Age Dependent Resistance to Transmissible Gastroenteritis of Swine (TGE) I. Clinical Signs and Some Mucosal Dimensions in Small Intestine

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ABSTRACT

Pigs were exposed to transmissible gastroenteritis (TGE) virus when three days old or when 21 days old. Diarrhea was earliest in onset, most frequent, most profuse and most prolonged in the youngest group. Pigs exposed when three days old also had a higher case fatality rate than those exposed when 21 days old. The histological response of both groups to exposure was atrophy of villi and hyperplasia of crypts in jejunum and ileum. However, from days three to seven post-exposure, when most fatalities occurred in the younger group, atrophy of villi was both more intensive and extensive in the younger group. Hyperplasia of crypts was also greater and more prolonged in the younger group. Regeneration of atrophic villi was more rapid in jejunum than ileum in both groups. Results were interpreted to indicate two populations, with different rates of regeneration, in the 21-day old group. Based on this interpretation, regeneration of villi was more rapid in one population from the 21-day old group than in the three-day old group.

The length of villi and depth of crypts in control pigs varied longitudinally (i.e. from site to site) in the intestine, within each age group. Length of villi and depth of crypts in control pigs also varied with age.

RÉSUMÉ

On a infecté des porcelets âgés respectivement de trois et de 21 jours, avec le virus de la gastro-entérite transmissible. La diarrhée apparut plus rapidement et plus souvent, elle s'avéra plus intense et dura plus longtemps chez les plus jeunes porcelets. On observa un taux de mortalité plus élevé, lorsqu'on infectait les sujets à l'âge de trois jours. La réponse histologique à l'infection se traduisit par une atrophie des villosités et une hyperplasie des cryptes du jéjunum et de l'iléon, au sein des deux groupes. Toutefois, de trois à sept jours après l'infection, alors que la mortalité atteignit son point culminant chez les sujets du plus jeune groupe, l'atrophie des villosités s'y avéra aussi plus intense et plus diffuse. Il en fut de même pour l'hyperplasie des cryptes. La régénération des villosités atrophiées s'effectua plus rapidement dans le jéjunum que dans l'iléon, chez les deux groupes. On interpréta ces résultats comme une indication de deux populations, avec des taux différents de régénération, chez les porcelets âgés de 21 jours. D'après cette interprétation, la régénération des villosités s'effectua plus rapidement chez certains des porcelets âgés de 21 jours que chez ceux de trois jours.

La hauteur des villosités et la profondeur des cryptes variaient d'un endroit à l'autre de l'intestin, au sein de chacun des deux groupes de porcelets témoins. Ces particularités variaient aussi selon l'âge des témoins.

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INTRODUCTION

Transmissible gastroenteritis of swine (TGE) is characterized by destruction of absorptive cells of villous epithelium in the small intestine, by virus (3, 6, 12, 14). As a result, villi shorten and broaden, and low cuboidal epithelium replaces the columnar absorptive cells on villi. This lesion is referred to as villous atrophy, and the resultant functional deficit is referred to as malabsorption (5, 6). Malabsorption occurs because both surface area and enzyme content are deficient in the flattened mucosa (3, 14). The clinical manifestation of malabsorption in TGE is diarrhea which usually leads to dehydration and death in newborn pigs. Villous atrophy is reversible as the mucosa regenerates in pigs which recover (6, 12). Such regeneration could result from the elongation of atrophic villi, the formation of new villi, or both. We do not know which mechanism is correct, but will refer to the process as the regeneration of villi.

Pigs of all ages are susceptible to TGE; however, the case fatality rate is high in newborn pigs and low after two to three weeks of age (4, 15). The work reported here was designed to study this difference in case fatality rate with age. Our hypothesis was based on the observation that three week old pigs normally replace villous absorptive cells in the small intestine about three times more rapidly than do newborn pigs (7). As diarrhea in TGE results from the loss of these cells, we hypothesized that during TGE, newborn pigs do not regenerate villi as rapidly as older pigs, thus resulting in a more prolonged diarrhea and a higher case fatality rate for the former. Our objective in this study was to compare clinical signs and the rates of regeneration of atrophic villi in pigs exposed to TGE virus at either three days or at three weeks of age.

MATERIAL AND METHODS

PIGS

Hysterectomy-derived, colostrum-deprived pigs were assigned to one of two groups. One group included 70 pigs that were three days old (Group A), and the second group included 38 pigs that were 21 days old (Group B). The experiments involving different lots (consisting of pigs from one to three litters) were conducted at different times over a 15 month period. Pigs were received during the first day of life, numbered by notching their ears, and following this, some pigs from each lot were assigned to Group A or B by random selection of their numbers. Similarly, pigs in each group to be principals (58 pigs in Group A and 29 pigs in Group B) or controls (12 pigs in Group A and nine pigs in Group B) were selected randomly. Furthermore, a random kill sequence was established for principal and control pigs in each age group. Tissues from pigs that died were not examined histologically, and the numbers for such pigs were removed from the random kill sequence after death. When a pig was to be killed, the surviving pig whose number was nearest the top of the random sequence, was killed.

EXPOSURE

When pigs in a lot were three days old, the principals in Group A were weighed and given (intragastrically, by stomach tube) 2.0 ml of a 10^{-5} dilution (in phosphate buffered saline) of the TGE virus preparation described by Tamoglia (13). This dose contained twice the lethal dose 50 percent (LD_{50}) for 72-hour old orphan pigs (13). When pigs in a lot were 21 days old, the principals in Group B were given a dose of virus preparation equal to the mean dose of virus/kg body weight that was given principals in Group A. The source, dilution and route of administration of the virus preparation were the same for all lots. Controls were not exposed and were held in an isolation room separate from principals.

NECROPSY

Twenty-five principals in each group were killed (sodium pentobarbital given intrathoracically) at 24 or 48 hr intervals from one to 15 days post-exposure (PE). Controls were killed at comparable ages (Fig. 1). Segments of intestine 10 cm long were prepared from five sites by intraluminal fixation with 10 percent formalin in 0.85 percent saline as previously (8). Site 1 was duodenum (the first 10 cm distal to the pylorus); Site 2 was 50-60 cm distal to Site 1; Site 3 was one-third, and Site 4 was two-thirds of the distance between

Sites 2 and 5. Site 5 was 15-25 cm proximal to the ileocecal valve. Sites 2 and 3 were called jejunum, and Sites 4 and 5 ileum. The remainder of the small intestine was removed from the mesentery, its contents discarded, and the tissue used for virus titration. The results of virus titration were reported separately (10).

Portions of fixed segments of intestine were embedded in paraffin; sections 7 μ thick were cut from these, stained with hematoxylin-eosin, and examined by light microscopy. Five well-oriented, complete crypts and villi in the sections from each site were measured with an ocular micrometer.

Serum osmolalities were determined by freezing point depression on samples from blood taken from the anterior vena cava just before pigs were killed.

RESULTS

Clinical Signs

Signs were compared between principal groups for the first seven days PE. Pigs killed prior to Day 7 PE were not included in order to simplify this comparison of signs. Some but not all pigs in each group were seen vomiting. All 43 pigs compared in Group A had diarrhea (mean time of onset, Day 1.9 PE; range time of onset, Day 1-4 PE). However, only 14 of 16 pigs compared in Group B had diarrhea. The mean time of onset in these 14 was Day 3.3 PE (range, Day 1-6 PE). Diarrhea was more profuse and dehydration (judged by decreased skin pliability and prominence of bony processes of scapula, pelvis and orbit) more severe in Group A than Group B. The striking difference between groups was that 28 of 43 pigs in Group A, and none of 16 in Group B died in the first seven days PE. The peak death loss in Group A was Day 4 PE when 14 pigs died. The sole death loss in Group B occurred after the comparison period on Day 9 PE.

There was also a difference between groups in the few principals available on Day 15 PE in that two of three pigs in Group A, and none of three pigs in Group B had diarrhea.

None of the controls in either group was seen vomiting; five had mild diarrhea of one or two days duration.

Serum osmolalities of control pigs tended

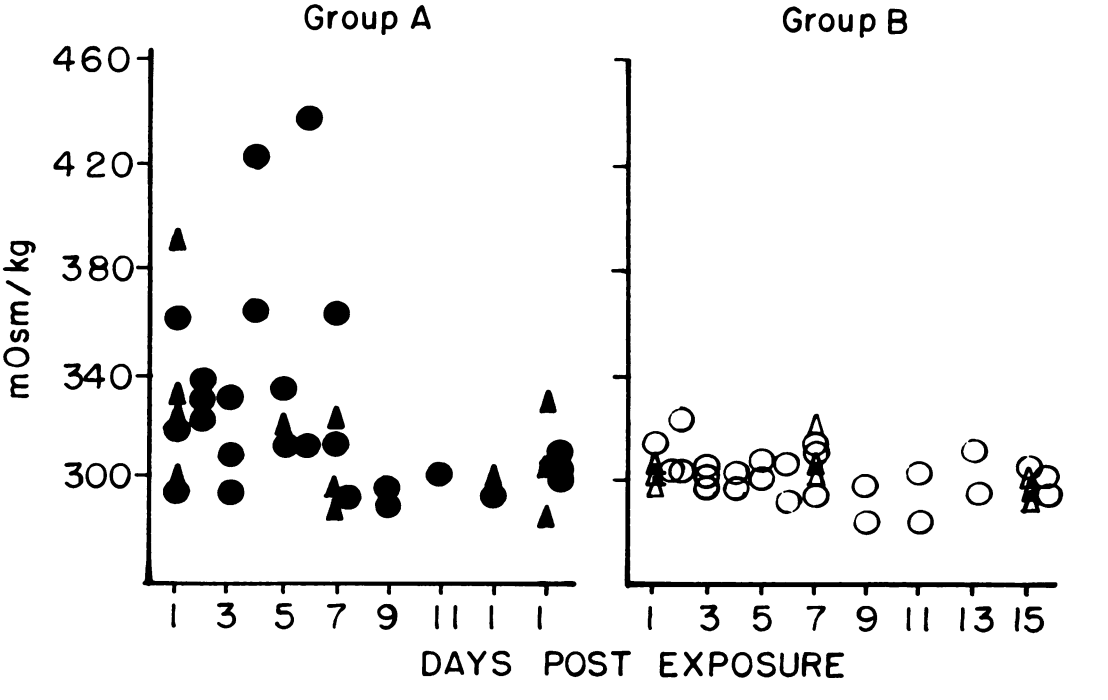

Fig. 1. Osmolality (milliosmoles/kg) of serum from control pigs (▲ or △) and principal pigs (● or ○). Principals in Groups A and B were three and 21 days old respectivly when exposed to TGE virus.

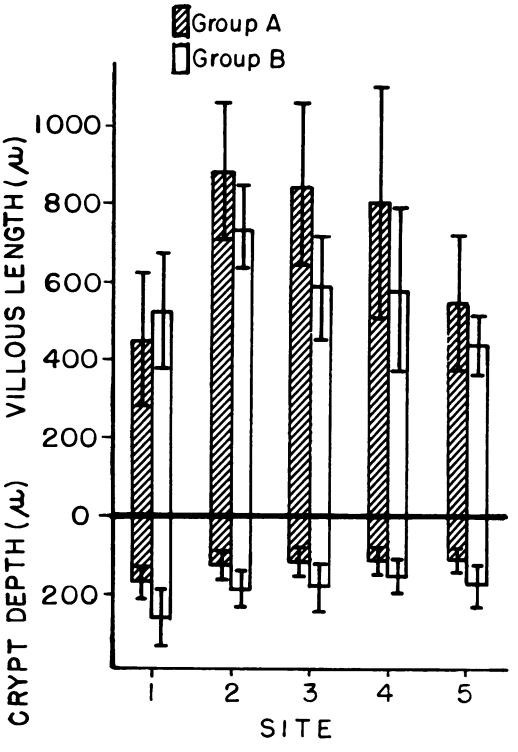

Fig. 2. Mean villous length and crypt depth in the small intestine of control pigs. Site 1 is duodenum, Sites 2 and 3 jejunum, Sites 4 and 5 ileum. Pigs in Groups A and B were four to 18 and 22 to 36 days old respectively when killed. Brackets represent the standard error.

to vary less and to decrease with age (Fig. 1). Serum osmolalities of principals in Group B were similar to controls. In contrast, serum of some principals in Group A was hypertonic on Days 4 to 7 PE, as compared to controls.

HISTOPATHOLOGY

In control pigs, there were significant ($P < 0.05$) differences between groups and among sites within pigs for both length of villi and depth of crypts. As shown in Figs. 2 and 4, pigs in Group B had shorter villi and deeper crypts than in Group A. In both groups, villi were shorter at Sites 1 and 5 than at 2, 3 and 4, and crypts were deeper at Site 1 than elsewhere. Villi were also significantly ($P < 0.05$) shorter and crypts significantly ($P < 0.05$) deeper as the experiments progressed in each control group (Fig. 4). Site 1 (duodenum) was not included in Fig. 4 because Site 1 was usually not affected in TGE (3, 5), and because villous atrophy was not observed at Site 1 in this study.

Data on jejunum and ileum from principals in both groups are compared with appropriate controls in Figs. 3 and 5. Considering Sites 2 to 5 combined as one, villous length in Group A was reduced to 25 percent of the control value compared with 55 percent in Group B. The nadir of villous length in Group A was Day 4 PE compared with Day 2 PE in Group B. The rates of regeneration from these low points did not appear different between groups. The increase in crypt depth in both groups was most noticeable one day after the nadir of villous length. The percent increase in crypt depth was greater in Group B than Group A. However, there were differences in villous length between sites and groups which were not apparent when Sites 2 to 5 were combined as in Fig. 5.

Villous atrophy occurred at all four sites in jejunum and ileum in both groups (Figs. 6 and 7). In Group A, return to control values occurred progressively earlier from distal to proximal small intestine. As a result, both incidence and degree of lesion were greater in ileum than jejunum of this group (compare Sites 2 and 5, Fig. 6). During the second week PE, villous atrophy at all sites in Group A was partial and probably would not have been recognized without a quantitative comparison with controls. There were differences between sites in Group B similar to those in Group A (compare Figs. 6 and 7). The greater incidence and degree of lesions in the ileum were most apparent in the first week PE in Group B but not until the second week PE in Group A. The maximum degree of villous atrophy was less in Group B than Group A, chiefly because villi in Group B controls were shorter. The response was more uniform in Group A than Group B. Data from both jejunum and ileum of Group B were clustered as if representing two different populations (Fig. 7). The two populations were distributed as if the rate of regeneration in the "higher population" was greater than for Group A, and comparable to or less than Group A in the "lower population." The five pigs which were killed on Days 4 to 13 and which comprised the "lower population", were from four different litters received in three different lots.

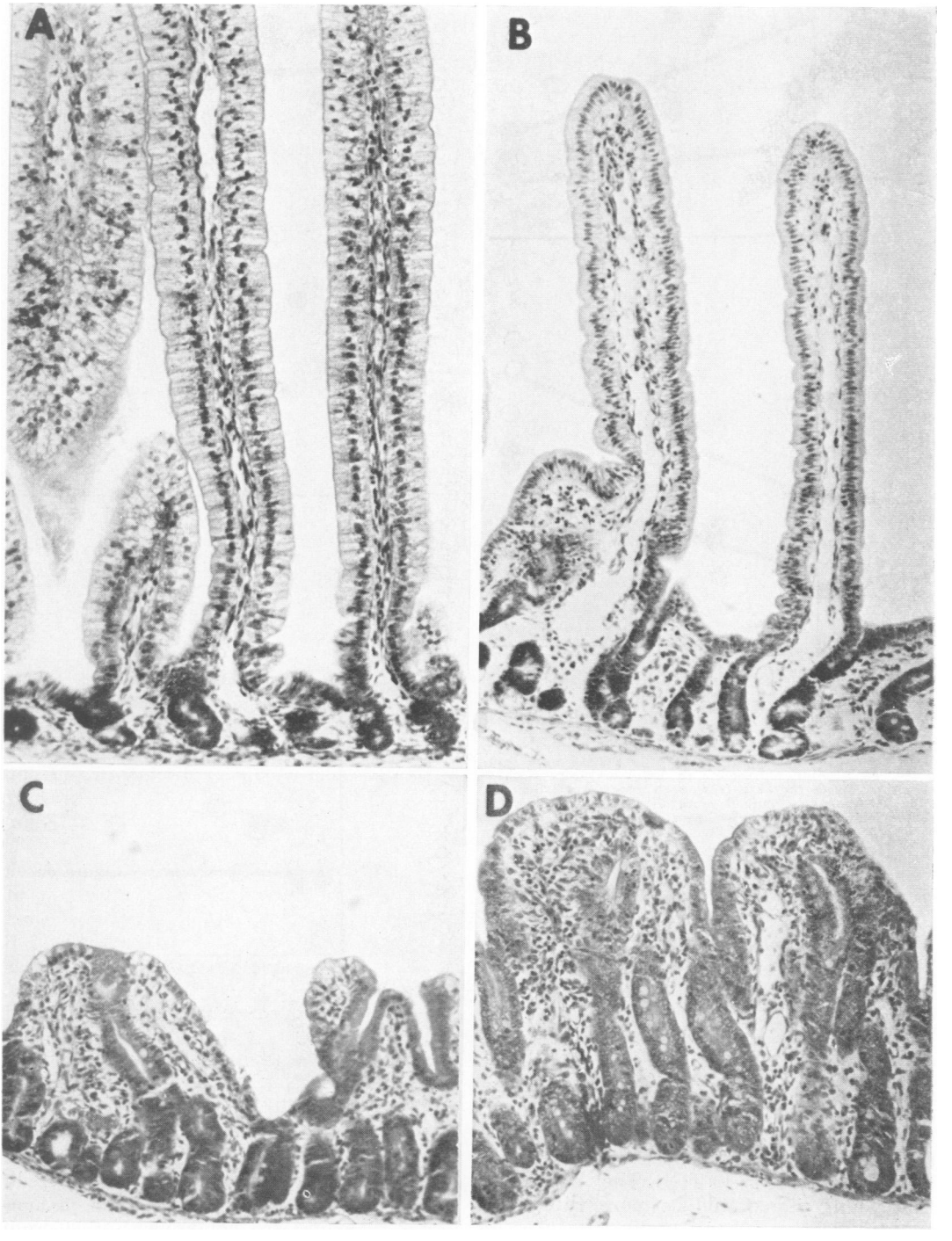

Fig. 3. Sections taken from the ileum (Site 4) of pigs one (controls) or three (principals) days after principals were exposed to TGE virus. A. Group A — control, B. Group B — control, C. Group A — principal, D. Group B — principal. Pigs in Groups A and B were three and 21 days old respectively when the experiment began. X400.

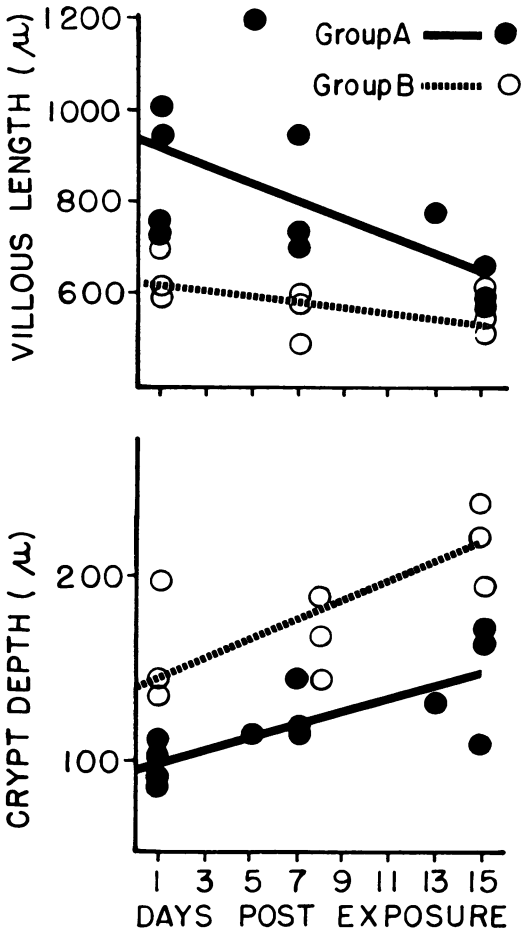

Fig. 4. Villous length and crypt depth in control pigs. Data from Sites 2 to 5 (jejunum and ileum) were combined, each point represents an individual pig. Regression lines fit to the data from each group were included. Pigs in Groups A and B were three and 21 days old respectively when the experiment began.

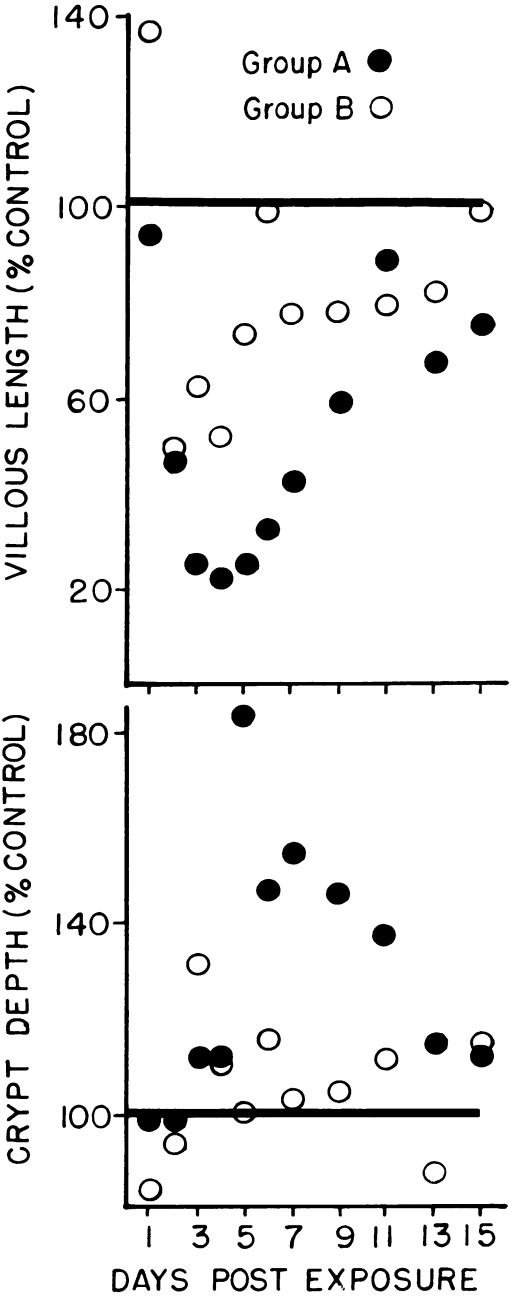

Fig. 5. Mean villous length and crypt depth in pigs for 15 days after exposure to TGE virus. Data from Sites 2 to 5 (jejunum and ileum) were combined and are expressed as a percent of the appropriate control value taken from the regression lines in Fig. 4. Pigs in Groups A and B were three and 21 days old respectively when exposed.

DISCUSSION

As anticipated, the case fatality rate was higher in pigs exposed to TGE virus when three days old (Group A) than in those exposed when 21 days old (Group B). This higher fatality rate correlated with a higher incidence, earlier onset, longer duration and more profuse diarrhea, as well as more severe dehydration, in the younger pigs. Cornelius *et al* (2) studied the consequences of these signs and discussed their relationship to fatality in TGE. They suggested that young pigs would be more vulnerable to dehydration than adults because of changes with age in body water content and distribution, as well as in renal electrolyte regulation. The ages at which such changes occur in swine are not known. If changes of this nature occurred during the 18 day interval between groups in this study, they

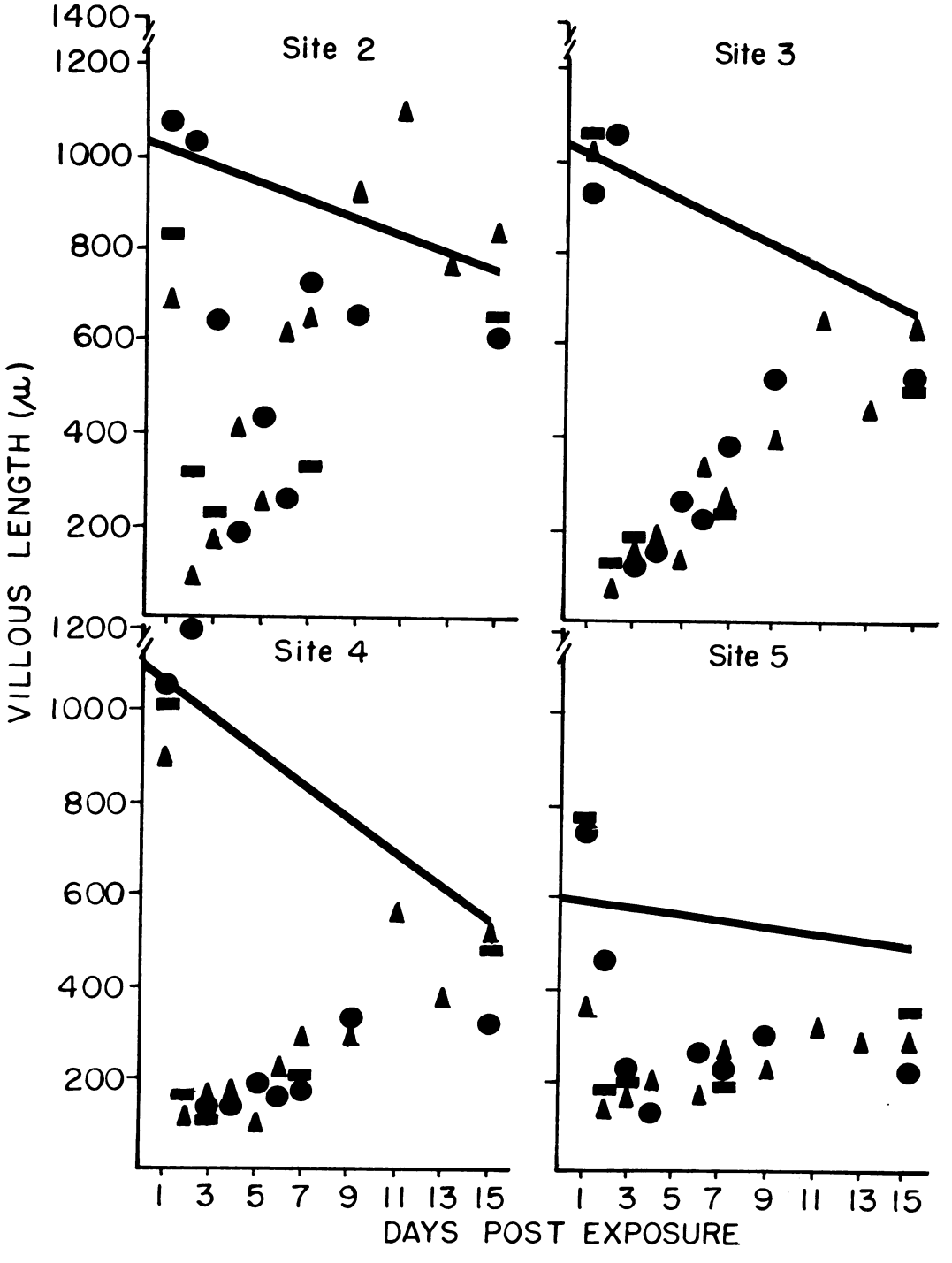

Fig. 6. Villous length in jejunum (Sites 2 and 3) and ileum (Sites 4 and 5) of pigs in Group A exposed, at three days of age, to TGE virus. Individual pigs for each day are identified by a triangle, circle or rectangle at each site. Regression lines representing the appropriate sites in control pigs during this period were included for comparison.

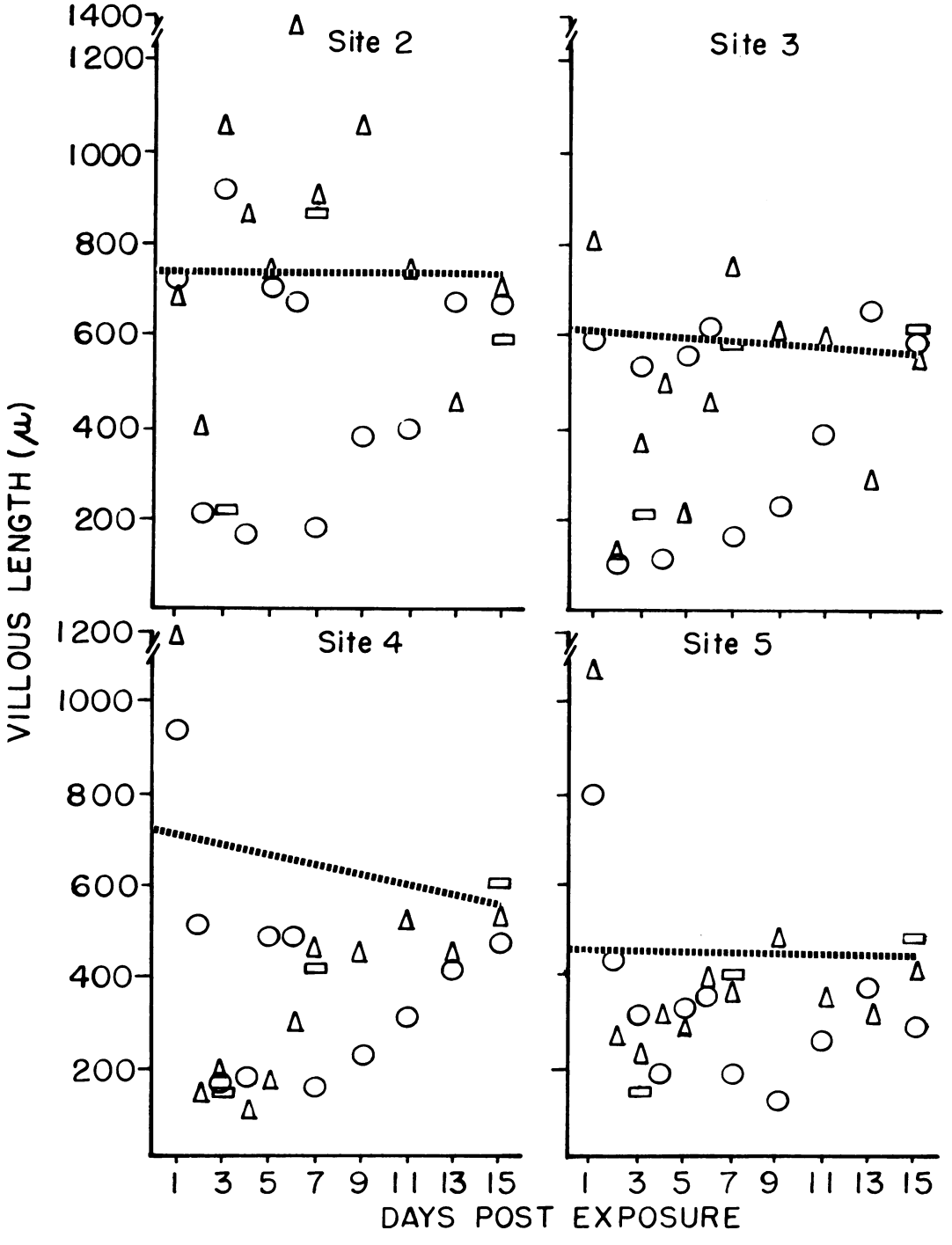

Fig. 7. Villous length in jejunum (Sites 2 and 3) and ileum (Sites 4 and 5) of pigs in Group B exposed, at 21 days of age, to TGE virus. Individual pigs, for each day, are identified by a triangle, circle or rectangle at each site. Regression lines representing the appropriate sites in control pigs during this period were included for comparison.

would have contributed to the differences in fatality rates between groups. Thus, the fatality rate may have been higher in the younger group both because they were less able to tolerate diarrhea and because more severe diarrhea occurred in this group than the other. The histological counterpart of the higher fatality rate in Group A was more intensive and extensive villous atrophy, particularly during Days 4 to 7 PE when most deaths occurred in Group A. Presumably, the differences in duration and profuseness of diarrhea between groups were the result of these quantitative differences in the intestinal lesions.

The histological differences in controls among sites and with time (Figs. 2-4, 6 and 7) are characteristic of pig small intestine during the first few weeks of life (6, 7, 8, 9). These patterns in controls were considered when histological comparisons between principals were made.

The interpretation of the histological results with regard to the initial hypothesis, predicting more rapid regeneration in Group B than in Group A, was complicated by the occurrence of two populations in Group B (Fig. 7). These populations were the result of differences in regeneration rates, or in maximum intensity of lesions, or rates of lesion development, or some combination of the above, among pigs in this group. Unfortunately, it was not possible to determine the explanation(s) for the different populations with certainty as each pig was examined histologically only once in this study. However, the occurrence of lesions of similar intensity as Site 4 in all pigs in this group killed on Days 3 and 4 PE, and later development of two populations at this site lead us to suggest different rates of regeneration as the most likely explanation. If this explanation is correct, then the upper population in Group B (Fig. 7) did regenerate more rapidly than Group A. If differences in maximal intensity of lesions are the correct explanation for the two populations, then comparisons between groups should be based on the lower population (Fig. 7). In that event, regeneration rates in ileum were similar for both groups, and jejunum regenerated more rapidly in Group A than in Group B.

In contrast to intestinal lesions, there was no apparent clustering of viral titers in the small intestine of Group B into two populations (10). However, Group A had earlier, more prolonged and higher peak titers of TGE virus than Group B. This difference between groups further complicated interpretation of the comparative rates of regeneration. In conclusion, the histological data neither support nor deny the initial hypothesis but indicate that it is an oversimplification.

In contrast to villous epithelium, crypt epithelium is not directly affected by TGE virus (12, 14); nevertheless, there was crypt hyperplasia in the principals as reported previously (6). Crypt hyperplasia was greater and more prolonged in Group A than Group B. Presumably, this is because the amount of villous epithelium to be regenerated was less and the size of the crypt population available to regenerate it was larger to start with in Group B than Group A.

Hooper and Haelterman (5) indicated that in pigs with TGE, the duodenum is usually spared from villous atrophy and that the lesion occurred less frequently in jejunum than ileum, particularly in two to three week old pigs. Bohl *et al* (1) also found that partially immunized pigs, which had comparatively mild clinical signs, had more severe lesions in posterior than anterior small intestine. Olson (11) reported that villous atrophy occurred in four-month old pigs with TGE but implied the lesion was less intensive than that characteristically seen in newborn pigs. Our observations support these reports. Apparently, there are gradients with age and with site in small intestine. The more proximal in the intestine and/or the older the pig, the less the chance of observing the lesion on any given day following exposure. Epithelial destruction was less frequent and less pronounced in duodenum than in jejunum following exposure to TGE (12); thus apparently explaining the lower incidence of villous atrophy at the former site.

A similar explanation may hold for jejunum as compared to ileum. However, based on Fig. 6 and (depending on interpretation) Fig. 7, more rapid regeneration in anterior than in posterior small intestine, following villous atrophy, may contribute to the lower incidence of lesions seen in the former area in this disease.

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