Changes in the Microflora and Physiology of the Anterior Intestinal Tract of Pigs Weaned at 2 Days, with Special Reference to the Pathogenesis of Diarrhea

P. A. BARROW, R. FULLER,* AND M. J. NEWPORT
National Institute for Research in Dairying, Shinfield, RG2 9AT, England

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The gastrointestinal microflora and gastric physiology of piglets weaned at 2 days was compared with that of piglets allowed to continue sucking the sow. Although there was a significantly higher count of *Escherichia coli* in the stomach, duodenum, and jejunum of the early-weaned compared with sow-reared pigs, these differences were not detectable in samples from the ileum. There were no qualitative differences in lactobacilli and in streptococci between the two treatments. *Lactobacillus fermentum, L. acidophilus, Streptococcus salivarius, S. bovis,* and related biotypes were isolated from both groups of pigs. *L. fermentum* and *S. salivarius* were isolated more frequently from sow-reared piglets. The weight of digesta in the stomach was greater in weaned than in sucking pigs and was even greater in scouring weaned pigs, suggesting that in scouring pigs there may be gastric stasis. The gastric pH was higher in the weaned pigs at 4 days of age, but gradually decreased up to 10 days, during which time the lactic acid concentration rose. In weaned pigs there was a highly significant negative correlation between pH and lactic acid concentration in the stomach digesta, and also a positive correlation between pH and number of *E. coli.* These correlations suggest that lactic acid, from bacterial fermentation, is the major component in the regulation of gastric pH in weaned pigs. Three of twenty sucking pigs, but none of the weaned pigs, were secreting HCl (chloride concentration > 3 mg/g, pH < 3.5). In sucking pigs there was an inverse relationship between the chloride and lactic acid concentrations in the digesta. In weaned scouring pigs there was a nonsignificant increase in pepsin concentration in the stomach tissue. There was a threefold increase in the total proteolytic activity of the stomach tissue.

Attempts to wean pigs at 2 days of age are frequently frustrated by a high incidence of diarrhea (scouring), which starts shortly after the pig is removed from the sow. Apart from the deprivation of antibodies and nonspecific antibacterial agents present in sow’s milk but not in the milk substitute, there was the possibility that changes in the intestinal microflora resulting from weaning were responsible for the increase in susceptibility of early-weaned pigs to enteritis. A comparison was therefore made of the intestinal microflora of baby pigs reared on the sow and on a milk substitute based on cow’s milk. Some aspects of gastric physiology that might be interrelated with the microflora were also investigated.

Our studies were restricted to the microflora of the stomach and small intestine because the signs of enteritis were visible in these sites and because we felt that if microbiological imbalances were responsible for the disease, they would be more easily detected in the anterior gut, where there is a relatively simple flora compared with that in the cecum and colon. In addition, since one of the long-term aims of this study was to try to manipulate the flora of early-weaned piglets to provide protection against enteric infection, knowledge of the commensal microorganisms normally found in the anterior gut—the part we wished to protect—was important.

**MATERIALS AND METHODS**

**Pigs.** Six litters were studied from the Institute’s herd of Large White pigs. Four pigs were weaned from each litter at 2 days of age, and the remainder of the litter was left on the sow. In each litter, one sucking pig was killed at 2 days of age, and one sucking and one artificially reared pig were killed at 4, 6, 8, and 10 days of age.

**Artificial-rearing technique.** Details of the housing of the pigs have been previously described (6). The room was thoroughly washed and fumigated with formaldehyde for 48 h after rearing each litter. Pigs were kept in individual cages of the type described
by Braude et al. (7). Pigs excreting feces of pasty or liquid consistency were assessed as scouring. Most scouring pigs had feces of liquid consistency.

The diet was prepared from a spray-dried powder containing 730 g of dry skim milk, 270 g of soybean oil, and 18 mg of butylated hydroxytoluene per kg. A mild heat treatment was used during the spray-drying to prevent the denaturation of the whey proteins (9). The spray-dried powder was reconstituted in water (200 g/liter), homogenized at 176 kg/cm², pasteurized at 72°C for 17 s, and stored at 4°C. The liquid diet was supplemented with Rovisol AD3E Oral, DL-a-tocopherol acetate (Roche Products Ltd., London), and manethystione (Koch-Light Laboratories Ltd., Colnbrook) to supply the following amounts per kilogram of dry matter: 0.6 mg of retinol; 5 μg of cholecalciferol; 1.65 μg of α-tocopherol, and 62 μg of menaphthone.

The pigs were fed at hourly intervals from an automatic feeder (7), using a scale of feeding based on live weight as described by Braude and Newport (8). The diet reservoirs and troughs were washed and sterilized daily in free-flowing steam and then refilled with the appropriate volume of diet, to which Formalin had been added (0.5 ml/liter).

Collection of samples. Sucking pigs were removed from the sow 1 h before killing. Artificially reared pigs were killed 1 h after a feed. Pigs were killed by an intracardiac injection of 5 to 10 ml of an aqueous solution of sodium pentobarbitone (30 mg/ml). After the body cavity was opened, the intestine was ligated with artery forceps to isolate the stomach, a 15-cm-long section of the duodenum 15 cm from the stomach, a 15-cm-long section in the middle of the small intestine, and a 15-cm-long section of the ileum 15 cm from the ileocecal junction. The stomach and the three sections of the small intestine were excised. The pH values of the esophageal and pyloric ends of the stomach were measured by inserting a microelectrode (Activion Glass Ltd., Halstead, Essex) into incisions made through the stomach wall. The contents of the stomach and small intestine segments were removed and weighed, and samples were taken for bacteriological analysis.

Bacteriological analysis. Decimal dilutions of the samples were made in boiled reinforced clostridial medium broth (18) and surface inoculated onto the following media:

(i) Reinforced clostridial medium agar containing horse blood (4%, vol/vol) and menadione (0.5 μg/ml) incubated anaerobically for 2 days. Plates were always pored the day before use and stored overnight in an anaerobic jar filled with 10% CO₂ in H₂. Anaerobiosis was established by evacuating the jar containing a cold catalyst, filling with a mixture of 10% CO₂ in H₂, and repeating the procedure (10, 34). This method has been shown to be as efficient as an anaerobic cabinet for counting anaerobic bacteria in human feces (34). This procedure was designed to be nonselective and detect organisms that might possibly be present but unable to grow on the selective media. The efficiency of recovery by this method was assessed by comparing the viable counts with total microscopic counts made by using a bacterial counting chamber. Only samples from the small intestine were compared in this way. It was not possible to do this satisfactorily with stomach samples because the presence of milk clots made the microscopic count unreliable.

(ii) Acetate agar (28) incubated anaerobically for 2 days for detection of lactobacilli.

(iii) For the detection of streptococci, mitis-salivarius agar modified by adding nalidixic acid (15 μg/ml) instead of potassium tellurite to basal medium CM157 (Oxoid Ltd., London). This modification was found to be necessary because in preliminary trials coliform organisms grew in the presence of potassium tellurite. It was incubated aerobically for 1 day. Tests using a wide range of group D streptococci showed that this medium, although originally devised for the isolation of viridans streptococci, was the most suitable for detecting the bouis-equinus types often encountered in pigs (14) as well as the typical enterococci.

(iv) Nutrient agar containing sheep blood (4%, vol/vol) and penicillin (1 IU/ml) incubated for 1 day. Initially a penicillin concentration of 0.1 IU/ml (31) was used, but this allowed the growth of streptococci. Increasing the concentration of penicillin eliminated the streptococci without affecting the coliform count.

All media were incubated at 37°C. After incubation for the prescribed period, colony counts were made and representatives of each colony type were transferred to appropriate broth media. These cultures were purified and then subjected to a range of physiological tests.

Characterization of coliforms. The hemolysis of sheep blood was noted on primary isolation plates. Isolates were tested for growth and acid and gas production in MacConkey broth at 44°C.

Characterization of lactic acid bacteria. The tests for production of gas from glucose (15) and of NH₃ from arginine and growth at various temperatures were done by conventional methods. All other physiological tests were done by using the media and miniaturized methods of Jayne-Williams (19, 20). Streptococci were grouped by using HCl extracts (22) concentrated by the method of Shatlock (29) and commercial antisera (Difco Ltd.).

Chemical analysis. Lactic acid was estimated by the method of Barker and Summerson (3). Pepsin was assayed by using denatured hemoglobin as the substrate (1). The absorbance of the trichloroacetic acid-soluble hydrolysis products was measured at 280 nm. One unit of activity was defined as an absorbance of 0.001 U/min at 37°C. Chloride was estimated by using a chloride meter (Evans Electroselenium Ltd., Halstead, Essex).

Statistical treatment of results. The experiment was designed to be balanced for litter effects. However, there was some mortality among the weaned pigs, which unbalanced the design. Analyses of the litter effect in the sucking group showed that it was not significant (P > 0.05), and therefore the number of pigs at each age in the weaned pigs does not bias any age effects. Comparisons between the three groups (sucking, healthy weaned, and scouring weaned pigs) were made by using linear regression analysis. Comparisons between means were made by t-tests. Correlations were obtained from linear regression analysis.

RESULTS

Efficiency of isolation techniques. Of the 71 comparisons of total microscopic counts with
the viable count on the nonselective medium, 39.6% showed a complete recovery and 94.4% showed a recovery of 20% or more.

The total recovery of organisms on the various selective media was compared with the numbers recovered on the nonselective medium. Of the 185 comparisons made, 47.0% showed complete recovery and 94.1% showed a recovery of 20% or more.

Quantitative bacteriology. Weaning at 2 days of age had a marked effect on the counts of *Escherichia coli* in the anterior gut (Fig. 1). Counts in the stomach and duodenum were significantly different between the two groups at 4 and 6 days and in the jejunum at 4, 6, and 8 days. The counts in the ileum were not significantly different, although the mean values were always slightly lower in the suckled pigs.

Hemolytic *E. coli* were frequently found in early-weaned pigs (6 of 21 compared with 3 of 28 in sucking pigs) but always formed only a small proportion of the total *E. coli* population. The mean log₁₀ count of hemolytic *E. coli* was 5.10, whereas that of nonhemolytic *E. coli* in these same pigs was 6.77.

The counts of lactobacilli and streptococci are shown in Fig. 2 and 3. Significant differences were not found at any age or on either treatment.

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**Fig. 1.** Viable counts of coliform organisms in (a) stomach, (b) duodenum, (c) mid small intestine, and (d) ileum of suckling (○) and early-weaned (△) pigs up to 10 days old. Each point is the mean of four to six values. Vertical bars denote standard error of the mean.

**Fig. 2.** Viable counts of lactobacilli in (a) stomach, (b) duodenum, (c) mid small intestine, and (d) ileum of suckling (○) and early-weaned (△) pigs up to 10 days old. Each point is the mean of four to six values. Vertical bars denote standard error of the mean.
In nearly all cases, the mean values for lactobacilli were lower in the early-weaned pigs. The pooled counts for pigs between 4 and 10 days of age are shown in Table 1, with the healthy and scouring early-weaned pigs analyzed separately. In the stomach, the E. coli count was significantly ($P < 0.05$) lower in weaned scouring pigs compared with weaned healthy pigs. However, there was a tendency for the counts of E. coli to increase in the small intestine of scouring pigs, whereas they decreased in healthy pigs so that in the jejunum the count of E. coli was significantly higher in scouring pigs. This multiplication of E. coli in the small intestine is characteristic of scouring caused by this organism, although the counts in the stomach are usually lower than those observed by us (30).

Scanning and transmission electron microscopy of the duodenal mucosa failed to reveal any adherent bacteria in scouring pigs. The high stomach count could be due, at least in part, to the large number of organisms ingested with diet. Counts on the artificial diet in the trough showed that there were about $10^6$ viable E. coli per ml, whereas in sow’s milk there was none. However, it is difficult to assess the contribution that E. coli ingested with bedding and feces might make to the population in the stomach of sow-reared pigs.

The pooled results (Table 1) also revealed significant ($P < 0.05$) differences in the lactobacillus counts between the sucking and healthy weaned pigs. Although these differences are small, they may represent the reduction or elimination of a particular species or strain.

The streptococcal counts were much more viable, and even after pooling (Table 1) the only significant difference was in the stomachs of sucking and healthy weaned pigs.

**Qualitative bacteriology.** Of the 84 strains of E. coli isolated from suckled pigs, 79 produced acid and gas in MacConkey broth at $44^\circ$C; the other five strains produced acid only. All 75 isolates from early-weaned pigs produced acid and gas at $44^\circ$C.

Strains identical with or closely resembling Lactobacillus fermentum, L. acidophilus, L. salivarius (Table 2), Streptococcus salivarius, and S. bovis (Table 3) were isolated from both suckled and early-weaned pigs. The three isolates of S. salivarius tested against group K antiserum were negative, although the serum precipitated with an extract from a known strain (NCTC 2076). L. fermentum and S. salivarius were isolated more frequently from suckled pigs (Tables 2 and 3).

**Effect on stomach physiology.** The consistency of the stomach contents was different in the two groups of pigs. Suckled pigs had a soft, crumbly milk clot in the stomach, whereas the stomach contents of early-weaned pigs tended to be harder and in extreme cases took up the shape of the stomach and did not collapse when the stomach wall was removed. In some cases it was possible to see a series of concentric shells, indicating the formation of separate clots with each successive feed entering the stomach.

Figure 4 shows the effect of weaning and age on gastric physiology. Except at 10 days of age, the weight of the stomach contents was greater in weaned than in suckled pigs, but only at 6 days was the difference significant ($P < 0.05$).
The pH value of the contents was considerably greater at 4 days of age in weaned pigs (P < 0.05), but from 6 to 10 days the gastric pH was similar in both suckled and weaned pigs. The lactic acid concentration in the contents was lower, but not significantly so (P > 0.05), in the weaned pigs. Lactic acid concentration did not alter consistently with age, although in the suckled pigs the concentration at 8 days was greater (P < 0.05) than at 2 days. Age also had little effect on the chloride concentration of the stomach contents, but the concentration at 10 days was greater (P < 0.05) than at 4 days of age in the weaned pigs, and only at 10 days were the values greater (P < 0.05) in the weaned compared with suckled pigs. In the contents, pepsin concentrations paralleled those for chloride. In the stomach tissue, the pepsin concentrations showed some tendency to increase with age in the weaned pigs, but neither in the weaned nor suckled pigs were any of the differences significant (P > 0.05).

The weight of stomach tissue at 2 days of age was significantly less (P < 0.05) than at any other age (Fig. 4g). In suckled pigs, the weight at 8 or 10 days of age was significantly greater (P < 0.05) than at 4 days, and in weaned pigs the weight was greater than at either 4 or 6 days. The difference between the weaned and suckled pigs was not significant (P > 0.05), although the stomachs of weaned pigs tended to be heavier at 8 and 10 days of age.

The pooled results for pigs aged 4 to 10 days are shown in Table 4, with results for the healthy and scouring early-weaned pigs treated as separate groups. The amount of contents was significantly (P < 0.05) increased in the scouring weaned pigs compared with both the sucking and the healthy weaned pigs. The increases in pH observed in weaned pigs were not significant. There was a tendency for chloride to be increased in weaned pigs, and this was significant (P < 0.05) in scouring pigs. Pepsin was significantly (P < 0.05) increased in healthy weaned pigs compared with both sucking and scouring weaned pigs.

Statistical relationships between bacterial and physiological parameters. All statistical relationships were examined, and significant (P < 0.05) relationships for suckled or weaned pigs are shown in Fig. 5. The correlations for weaned pigs were also examined separately for the healthy and scouring pigs. In the stomach...
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FIG. 4. Biochemical analyses of stomach contents and stomach tissue from suckling (●) and early-weaned (△) pigs up to 10 days old. (a) Weight of contents; (b) pH of contents; (c) lactic acid concentration of contents; (d) chloride ion concentration of contents; (e) pepsin activity of contents; (f) pepsin activity of tissue; (g) weight of stomach tissue. Each point is the mean value of four to six values except pepsin in suckled pigs at 10 days, which is based on three values. Vertical bars denote standard error of the mean.

TABLE 4. Effect of weaning and scouring on the physiology of the stomach of pigs aged 4 to 10 days*

<table>
<thead>
<tr>
<th>Method of rearing</th>
<th>Health status</th>
<th>Wet weight (g)</th>
<th>pH</th>
<th>Lactic acid (mg/g)</th>
<th>Chloride (mg/g)</th>
<th>Pepsin (mg/g)</th>
<th>Tissue pepsin* (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suckled Healthy</td>
<td>37.85 ± 7.22</td>
<td>4.13 ± 0.18</td>
<td>8.56 ± 1.25</td>
<td>1.65 ± 0.17</td>
<td>55.19 ± 8.54</td>
<td>149.41 ± 21.48</td>
<td></td>
</tr>
<tr>
<td>Weaned Healthy</td>
<td>54.78 ± 10.11</td>
<td>4.43 ± 0.09</td>
<td>6.04 ± 0.84</td>
<td>2.02 ± 0.32</td>
<td>87.76 ± 12.50</td>
<td>113.61 ± 28.98</td>
<td></td>
</tr>
<tr>
<td>Weaned Scouring</td>
<td>99.05 ± 8.87</td>
<td>4.22 ± 0.13</td>
<td>7.84 ± 1.37</td>
<td>2.54 ± 0.35</td>
<td>45.51 ± 7.85</td>
<td>209.04 ± 52.08</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ages of the pigs on each treatment were similar to those in Table 1.
* Mean values ± standard errors based on 16 to 20 observations (suckled pigs), 9 to 12 observations (healthy weaned pigs), and 9 observations (scouring weaned pigs).
* Sucking versus weaned scouring pigs significantly different (P < 0.05).
* Sucking versus healthy weaned pigs significantly different (P < 0.05).
* Scouring versus healthy weaned pigs significantly different (P < 0.05).

contents of sucking pigs, chloride and pepsin concentrations were positively correlated (Fig. 5a). A negative correlation was found between chloride and lactic acid (Fig. 5b). In the weaned pigs there was a positive correlation of pepsin with chloride concentration (Fig. 5c) in scouring pigs. The E. coli count was positively correlated with the pH value of the digesta (Fig. 5d) and
negatively correlated with the chloride concentration in the digesta (Fig. 5e). When the data for healthy and scouring weaned pigs were considered separately, the correlation of E. coli and chloride was significant (P<0.05) only for healthy pigs, and that of E. coli with pH was significant (P<0.001) only for scouring pigs. Similarly, pH was highly correlated with the lactic acid concentration (P<0.001) when all weaned pigs were considered (Fig. 5f), but was significant only for the scouring pigs (P<0.05) and not for the healthy ones when treated separately.

**pH of small intestine.** The mean (± standard error) of the pooled values for all ages for 22 sucking pigs was 6.09 (± 0.066) in the upper, 6.32 (± 0.041) in the middle, and 6.65 (± 0.028) in the lower small intestine. The corresponding values for 21 early-weaned pigs were 5.81 (± 0.089), 6.47 (± 0.020), and 6.69 (± 0.060). Between treatments, the differences were significant for the upper (P<0.05) and middle (P<0.01) but not for the lower small intestine.

**DISCUSSION**

The total viable count of bacteria in the small intestine represented in the majority of cases 20% or more of the total microscopic count. There is little information with which to compare these results, but these figures are similar to those obtained for stomach samples from colobus monkeys (25). Comparisons with previously published results would be difficult anyway because there may well be differences between hosts and even between different sites in the alimentary tract of the same individual. For example, the number of dead organisms may be high in the stomach due to acidity. These dead organisms would pass into the small intestine and be counted by the microscopic method but not by the viable counting technique. The recovery of bacteria on the selective media represented a large proportion of the total numbers recovered on the nonselective medium. Given the inherent inaccuracy of the bacterial counting...
procedure and the fact that selective media often have a slight inhibitory effect on the group of organisms selected for, we have concluded that lactobacilli, streptococci, and coliforms were the only major groups of organisms present in the anterior part of the intestinal tract. Smith and Jones (30) also failed to isolate significant numbers of bacteria other than lactobacilli, streptococci, or coliforms from the anterior gut of pigs over 1 day old.

Although the counts of lactic acid bacteria were very similar in the two groups of pigs, _L. fermentum_ and _S. salivarius_ were isolated more intestinal tract. In the past, much interest has focused on the bifidobacteria, which Tissier (32) showed to be predominant in the feces of human breast-fed infants, and a role in protection against enteric infections has been implied. However, more recent work has cast grave doubts on this relationship, since large numbers of _E. coli_ and bifidobacteria can coexist in human infant feces (13, 17, 36). The relevance of the fecal flora to protection against intestinal disease is, anyway, questionable, because as Hewitt and Rigby (17) pointed out, it is more likely that the protective effect is exerted in the small intestine. It is interesting in this context to note that in our experiments the differences that were obvious in the stomach and duodenum were not detected in the ileum and presumably would also have been missed had feces been used.

Although the counts of lactic acid bacteria were very similar in the two groups of pigs, _L. fermentum_ and _S. salivarius_ were isolated more frequently from suckled pigs. The possible involvement of these two species in protection of sucking pigs against scouring would be worth investigating. There are already many reports of the beneficial effects that follow administration of lactic acid bacteria to animals (see 28 for review).

The only studies directly comparable with those reported here are those of White et al. (35). Their pigs were also weaned at 2 days and onto a similar diet. In their case, weaning had no effect on the numbers of _E. coli_ in the stomach, but it is worth noting that the counts of _E. coli_ were higher in their control pigs than in ours.

The importance of high counts of _E. coli_ in relation to diarrhea in our pigs is still undecided. High counts of _E. coli_ and gastric stasis occurred whether or not the pigs scoured. One puzzling feature of the microbial ecology of the stomach is the apparent multiplication of bacteria in an environment with a pH of 4.5 and below. The possibility that local multiplication is occurring in microenvironments with higher pH values should be considered.

Smith and Jones (30) claimed that in order to establish a valid diagnosis of _E. coli_ diarrhea, large numbers of _E. coli_ must be shown to be present in the anterior small intestine. Although many of our scouring pigs fulfilled this criterion, the counts in the stomach were also high, whereas Smith and Jones (30) reported low counts in the stomach. Moreover, the counts were high whether or not the pigs scoured. Although the serotypes usually associated with neonatal diarrhea of pigs were not found in the present experiment (Wray, Barrow, and Fuller, unpublished data), it is still possible that as yet untypable pathogenic strains are present in the scouring early-weaned pigs but not in the healthy ones. More information on the antigenic structure and enterotoxigenicity of these isolates might clarify this point.

Gastric stasis in early-weaned scouring pigs has been reported previously (35). Similarly, in our experiments, the increased amount of digesta in the stomach of the scouring weaned pigs indicated that gastric stasis, or at least a reduction in the rate of stomach emptying, had occurred. The reduced amount of digesta in the stomach of the sucking pig suggests that sow's milk is more amenable to enzymatic attack. Reduced intake is unlikely to be the cause of this difference, because the amount of milk substrate given to weaned pigs was based on the amount of milk consumed by sucking pigs (2).

The observed pH values for stomach contents are similar to those reported for suckling pigs by Walker (33) and for weaned pigs by Manners (23). The pH value of the digesta may also be influenced by secretion of HCl, indicated by high chloride concentrations. The time of onset of gastric secretion of HCl seems to be variable. Although Kutas and Szabó (21) reported that nearly all pigs examined at 7 days of age were achlorhydric, Cranwell and Titchen (12) demonstrated HCl secretion in pigs within 1 day of birth. Cranwell et al. (11) demonstrated HCl secretion in some suckling pigs as early as 2 days of age and found chloride concentrations of at least 3 mg/g together with pH values of 3.5 or less. Using this criterion, only 3 of the 20 suckling pigs and none of the 21 weaned pigs in our experiment were secreting HCl. The tendency for chloride concentrations to be slightly higher in the weaned pigs was probably a reflection of the greater chloride concentration in the milk substitute (2 mg/ml) compared with sow's milk (1 mg/ml).

The negative correlation of chloride with lactic acid concentration in the digesta of suckling
pigs agrees with the data of Cranwell et al. (11), who found that lactic acid concentrations in the digesta were usually considerably greater when chloride concentrations were low. In the weaned pigs, the highly significant negative correlation ($P < 0.001$) of pH with lactic acid concentration (Fig. 5f) suggests that lactic acid is the major component regulating stomach pH in weaned pigs between 4 and 10 days of age. The data from healthy and scouring weaned pigs were analyzed independently, and this correlation was significant ($P < 0.05$) only for the scouring pigs. However, in view of the small number of observations available, the biological interpretation of any statistically significant differences should be viewed with caution.

A reduction in the pH value of the stomach digesta with age was found in the weaned pigs. This may have a significant effect on bacterial growth in the stomach. The high pH values of 4 days of age may mean that the gastric “barrier” against potential pathogens is ineffective until 10 days of age, when the pH is lower. Although not as high as those found in HCl-secreting pigs by Cranwell et al. (11), the greater chloride concentrations in the contents at 10 days of age might signal the onset of HCl secretion. The decline in pH with age might be accounted for by the increase in lactic acid, with possibly a small effect of HCl secretion at 10 days of age. The stomach pH in sucking pigs remained constant, with a tendency for lactic acid to increase and chloride concentration to fall.

There was a tendency for the stomach tissue concentration of pepsin to be greater in the weaned pigs. Hartman et al. (16) have reported that weaning at 7 days of age reduced proteolytic enzyme activity, but this may have been associated with a reduction in feed intake after weaning. The greater concentration of pepsin in the stomach tissue in scouring compared with healthy pigs (Table 4), although not significant ($P > 0.05$), may indicate partial inhibition of pepsin secretion as a result of the reduction in rate of stomach emptying or gastric stasis.

There was no significant change from 2 to 10 days of age in the pepsin concentration in the digesta or stomach tissue. These results agree with those of other workers (5, 16; P. D. Cranwell and D. A. Titchen, Proc. Nutr. Soc. 35:28A, 1976) who found that pepsin activity increased only after the first 2 weeks of life. There was approximately a threefold increase in the weight of stomach tissue between 2 and 10 days of age (Fig. 4g), resulting in a corresponding increase in the total proteolytic capacity of the stomach.

At the moment, the evidence for $E. \text{coli}$ as the sole causative agent of diarrhea in these early-weaned pigs is not convincing. Failure to demonstrate $E. \text{coli}$ attached to the duodenal mucosa, although not conclusive, also suggests a different etiological agent. Further doubt is cast on the involvement of $E. \text{coli}$ in this disease by the finding that similar scouring pigs in other trials often failed to respond to antibiotics shown to be active against $E. \text{coli}$ in their feces (Barrow, Fuller, and Newport, unpublished data).

The possibility of a virus being involved must now be considered. There is growing evidence in pigs and other animals that diarrhea previously thought to be due to $E. \text{coli}$ is in reality caused by a virus (4, 24, 37). In particular, one report from Australia (26) describes the recovery of a reovirus-like agent from three pigs that scoured shortly after being transferred from the sow to a liquid cow’s milk diet. Even if virus is shown to be the primary cause of diarrhea in early-weaned pigs, exacerbation of the syndrome by $E. \text{coli}$ remains possible.

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LITERATURE CITED


