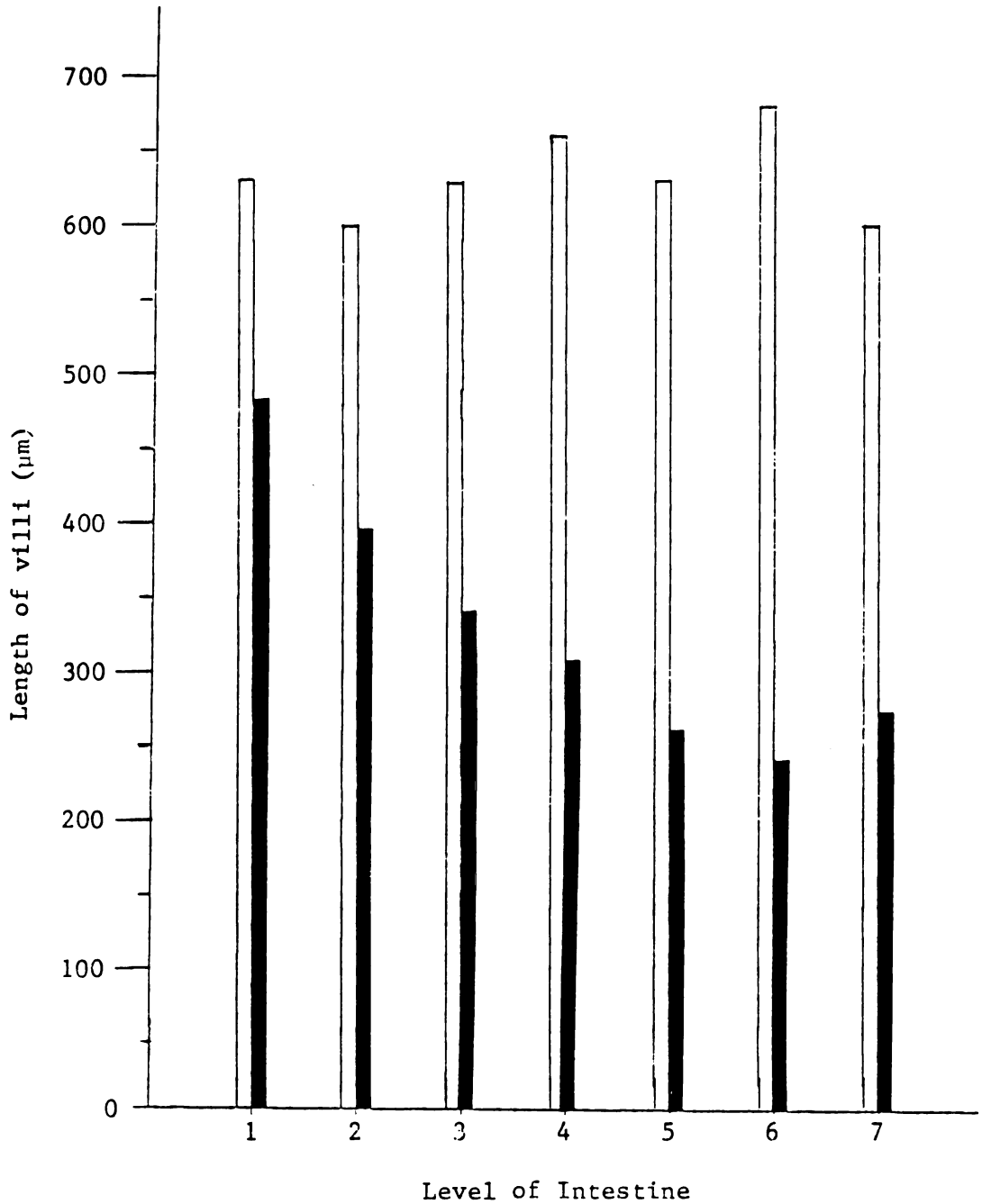


Figure 6

Figure 7. Length of intestinal villi of pigs exposed to TGE vaccine virus and unexposed pigs.



Level 1 = 5 cm from pylorus; Level 7 = 5 cm from ileo-cecal junction; Levels 2 through 6 equally spaced between Levels 1 and 7.

Control ☐ Exposed ■

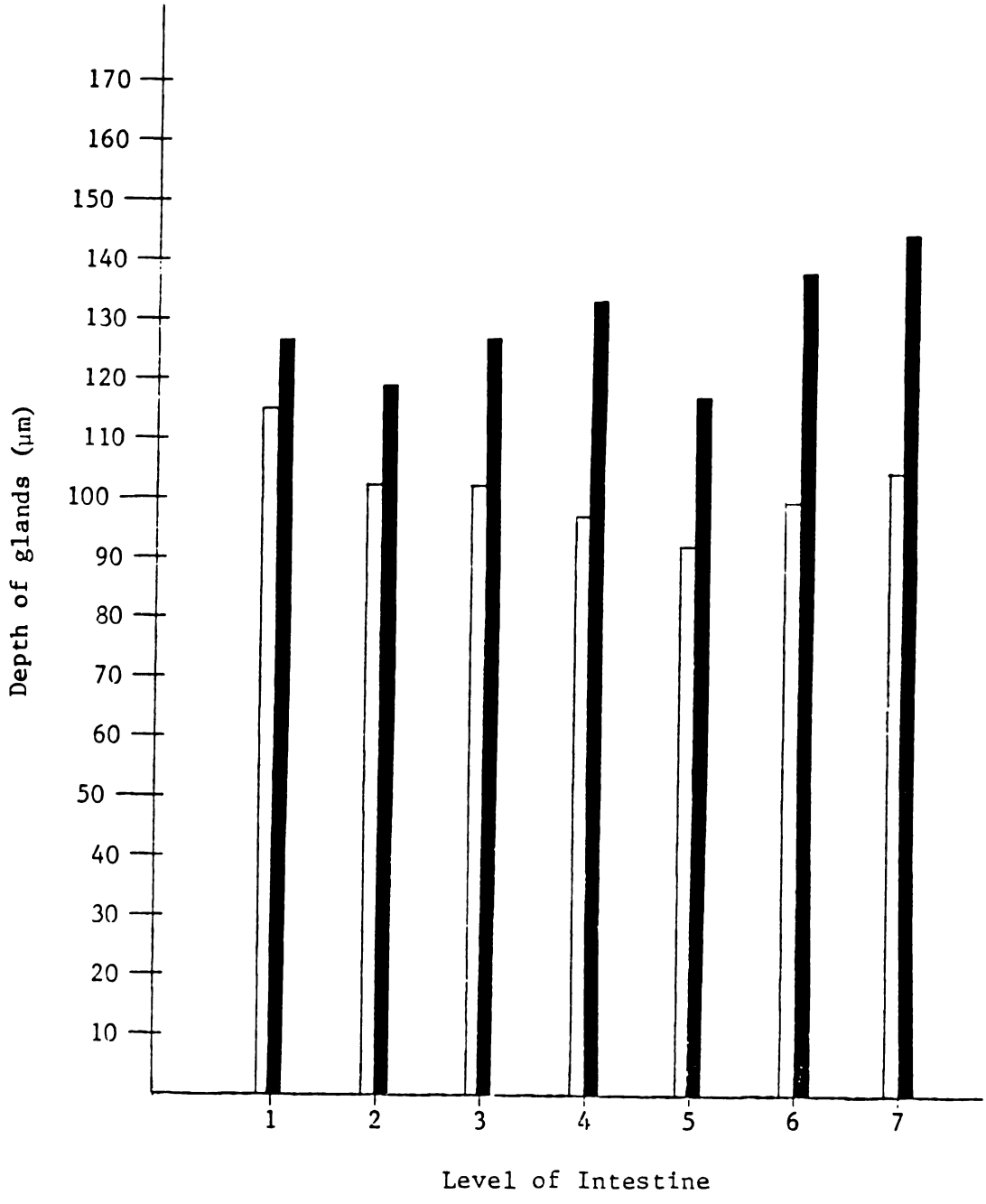
analysis of variance ($p<0.02$). In a similar fashion, there was a trend, in exposed pigs, toward a greater increase in the depth of glands in the posterior portion of the small intestine (Figure 8). For the glands there was an interaction of treatment and level in the analysis of variance ($p<0.04$).

When the effects of pig-to-pig passage of the TGE vaccine virus were analyzed statistically by analysis of variance, there were significant differences in the length of intestinal villi ($p<0.01$) and the depth of intestinal glands ($p<0.0005$) among passages. When these differences were compared by students' t test, the decrease in the length of the villi and increase in the depth of the glands when compared to unexposed values was statistically significant ($p<0.01$) at all 3 passage levels (Tables 7 and 8). Application of students' t test to differences among passages indicated that differences between passages 1 and 2 were significant ($p<0.01$) but that the differences between passages 2 and 3 were not significant ($p<0.1$).

Immunofluorescence

When anti-TGE conjugate was applied to intestinal sections from pigs in these litters, there was no immunofluorescence in sections from control pigs. All of the intestinal sections from pigs exposed to the vaccine virus had some degree of positive immunofluorescence. Also, sections from pigs of the second and third passages had variable degrees of positive immunofluorescence (Table 9).

Figure 8. Depth of intestinal glands of pigs exposed to TGE vaccine virus and unexposed pigs.



Level 1 = 5 cm from pylorus; Level 7 = 5 cm from ileocecal junction; Levels 2 through 6 equally spaced between Levels 1 and 7.

Control □ Exposed ■

Table 7. Effect of Serial Passage of Attenuated Transmissible Gastroenteritis Virus on Length of Intestinal Villi in Gnotobiotic Pigs.

Passage No.	Length of Intestinal Villi (μm)*	
	Virus Exposed	Unexposed
1	415.4 ± 44.7**	640.2 ± 73.0
2	212.5 ± 44.7**	631.8 ± 73.0
3	324.7 ± 51.6**	631.1 ± 73.0

* ± Standard error of mean.
**Differences between Passages 1 and 2 significant ($p<0.01$).
Differences between Passages 2 and 3 not significant ($p>0.1$).

Table 8. Effect of Serial Passage of Attenuated Transmissible Gastroenteritis Virus on Length of Intestinal Villi in Gnotobiotic Pigs.

Passage No.	Depth of Intestinal Glands (μm)*	
	Virus Exposed	Unexposed
1	111.0 ± 5.9**	90.8 ± 9.7
2	139.0 ± 5.9**	107.9 ± 9.7
3	140.1 ± 6.9**	102.1 ± 9.7

* ± Standard error of mean.
**Differences between Passages 1 and 2 significant ($p<0.01$)
Differences between Passages 2 and 3 not significant ($p>0.1$)

Table 9. Effect of Serial Passage of TGE Vaccine Virus on Viral Antigen in Intestinal Epithelial Cells as Determined by Immunofluorescence.

Litter No.	Pig No.	Passage No.	Intestinal Level* 1	3	5	7
1	1	I 1	-	+	+	++
	2	I 1	++	++++	++	++++
	3	I 1	++	++++	++++	++++
	4	C 1	-	-	-	-
	5	I 2	+	++++	++++	++++
	6	I 2	+	++++	++++	++++
	7	I 2	+	++++	++++	++++
	8	C 2	-	-	-	-
	9	I 3	+	++++	++	+++
	10	I 3	+	++++	++++	+++
	11	C 3	-	-	-	-
2	12	I 1	-	-	+	++
	13	I 1	-	+	++	+++
	14	I 1	+	++	++++	+++
	15	C 1	-	-	-	-
	16	I 2	+	+++	+++	++++
	17	I 2	+	++	+++	++++
	18	I 2	++	++++	++++	++++
	19	C 2	-	-	-	-
	20	I 3	-	++	++++	+++
	21	I 3	-	++	++++	+++
	22	C 3	-	-	-	-
3	23	I 1	-	++	++++	+++
	24	I 1	-	-	-	++
	25	C 1	-	-	-	-
	26	I 2	++	++	+	++++
	27	I 2	-	++++	+++	++++
	28	C 2	-	-	-	-
	29	I 3	-	+	+++	++++
	30	I 3	-	+++	+++	+++
	31	C 3	-	-	-	-

*Level 1 = 5 cm from pylorus; Level 7 = 5 cm from ileocecal junction; Levels 3 and 5 are equally spaced between Levels 1 and 7.
I = Infected; C = Control
1 = Passage number 1; 2 = Passage number 2; 3 = Passage number 3
- = No fluorescing cells; + = 1-10 fluorescing cells/slide;
++ = 11-20 fluorescing cells/slide; +++ = 21-30 fluorescing cells/slide;
++++ = More than 30 fluorescing cells/slide

Microbiologic Examination

At the end of the experiments, the material for culture from litters 1 and 2 was negative for the growth of bacteria and mycoplasma with one exception. From Isolator 4, litter I, a gram-positive coccus (*Staphylococcus epidermis*) was isolated. In litter 3, all isolators contained a rod-shaped organism (*Bacillus sp.*).

DISCUSSION

Clinical Signs

None of the control pigs had any clinical signs of TGE or other illness. There was evidence that exposed pigs had clinical signs (anorexia, diarrhea, dehydration, and rough hair coat) which indicated they were infected with the vaccine virus of TGE. Many of the clinical signs in the exposed pigs were similar to those previously described for TGE (Hooper and Haelterman, 1966a, 1969; Bohl, 1975). Differences in the clinical signs (anorexia, diarrhea, dehydration) among litters and passages in the same litter were seen (Table 3). Morehouse (1975) reported that serial passage of the vaccine virus in young conventional pigs resulted in marked increase in mortality and severity of lesions. This was not confirmed in gnotobiotic pigs in the present work. Also, an additional report by workers in Missouri, Larson et al. (1980), indicated that a strain of TGE virus of low virulence was isolated from 14-day-old pigs suckling sows vaccinated with an attenuated TGE vaccine. Experimentally, both the field isolate and vaccine strain were infective, and in some instances lethal, for 2-day-old pigs exposed orally; however, neither strain was as virulent as the Purdue strain. In this study we did not have any mortality in gnotobiotic pigs. However, if the pigs had been permitted to live longer we may have had some mortality.

At the time of surgical delivery, all pigs from the 3 litters appeared to be in good health. One pig in litter 2 had evidence of weakness 3 days

after birth. It consumed no food after that time and died 2 days later. One pig in litter 3 was weak and died at 2 days of age. There were no gross or histological lesions found in these pigs to explain the cause of death.

A possible explanation for the death of these 2 pigs is that they may have had hypoglycemia. Swine have been found to have a low tolerance for glucose. Goodwin (1957) observed that a newborn pig soon dies when fasted. Graham et al. (1941) reported spontaneous hypoglycemia in newborn pigs. Schaffer et al. (1963, 1965) encountered high morbidity and mortality in pigs during the first 48 hours of life. Their diet consisted of unsupplemented cow's milk, and they attributed the losses to hypoglycemia. Although the diet fed in the present study was apparently adequate, the weakness and anorexia experienced by the 2 pigs probably led to hypoglycemia.

Gross Lesions

Many of the gross lesions in exposed pigs in the present study were consistent with those reported earlier by Trapp et al. (1966), Hooper and Haelterman (1969), Olson et al. (1973, Bay (1952) and Spotts (1974). These results suggest that the vaccine virus of TGE has the capability of infecting the intestine of gnotobiotic pigs and of producing lesions similar to those described in both conventional and gnotobiotic pigs infected with the virulent virus as found in the field.

In the present study there was no increase in the severity of the gross lesions as the vaccine virus passed from one pig to the other.

One exposed pig in litter 3 had perirenal edema. This pig was in the third passage group. The cause of this lesion was not determined.

Microscopic Lesions

In control pigs, there was no evidence of any microscopic lesions in the small intestine. This is in contrast to the exposed pigs from all 3 litters in which most had evidence of microscopic lesions similar to those of TGE in the small intestine.

Microscopic lesions in exposed pigs in this study were similar to those previously reported by Trapp et al. (1966), Olson et al. (1973) and Larson (1980).

Measurements made on the histologic sections of the small intestine revealed rather consistent decreases in the length of villi and increase in the depth of glands in pigs exposed to the vaccine virus when compared to control animals (Tables 4, 5 and 6). The extent of these changes could not be related to the number of pig passages.

Although there was evidence that the virulence of the TGE vaccine virus, as measured by decreased length of villi and increased depth of glands, increased between passage 1 and passage 2, this was not true between passage 2 and passage 3. The cause of this effect could not be explained because of the limited number of pig passages. Further research in gnotobiotic pigs is needed to determine if additional passages would produce significant effects on the length of the villi and depth of glands.

Immunofluorescence

There was no evidence of increase or decrease in the amount of viral antigen in intestinal epithelial cells, as determined by immunofluorescence, with passage of the vaccine virus (Table 9). This would suggest that the virus was not becoming more infectious with pig-to-pig passage.

Microbiologic Examination

Gnotobiotic conditions were maintained in litters 1 and 2 with the exception of one isolator in litter 1 which became contaminated. This was the isolator that contained the control pigs. The bacterium Staphylococcus epidermidis probably was a skin contaminant from a tear in the glove of the isolator. In litter 3, where all isolators were contaminated by a rod-shaped organism (Bacillus sp.), it is assumed that the most likely cause was improper sterilization of some of the isolator equipment or supplies since the contaminant was a spore forming organism. There was no evidence that the bacterial contaminant altered the course of the disease or had any effect upon this study.

The results of this study indicated that the attenuated strain of the TGE vaccine virus is fairly stable after 3 pig passages. In the gnotobiotic pigs, the virus retains its ability to produce lesions, but there is no evidence that it consistently increases in virulence as a result of pig-to-pig passage.

SUMMARY

In a study of the effects of serial passage of transmissible gastroenteritis (TGE) vaccine virus through gnotobiotic pigs, 3 litters of pigs were used. In each litter, the vaccine virus was given orally to 2 or 3 pigs at 3 days of age. Intestinal material was then collected at necropsy 3 days later and given orally to additional pigs. The virus was thus passed through 3 groups of pigs in each litter. At the same time, 1 pig received sterile diluent in the same manner and acted as a control.

Although clinical signs such as diarrhea, dehydration, and rough hair coat were seen in some pigs, they were not marked, and there was no evidence of increase or decrease in severity of clinical signs with passage of the virus. Similarly, decrease in the thickness of the wall of the small intestine was seen at necropsy, but the degree of this change could not be correlated with passage of the virus.

Measurements made on histologic sections of the small intestine revealed a consistent decrease in the length of villi and a consistent increase in the depth of glands of pigs exposed to the vaccine virus when compared to control animals. The decrease in villus length and increase in gland depth between passages 1 and 2 were statistically significant ($p<0.01$), but the changes between passages 2 and 3 were not significant ($p>0.1$).

When the intestine was examined by immunofluorescence, there was no evidence of decrease in the amount of viral antigen in intestinal

epithelial cells with passage of the vaccine.

The results of this study indicated that the attenuated strain of the TGE vaccine virus is fairly stable through 3 pig passages. In the gnotobiotic pig, the virus retains its ability to produce lesions, but its virulence does not appear to increase in 3 pig passages.

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