Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Colonic Compensation in Transmissible Gastroenteritis of Swine

R. A. ARGENZIO, H. W. MOON, L. J. KEMENY, and S. C. WHIPP
National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa

Absorption of water and electrolytes by the small and large intestine was examined using a nonabsorbable marker technique in 3-day-old and 3-wk-old pigs. One-half of the pigs in each group were orally infected with transmissible gastroenteritis virus; the remaining pigs served as controls. Three-day-old control pigs concentrated the nonabsorbable fluid marker twelvefold along the small and large intestine, indicating an efficiency of about 95% in absorption of the exogenous daily fluid load presented to the intestine. In contrast, the marker concentration in infected pigs showed no change whatsoever along either the small or large intestine, indicating a complete absence of net fluid absorption or secretion in these animals. Three-week-old control pigs concentrated the marker similarly to the 3-day-old group, with the bulk of the fluid absorption occurring in the small intestine. Infected pigs in the 3-wk-old group had marked net fluid secretion in the proximal small intestine, so that about twice the fluid load was presented to the large intestine of the 3-wk-old infected pigs as compared to the 3-day-old infected group. However, in contrast to the 3-day-old infected group, the large intestine of the 3-wk-old infected pigs increased fluid absorption some six times over the control, and this compensatory response prevented diarrhea in these older animals. Analysis of luminal contents indicated that in the older pigs, unabsorbed carbohydrate was almost completely fermented to short-chain fatty acids in the colon, whereas in the younger pigs the carbohydrate passed through the colon unchanged. These results demonstrate that development of microbial digestion, together with rapid short-chain fatty acid absorption, is a primary feature responsible for the colonic compensation in the older pigs with transmissible gastroenteritis.

Transmissible gastroenteritis (TGE) is a coronavirus infection of absorptive epithelial cells in the small intestine of pigs. The disease is characterized by atrophy of intestinal villi and acute diarrhea. Swine of all ages are susceptible to this infection. The case fatality rate approaches 100% in pigs that become infected during the first week after birth; however, it is comparatively low (≤2%) in pigs that become infected when they are older (≥3 wk old). Villous atrophy and diarrhea are less severe and of shorter duration in older pigs than in newborn pigs. The reason for this age-dependent resistance is unclear; it has been ascribed, however, at least in part, to an accelerated rate of epithelial cell replacement in older pigs (1–3). Normal newborn pigs require three times as long as normal 3-wk-old pigs to replace villous absorptive cells (4). The comparatively short-lived villous absorptive cells of older pigs also tend to produce lower titers of TGE virus than those in newborn pigs. Villous absorptive cells destroyed by TGE virus are replaced by cells that migrate from the crypts before they are completely differentiated. Crypt epithelium and the incompletely differentiated cells covering atrophic villi are resistant to attack by the TGE virus (2,3,5).

Previous studies on the pathophysiology of this disease have been limited to pigs >3 wk old and to isolated segments of small intestine or to in vitro mucosal sheets perfused with balanced electrolyte solutions (6–8). These studies have shown that disaccharidase activity of the small bowel mucosa is reduced and that the operation of the coupled Na+

Abbreviations used in this paper: PFU: plaque-forming units; SCFA: short-chain fatty acids; TGE: transmissible gastroenteritis.
glucose transport mechanism is impaired. Secretory function of the small bowel, however, appears to be intact. These results correlate well with the villous atrophy and crypt cell hyperplasia observed histologically.

Although such studies can elucidate abnormalities in epithelial ion transport mechanisms or mucosal enzyme activity, they do not account for the effect of digestive contents that may have a considerable impact on transmural ion and water transport in malabsorptive diseases. For example, it is assumed that unabsorbed carbohydrate, besides exerting an appreciable effective osmotic pressure in the small bowel, is rapidly fermented to short-chain fatty acids (SCFA) in the colon, thereby increasing further the osmotic driving force for net fluid secretion (9). Alternatively, the colon may be compensating for small bowel malabsorption to some degree by rapidly absorbing these SCFA, as observed in human jejunoileal bypass patients (10).

In TGE, neither morphological changes nor viral infection is observed in the colon and, thus, functional changes in the colon (if any) may be solely related to the abnormal contents presented to it from the small bowel. Therefore, the present study was undertaken to determine the significance of colonic function in this disease and if colonic function contributes to the age-dependent resistance observed both clinically and experimentally. A fluid marker technique was used which allowed the estimation of the effect of normal digestive components on net water movements in both the small and the large intestine.

**Materials and Methods**

**Pigs**

Thirty-two hysterectomy-derived, colostrum-deprived, new born pigs were randomly assigned to two equal groups. One group was designated 3-day-old pigs and the other designated 3-wk-old pigs. Eight pigs were selected randomly from each group to be infected. All pigs were raised in the laboratory in isolation to prevent inadvertent exposure to the virus. Pigs were fed 100 ml of sterilized cow’s milk [supplemented with vitamins, minerals, and eggs (11)] twice a day into which was incorporated the water-soluble marker polyethylene glycol-4000 (PEG) at a concentration of 5 g/L. Pigs maintained for 3 wk were also given 15 ml of a ground, grain-based pig starter ration twice a day beginning at week 2, and this was increased to 30 ml by week 3. Feed intake was restricted to insure complete consumption of the meal. Both control and infected pigs readily consumed the total amount of feed given at each feeding.

Steady-state marker conditions were approximated as described by Hamilton and Roe (12). On the day of necropsy, the daily feedings were divided into eight equal portions and fed at hourly intervals for 3 h and at 30-min intervals for a remaining 2 h. Although Hamilton and Roe (11) had established that a relatively constant concentration of PEG was obtained by 5 h with this schedule by means of a fistula near the ligament of Treitz, it was questioned whether or not steady-state marker conditions would be obtained throughout the digestive tract, especially in the more distal segments. We define the steady state as a constant perfusion of PEG in amount (CV) per unit time (t) throughout the digestive tract, where C is PEG concentration and V is volume flow. Then, \( C_V \frac{t}{t} = C_i V_i \frac{t}{t} \), where i and o refer to the input and output, respectively, to any segment of the digestive tract. Therefore, the flow rate past a sampling point is \( V_i t = C_i V_i o \). These conditions imply that in the steady state, C and V must be constant in any given segment as a function of time. In order to test the assumption that relatively stable PEG concentrations were obtained in each segment, preliminary experiments were conducted with control pigs of each age group. These pigs were fed their meals according to the above schedule and were necropsied in groups of three at 3, 4, 5, and 6 h following the initial morning feeding. This procedure permitted examination of the change in marker concentrations with time in various segments of the bowel. These studies showed that relatively constant marker concentrations were established by 5 h in all segments of the tract examined (see Results), and, therefore, 5 h appeared sufficient to approximate steady-state conditions in the main experiment. The constancy of V is only assumed, but appears reasonable in view of the small, frequent feedings.

**Inoculum**

The virus preparation was from the same stock as used previously (3). Pigs in the 3-day-old group were infected intragastrically on day 3 with 5 ml of a 1:20,000 dilution of the stock virus suspension containing \( 1.5 \times 10^6 \) plaque-forming units (PFU)/ml. Pigs in the 3-week-old group were given 12 ml of the same dilution of virus on day 21 to compensate for their greater body weight.

**Necropsy**

The 3-day-old pigs were randomly assigned to necropsy at days 5 and 8 for controls and days 6 and 7 for infected animals. Three-week-old pigs were necropsied on days 23 and 26 for controls and days 24 and 25 for infected animals.

Pigs were killed with pentobarbital Na and the stomach and intestinal sites were immediately ligated. These sites included the stomach, two equal lengths of small intestine, the cecum plus the first loop of spiral colon, and the remainder of the colon. Two 10-cm segments of small intestine were fixed in situ by injecting a 10% formalin solution intraluminally. These segments were from sites located 1 m distal to the ligament of Treitz and 1 m proximal to the ileocecal junction. Segments of cecum and colon were also fixed in 10% formalin. Formalin-fixed segments were embedded in paraffin, cut into sections 7 \( \mu \)m thick, and examined with a light microscope equipped
June 1984

COLONIC FUNCTION IN TGE 1503

with an ocular micrometer. Five well-oriented villi from each segment of small intestine were measured to determine mean villous height for the jejunum and ileum in each pig. Mucosal depth was measured in the cecum and colon of all pigs (five measurements per site per pig).

Intestinal contents were collected from each ligated segment and the pH was measured immediately. A portion of contents was centrifuged at 20,000 rpm and the supernatant was collected, diluted 1:5 with distilled water, and frozen. The remainder was frozen immediately and was pooled from all pigs in the group. The small intestine was ground and virus titer was determined, as previously described (3).

Chemical Analysis

Diluted samples were analyzed for PEG by the method of Hyden (13), osmolality by freezing-point depression, Na and K by flame photometry, and Cl by the method of Schales and Schales (14). Pooled samples were thawed, mixed, centrifuged at 4°C, and analyzed for total carbohydrate (15) and for volatile fatty acids by gas chromatography (16).

Results

Virology

Small intestinal tissue from all control pigs was negative for TGE virus. Pigs exposed to virus at day 3 and necropsied 3 and 4 days after being infected had virus titers of $4.07 \pm 2.7$ (SE) $\times 10^5$ PFU/ml of intestinal homogenate. Three-week-old pigs also necropsied 3 and 4 days after being infected displayed significantly lower virus titers of $4.45 \pm 2.6 \times 10^4$ PFU/ml of homogenate, even though these pigs were inoculated with 2.5 times the dose given to the 3-day-old group.

Histopathology

Results of the histopathologic studies of intestinal mucosa, expressed as villous length (jejunum, ileum) or mucosal depth (cecum, colon), are shown in Figure 1. Three-day-old pigs exposed to the virus had marked villous atrophy of the jejunal and ileal mucosa with a mean villous length of <25% of the control tissues (Figures 1 and 2). The degree of villous atrophy was remarkably constant with little variation among pigs. The mucosal depth of the cecum and colon of exposed pigs, however, was unaffected, nor were there any other morphological alterations seen in these tissues when compared to the controls.

In contrast, the height of jejunal and ileal villi in the 3-wk-old infected group was not significantly different from that in the control group ($p > 0.10$). Large variations were present in the jejunum of these older infected pigs, as shown by the relative size of the standard error (Figure 1). Three of the 8 pigs had a normal jejunal villous length of $637 \pm 99$ µm, and the remaining 5 pigs had a mean jejunal villous length of $222 \pm 4$ µm (Figure 2). Such variation in older pigs has been demonstrated previously and was expected. However, no correlation between the degree of villous atrophy and virus titer was present. As in the 3-day-old group, there were no structural alterations of the cecum and colon in the older pigs.

Net Water Movement

Preliminary experiments. The PEG concentration in the various segments of the digestive tract examined as a function of time are shown in Figure 3. Although large individual variation was present, relatively stable PEG concentrations were obtained for the young pigs by 5 h after the initial morning feeding. Large individual variation was also present in the older pigs; however, changes in PEG concentration with time for each site were nonsignificant. Inasmuch as large changes in PEG concentration between certain segments were obtained, it appeared reasonable to expect that changes in various segments of the bowel as a result of the infection could be established with confidence using the 5-h necropsy schedule. Using this single time period also
allowed the number of pigs to be increased in each group from 3 to 8, thus reducing the error due to individual variation. However, it should be emphasized that large errors are possible with the present method. The overall variation in PEG concentration among the pigs in a given segment approached ±34% and was as high as ±60% in the distal colon. Coupled with this, a variation as high as ±20% could be expected from non-steady-state conditions, leading to an overall uncertainty in estimating the net flow of water in control segments of ±40%. We also assume a relatively constant marker release from the stomach and a regular propulsion of marker through the small intestine; conditions which may very well be altered in infected animals. Nevertheless, the use of this method allowed a noninvasive and more physiologic examination of net water movement in the entire digestive tract and, thus, would be more...
Figure 3. Concentration of PEG in gut segments from 3-day-old and 3-wk-old control pigs as a function of time after initial AM feeding. Segments are designated as follows: stomach, ●; jejunum, ○; ileum, □; cecum, △; and colon, ▲. Mean ± SE, n = 8.

suitable for assessing the effect of normal digestive components on net water movement than the conventional perfusion methods.

Control and infected pigs. The concentration of PEG in the various segments of the gastrointestinal tract is shown in Figure 4. The marker was concentrated fivefold by the time ingesta reached the ileum in 3-day-old control pigs, and was further concentrated in the large intestine to a value nine times that found in gastric contents. In striking contrast, no change in PEG concentration was observed along the entire gastrointestinal tract in the 3-day-old pigs with TGE.

Similar data recorded for the 3-wk-old group are shown in the lower portion of Figure 4. Again, the control pigs concentrated the marker severalfold during passage along the small and large intestine. The infected pigs were unable to concentrate the marker in the small intestine to the same degree; however, unlike the 3-day-old group, the large intestine of these 3-wk-old infected pigs did concentrate the nonabsorbable marker.

Examination of the relative PEG concentrations along the gastrointestinal tract may be quantitatively misleading, because a small change in marker concentration in the upper tract, where the flow rate is high, indicates relatively large volume changes. Conversely, although the marker is concentrated severalfold in the distal colon, very little net movement of water may actually be present. Therefore, the data are replotted in Figure 5 as the percentage of fluid intake passing the midpoint of each of the gastrointestinal segments listed. In expressing the data in this manner, we must assume that a steady state in regard to feed and PEG intake is present. Although we are aware that we have not proven this assumption, the large differences in marker concentration observed between individual segments and between control and infected pigs should allow an estimate of the relative quantitative contribution of each of the segments to net water movement.

Several important aspects are revealed in Figure 5 which would not be evident from an examination of PEG concentrations alone. This is especially true for the large intestine. For example, the upper portion of Figure 5 shows that in the 3-day-old control pigs, the actual quantitative contribution of the large intestine to net water absorption is minimal. However, the actual percentage of fluid intake entering the large intestine is only ~10%–12%. Similarly, the percentage of fluid intake in the 3-wk-old control pigs, shown in the lower portion of Figure 5, has been reduced to ~15%–20% by the time the ingesta enter the large intestine. Thus, these figures would indicate that in normal pigs at both of these ages, the small intestine accounts for the majority of net water absorption.

The most striking differences in the infected pigs were observed in the large intestine. The colons of

Figure 4. Concentration of PEG in gut segments from 3-day-old and 3-wk-old control or infected pigs. St, stomach; Sl, proximal one-half of small intestine; Sl2, distal one-half of small intestine; Ce, cecum and proximal loop of spiral colon; C, distal colon. Mean ± SE, n = 8.
control and infected 3-wk-old pigs was markedly lower than that seen in the 3-day-old pigs.

The osmolality of gastrointestinal contents for 3-day-old pigs varied little throughout the tract and was similar to the milk osmolality. Considerably higher values were present in gastric contents of 3-wk-old pigs. These values decreased markedly in the small intestine of infected pigs, whereas in the controls, a marked decrease occurred in the colon.

The concentrations of Na, K, and Cl are shown in Figure 7. The concentration of Na increased along the small intestine to a greater degree in the 3-day-old control group than in the infected pigs, and similarly decreased in the colon to a greater degree in the control pigs. This apparently reflects a decrease in surface area for diffusion in infected pigs; however, no apparent differences in Na concentration were noted in the older pigs. The reasons for these differences in age are unclear, but it should also be noted that the substantial net secretion present in the jejunum of the older infected pigs may have contributed to their luminal Na concentration. Small differences in the concentration of Cl between control and infected pigs were noted; however, gastric contents of older pigs had Cl concentrations that were about twice the values shown in the younger pigs.

Electrolyte Changes

The pH and osmotic pressure of the gastrointestinal contents for all groups of pigs are shown in Figure 6. The pH was lower in the large intestinal contents of infected animals and this was especially true in the 3-day-old group. The cecal pH of both control and infected 3-wk-old pigs was markedly lower than that seen in the 3-day-old pigs.

The pH and osmotic pressure of the gastrointestinal contents for 3-day-old pigs varied little throughout the tract and was similar to the milk osmolality. Considerably higher values were present in gastric contents of 3-wk-old pigs. These values decreased markedly in the small intestine of infected pigs, whereas in the controls, a marked decrease occurred in the colon.

The concentrations of Na, K, and Cl are shown in Figure 7. The concentration of Na increased along the small intestine to a greater degree in the 3-day-old control group than in the infected pigs, and similarly decreased in the colon to a greater degree in the control pigs. This apparently reflects a decrease in surface area for diffusion in infected pigs; however, no apparent differences in Na concentration were noted in the older pigs. The reasons for these differences in age are unclear, but it should also be noted that the substantial net secretion present in the jejunum of the older infected pigs may have contributed to their luminal Na concentration. Small differences in the concentration of Cl between control and infected pigs were noted; however, gastric contents of older pigs had Cl concentrations that were about twice the values shown in the younger pigs.

Electrolyte Changes

The pH and osmotic pressure of the gastrointestinal contents for all groups of pigs are shown in Figure 6. The pH was lower in the large intestinal contents of infected animals and this was especially true in the 3-day-old group. The cecal pH of both control and infected 3-wk-old pigs was markedly lower than that seen in the 3-day-old pigs.

The osmolality of gastrointestinal contents for 3-day-old pigs varied little throughout the tract and was similar to the milk osmolality. Considerably higher values were present in gastric contents of 3-wk-old pigs. These values decreased markedly in the small intestine of infected pigs, whereas in the controls, a marked decrease occurred in the colon.

The concentrations of Na, K, and Cl are shown in Figure 7. The concentration of Na increased along the small intestine to a greater degree in the 3-day-old control group than in the infected pigs, and similarly decreased in the colon to a greater degree in the control pigs. This apparently reflects a decrease in surface area for diffusion in infected pigs; however, no apparent differences in Na concentration were noted in the older pigs. The reasons for these differences in age are unclear, but it should also be noted that the substantial net secretion present in the jejunum of the older infected pigs may have contributed to their luminal Na concentration. Small differences in the concentration of Cl between control and infected pigs were noted; however, gastric contents of older pigs had Cl concentrations that were about twice the values shown in the younger pigs.

Electrolyte Changes

The pH and osmotic pressure of the gastrointestinal contents for all groups of pigs are shown in Figure 6. The pH was lower in the large intestinal contents of infected animals and this was especially true in the 3-day-old group. The cecal pH of both control and infected 3-wk-old pigs was markedly lower than that seen in the 3-day-old pigs.

The osmolality of gastrointestinal contents for 3-day-old pigs varied little throughout the tract and was similar to the milk osmolality. Considerably higher values were present in gastric contents of 3-wk-old pigs. These values decreased markedly in the small intestine of infected pigs, whereas in the controls, a marked decrease occurred in the colon.

The concentrations of Na, K, and Cl are shown in Figure 7. The concentration of Na increased along the small intestine to a greater degree in the 3-day-old control group than in the infected pigs, and similarly decreased in the colon to a greater degree in the control pigs. This apparently reflects a decrease in surface area for diffusion in infected pigs; however, no apparent differences in Na concentration were noted in the older pigs. The reasons for these differences in age are unclear, but it should also be noted that the substantial net secretion present in the jejunum of the older infected pigs may have contributed to their luminal Na concentration. Small differences in the concentration of Cl between control and infected pigs were noted; however, gastric contents of older pigs had Cl concentrations that were about twice the values shown in the younger pigs.

Electrolyte Changes

The pH and osmotic pressure of the gastrointestinal contents for all groups of pigs are shown in Figure 6. The pH was lower in the large intestinal contents of infected animals and this was especially true in the 3-day-old group. The cecal pH of both control and infected 3-wk-old pigs was markedly lower than that seen in the 3-day-old pigs.

The osmolality of gastrointestinal contents for 3-day-old pigs varied little throughout the tract and was similar to the milk osmolality. Considerably higher values were present in gastric contents of 3-wk-old pigs. These values decreased markedly in the small intestine of infected pigs, whereas in the controls, a marked decrease occurred in the colon.

The concentrations of Na, K, and Cl are shown in Figure 7. The concentration of Na increased along the small intestine to a greater degree in the 3-day-old control group than in the infected pigs, and similarly decreased in the colon to a greater degree in the control pigs. This apparently reflects a decrease in surface area for diffusion in infected pigs; however, no apparent differences in Na concentration were noted in the older pigs. The reasons for these differences in age are unclear, but it should also be noted that the substantial net secretion present in the jejunum of the older infected pigs may have contributed to their luminal Na concentration. Small differences in the concentration of Cl between control and infected pigs were noted; however, gastric contents of older pigs had Cl concentrations that were about twice the values shown in the younger pigs.
pigs. These concentrations were reduced substantially in the lower small bowel. Potassium concentrations displayed a reciprocal relationship with Na, but no marked differences were seen among the different groups.

The composite results of SCFA and total carbohydrate concentrations in the cecum and colon contents of these pigs are shown in Table 1. Concentrations of SCFA were much lower in the 3-day-old infected group than in the control group, whereas in the older pigs, similar and higher total SCFA concentrations were present in both groups. An increase in lactate concentration was noted in the older infected group of pigs.

In contrast, total carbohydrate concentrations were much higher in the 3-day-old infected group than in the controls. In fact, the concentrations of carbohydrate in the colon of the infected pigs would account for >40% of the total osmolality of the contents (cf. Figure 6 and Table 1) if all of the contents were in the form of lactose. However, in the older pigs, similar low concentrations of total carbohydrate were seen in both groups, and the carbohydrate concentration in the colons of the 3-wk-old infected pigs was only about one-sixth that of the young infected group.

Although the much lower SCFA concentrations in 3-day-old pigs suggested undeveloped microbial populations in the large bowel, it was questioned whether this observation would also apply to conventionally reared pigs. For example, the hysterectomy-derived animals maintained in a laboratory environment may not obtain a lush microbial population as quickly as pigs reared on the sow. Therefore, 3 conventional pigs were removed from the sow on day 5 of their life and contents from the cecum and colon were collected and analyzed for SCFA, as described above. These results showed that SCFA concentrations ranged from 51.6 to 132 mM in the proximal colon and from 31.8 to 60.6 mM in the distal colon. Thus, these concentrations ranged from those observed in the same age group of hysterectomy-derived control pigs to nearly as high as those in the 3-wk-old control group. Therefore, it is probable that the artificial experimental conditions uniformly delayed the development of a microbial population to some degree.

### Table 1. Short-Chain Fatty Acids (SCFA) and Total Carbohydrate Concentrations (CHO)

| Group/segment | SCFA [mEq/L] | Total CHO [g/100 ml] |
|---------------|--------------|----------------------|
|               | C_1  | C_2  | C_3  | C_4  | Lactate | Total^a |                     |
| 3-Day control |      |      |      |      |         |         |                     |
| Cecum         | 1.6  | 26.3 | 0.4  | 1.8  | 0.5     | 38.2    | 0.94                |
| Colon         | 1.2  | 22.5 | 0.7  | 1.1  | 0.0     | 32.7    | 0.79                |
| 3-Day infected|      |      |      |      |         |         |                     |
| Cecum         | 6.3  | 5.2  | 0.0  | 1.1  | 2.5     | 18.2    | 3.63                |
| Colon         | 9.5  | 4.3  | 0.0  | 0.8  | 1.9     | 19.2    | 4.94                |
| 3-Wk control  |      |      |      |      |         |         |                     |
| Cecum         | 0.0  | 81.5 | 6.6  | 22.0 | 0.9     | 125     | 0.64                |
| Colon         | 0.6  | 46.6 | 4.7  | 8.8  | 0.0     | 70.5    | 1.07                |
| 3-Wk infected |      |      |      |      |         |         |                     |
| Cecum         | 2.4  | 51.7 | 10.9 | 15.9 | 20.3    | 114     | 1.26                |
| Colon         | 3.9  | 36.5 | 9.8  | 6.4  | 23.7    | 91.6    | 0.79                |

^a Total SCFA includes 14 mono- and dicarboxylic acids (15). Only the major SCFA have been listed individually. Values are from pooled samples of intestinal contents from each group of 8 pigs. C_1-C_4 are formate, acetate, propionate, and butyrate, respectively. Lactate includes both D and L isomers.
Discussion

Villous atrophy, associated with decreased levels of mucosal disaccharidases, has been uniformly demonstrated in studies of TGE (1,6). These changes have suggested that unabsorbed carbohydrate may be responsible for osmotic retention of water in the small bowel lumen or even the induction of net secretion because of the increase in effective osmotic pressure of the luminal contents (17).

The present results concerning the small intestine of infected pigs demonstrated zero net fluid movement, or even net fluid secretion into the jejunum in the case of the older pigs. This latter effect appeared to be the result of dilution of the hypertonic gastric contents with a hypotonic fluid, the osmolality of which can be calculated to be \( \sim 185 \text{ mosm/L} \). Thus, these results in the small intestine are consistent with the hypothesis of small bowel malabsorption and fluid accumulation as a result of osmotic forces; however, an active secretory process by the small intestine cannot be entirely ruled out.

The response of the large intestine to TGE infection differed markedly in the two age groups of pigs. The colon of the 3-day-old pigs was incapable of net fluid absorption, whereas the 3-wk-old colon increased absorption some sevenfold over the control and this compensatory response prevented a significant increase in the fecal output of water.

Several possible reasons for this change in colonic function should be considered. First, it is possible that in the young pigs there may be a defect in colonic transport mechanisms caused directly by the virus. However, this seems unlikely because the virus does not invade epithelial cells or cause histologic changes in the large intestine. Furthermore, a significant fraction of the osmotic activity entering and leaving the colon of these young pigs was in the form of carbohydrate (presumably lactose) which, as such, cannot be absorbed by the colonic mucosa.

Second, pigs of this age group may have undeveloped colonic transport mechanisms or colonic absorptive capacity (e.g., surface area). There is now evidence that, in fact, active Na absorption by in vitro pig colonic mucosa reaches very high and near maximal rates by day 1 of life due to high aldosterone secretion rates at this time (18,19). Therefore, it is unlikely that the transport capacity of this tissue represents a limiting factor in colonic absorption. However, the colon of these younger pigs is proportionally much smaller in size than in older pigs and the possibility of total surface area or transit time as a limiting factor needs to be explored. Nevertheless, the same argument can be invoked as with the first alternative; namely, a major proportion of the contents was in the form of carbohydrate which cannot be absorbed by colonic mucosa regardless of the surface area involved.

Finally, a most likely explanation of the differences in function concerns carbohydrate metabolism by colonic bacteria. In fact, it is precisely an overactive bacterial fermentation of carbohydrate to short-chain organic acids that has been postulated as being responsible for luminal acidification and osmotic diarrhea involving the colon; i.e., fermentative diarrhea (9). Fermentation of carbohydrate did produce a degree of acidification and high levels of organic acids in the older pigs, and in the case of the older infected pigs, a proportion of this was in the form of \( \alpha \)-and \( \beta \)-lactic acid. If the feed intake had not been restricted, it is likely that large amounts of lactic acid and acidification of the contents would have resulted in an osmotic diarrhea. However, under these experimental conditions, colonic absorption in the older pigs was unimpaired. Thus, the evidence suggests the alternative hypothesis; namely, that the fermentation process itself is the rate-limiting factor in reducing the osmotic load in the younger pigs. A similar conclusion was reached in human studies involving carbohydrate infusions into the intact colon (20).

Figure 8 summarizes the flow of carbohydrate in grams per day into and out of the large intestine together with the amount of carbohydrate disappearing from the colon, presumably fermented to SCFA. Also shown in parentheses is the number of millimoles per day entering and leaving the large intestine that were not accounted for by the product \( \text{(Na} + \text{K}) \times 2 \). This osmotic fraction would presumably include carbohydrate and peptides. This fraction was calculated for individual pigs instead of the pooled result for carbohydrate and, thus, may be a more accurate description of unabsorbed carbohydrate. In addition, these values are shown for only the 5 pigs with villous atrophy in the 3-wk-old infected group, whereas the pooled carbohydrate sample includes all pigs.

Clearly, the colon of the older infected pigs was capable of effectively disposing of the unabsorbed carbohydrate, whereas in the younger infected pigs, nearly all of the dietary carbohydrate passed through the colon unchanged. It is now well established that SCFA are rapidly absorbed by colonic mucosa and utilized as a source of energy. Therefore, the microbial process converts unabsorbable and, therefore, osmotically active material to rapidly absorbed SCFA, thereby reducing the effective osmotic pressure of the colonic contents, and salvaging calories which would otherwise be unavailable. In addition, these SCFA, at concentrations observed in the 3-wk-old pigs, have been shown to augment Na and water...
absorption from the colon of several animal species, including humans (21,22). The development of microbial digestion, therefore, appears to play a central role in the changes in colonic function observed.

It is necessary to point out, however, that the artificial experimental conditions may have delayed the development of microbial digestion in the young pigs. The range of SCFA concentrations observed in the conventional pigs of this same age suggests a more rapid, but wide, variation in the rate of development of the process under those conditions. Thus, the environment and diet of the animal may be equally or even more critical a factor than age in the contribution of these factors to the development of microbial digestion in the young pigs. The range of SCFA concentrations observed in artificial experimental conditions may have delayed the development of microbial digestion in the young pigs. The range of SCFA concentrations observed in the conventional pigs of this same age suggests a more rapid, but wide, variation in the rate of development of the process under those conditions. Thus, the environment and diet of the animal may be equally or even more critical a factor than age in the inoculation of the bowel or the ability of the resident bacteria to ferment carbohydrate. Further study will be necessary to quantitatively assess the individual contribution of these factors to the development of colonic function.

References

1. Moon HW, Norman JO, Lambert G. Age-dependent resistance to transmissible gastroenteritis of swine (TGE). I. Clinical signs and some mucosal dimensions in small intestine. Can J Comp Med 1973;37:157–66.
2. Thake DC, Moon HW, Lambert G. Epithelial cell dynamics in transmissible gastroenteritis of neonatal pigs. Vet Pathol 1973;10:330–41.
3. Moon HW, Kemeny LJ, Lambert G, et al. Age-dependent resistance to transmissible gastroenteritis of swine. III. Effects of epithelial cell kinetics on coronavirus production and on atrophy of intestinal villi. Vet Pathol 1975;12:434–45.
4. Moon HW. Epithelial cell migration in the alimentary mucosa of the suckling pig. Proc Soc Exp Biol Med 1971;137:151–4.
5. Pensaert M, Haelterman EO, Burnstein T. Transmissible gastroenteritis of swine: virus-intestinal cell interactions. I. Immunofluorescence, histopathology and virus production in the small intestine through the course of infection. Arch Gesamte Virusforsch 1970;31:321–34.
6. Butler DG, Gall DG, Kelly MH, et al. Mechanisms responsible for diarrhea in an acute viral enteritis in piglets. J Clin Invest 1974;53:1335–42.
7. Kerzner B, Kelly MH, Gall DG, et al. Transmissible gastroenteritis: sodium transport and the intestinal epithelium during the course of viral enteritis. Gastroenterology 1977;72:457–61.
8. Shephard RW, Gall DG, Butler DG, et al. Determinants of diarrhea in viral enteritis. The role of ion transport and epithelial changes in the ileum in transmissible gastroenteritis in piglets. Gastroenterology 1979;76:20–4.
9. Phillips SF. Diarrhea: a current view of the pathophysiology. Gastroenterology 1972;63:405–518.
10. Bond JH, Currier BE, Buchwald H, et al. Colonic conservation of malabsorbed carbohydrate. Gastroenterology 1980;78:444–7.
11. Amtower WC, Calhoun JR. A beta-propiolactone sterilized milk formula for specific pathogen-free pigs. Lab Anim Care 1964;14:382–7.
12. Hamilton DL, Roe WE. Electrolyte levels and net fluid and electrolyte movements in the gastrointestinal tract of weanling swine. Can J Comp Med 1977;41:241–50.
13. Hyden S. A turbidometric method for the determination of higher polyethylene glycols in biological materials. K Lantbrukshogsk Ann 1956;22:139–45.
14. Schales O, Schales SS. A simple and accurate method for the determination of chloride in biological fluids. J Biol Chem 1941;140:879–84.
15. Morris DL. Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 1948;107:254–5.
16. Salanitro JP, Muirhead PA. Quantitative method for the gas chromatographic analysis of short-chain monocarboxylic and dicarboxylic acids in fermentation media. Appl Microbiol 1975;29:374–81.
17. Fordtran JS, Ingelfinger FJ. Absorption of water, electrolytes, and sugars from the human gut. In: Code CF, Heidel W, eds. Handbook of physiology. Section 6, Alimentary canal. Vol. III. Washington DC: American Physiological Society, 1968, 74:1457–90.
18. Bentley PJ, Smith MW. Transport of electrolytes across the helicoidal colon of the newborn pig. J Physiol 1975;249:103–17.
19. Ferguson DR, James PS, Paterson JYF, et al. Aldosterone induced changes in colonic sodium transport occurring naturally during development in the neonatal pig. J Physiol 1979;292:495–504.
20. Saunders DR, Wiggins HS. How do single doses of carbohydrates such as lactulose cause diarrhea (abstr). Gastroenterology 1981;80:1271.
21. Argenzio RA. Short-chain fatty acids and the colon. Digest Dis Sci 1981;26:97–9.
22. Ruppin H, Bar-Meir S, Soergel KH, et al. Absorption of short-chain fatty acids by the colon. Gastroenterology 1980;78:1500–7.