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Effect of Dietary Regimen on Rotavirus-*Escherichia coli* Weanling Diarrhea of Piglets†

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Previously, we induced weanling diarrhea in piglets by infecting them with rotavirus followed by hemolytic enteropathogenic *Escherichia coli*. We postulated that rotavirus, by damaging the epithelium of the small intestines, produced an enteroenvironment which favored the selection and growth of enteropathogenic *E. coli*. Furthermore, diet might affect the enteroenvironment and influence the course of the disease. To test this, newly weaned 3-week-old piglets were assigned to one of four dietary regimens and infected with rotavirus followed 24 h later with enteropathogenic *E. coli*. The course of the disease was followed by monitoring the severity of diarrhea and the fecal shedding of rotavirus and enteropathogenic *E. coli* in these dually infected piglets. The dietary regimen designed to tax the digestive and absorptive capacities of the piglets (high nutrient intake fed three times a day) produced the most prolonged diarrhea, colonization of the gut by hemolytic enteropathogenic *E. coli*, and persistent shedding of rotavirus ($P < 0.01$). The same nutrient intake divided into 24 equal increments and fed hourly produced a less severe response ($P < 0.01$). The least severe response was seen in piglets fed one-third the nutrient intake either hourly or three times a day ($P < 0.01$). We conclude that dietary regimen plays an important role in rotavirus-*E. coli*-induced weanling diarrhea.

Piglets and other neonates often experience a diarrhea shortly after weaning called weanling diarrhea (1, 7, 15, 21). This syndrome, generally diagnosed as colibacillosis, is accompanied by a dramatic shift in the aerobic fecal flora, from one of mainly nonhemolytic *Escherichia coli* and enterococci to one of profuse hemolytic *E. coli* (2, 3, 5, 8, 9, 16, 20). These hemolytic *E. coli* strains usually produce enterotoxins and possess pili which specifically mediate the adherence of *E. coli* to enterocytes (6). Such strains are called enterotoxigenic or enteropathogenic *E. coli* and belong to a variety of serotypes (5, 21).

In 1973, we described the pathogenesis of a fatal diarrhea in colostrum-deprived neonatal piglets that were being reared artificially (4). Initially, this syndrome was diagnosed as colibacillosis. Subsequently, we reproduced the syndrome by infecting neonatal colostrum-deprived piglets with bacteria-free diarrhetic fluid containing rotavirus, a newly characterized virus that replicates in and destroys enterocytes (14). Later, we detected large numbers of rotaviruses in the feces of 3- to 4-week-old newly weaned diarrhetic piglets and assigned etiological significance to rotavirus in piglet weanling diarrhea (12). A shift in fecal flora from nonhemolytic *E. coli* to hemolytic enteropathogenic *E. coli* during diarrhea was also noted (10). However, it was not possible to reproduce colibacillosis (weanling diarrhea) as seen in the field in piglets infected per os with hemolytic enteropathogenic *E. coli* (serotype O157:K · :NM) unless the animals were concurrently infected with rotavirus. We concluded that rotavirus, by damaging the gut, provided an enteroenvironment which favored the selection, colonization, and growth of enteropathogenic *E. coli* in the small intestines (10).

Subsequently, we wondered whether dietary regimen could influence the enteroenvironment and thus the course of the disease. In this connection, we undertook the present investigation to determine whether dietary regimen could modify the severity of diarrhea and the fecal shedding of both rotavirus and hemolytic enteropathogenic *E. coli*. For this purpose, newly weaned 3-week-old piglets were assigned to one of the following liquid dietary regimens: (i) a diet containing high solids (18% dry matter) fed three

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times a day; (ii) the high-solids diet fed hourly; (iii) the high-solids diet diluted with water to 6% dry matter and fed three times a day (low solids); and (iv) the low-solids diet fed hourly. After a 1-week adjustment period to their respective dietary regimens, the piglets were infected with rotavirus and, 1 day later, with hemolytic enteropathogenic *E. coli*. Results reported herein show that at least three factors play an important role in weanling diarrhea. First is an infection with rotavirus, followed by an infection with enteropathogenic *E. coli*, and a dietary regimen that burdens the absorption capacity of the gut.

MATERIALS AND METHODS

Animals. "Sanitary" piglets were farrowed in an intensive care farrowing facility where they nursed for 3 weeks (10, 13). After 3 weeks, some of the piglets were weaned into an isolated, fumigated room containing an automatic feeding device (Autoweaner). Piglets were individually caged and fed hourly. Others were individually caged in isolated fumigated rooms where they were fed three times a day.

Diets. Diets consisted mainly of nonfat cow's milk solids, fat, vitamins, and minerals (11). Of the dry matter, 30% was protein, 40% lactose, and 20% animal fat (MS 12/50; Milk Specialties Co., Dundee, Ill.). The basic diet contained 18% solids (dry matter). This was called the high-solids diet. When this diet was diluted with water to 6% total solids, it was called the low-solids diet. During the course of the experiment, the volume of the diets fed in a 24-h period was calculated to be 30% of the body weight of the piglet (e.g., a 6-kg piglet was fed 1,800 ml). The daily volume was divided into 24 equal increments and fed hourly or into 3 equal increments and fed three times a day (at 9 a.m., 1 p.m., and 7 p.m.).

Piglets were fed according to four dietary regimens: (i) high solids three times a day; (ii) high solids hourly; (iii) low solids three times a day; and (iv) low solids hourly. Thus, piglets fed high solids received, proportional to their weight, the same total solids and volume in either 3 or 24 equal increments. Piglets assigned to the low-solids regimens were fed similarly except that the daily intake of solids (dispensed in either 3 or 24 equal increments) was one-third that of the high-solids regimen. The high-solids diet promotes high rates of weight gain and is near the digestive and absorptive capacities of the piglets (11). The low-solids diet promotes minimal weight gains.

Experimental. At weaning, piglets were randomly selected from 12 sows that had farrowed in an intensive care farrowing facility (13). These piglets were assigned to one of the four dietary regimens (five piglets per regimen). This design was repeated through three farrowing groups, i.e., 15 pigs per dietary regimen, with the exception of the high-solids group fed hourly. This particular regimen was repeated twice (10 pigs per dietary regimen).

Statistics. Data from the different farrowing groups were pooled and expressed as the percentage of piglets experiencing diarrhea, and as the percentage of piglets shedding rotavirus and hemolytic enteropathogenic *E. coli*-7 in their feces (see below).

The degree of diarrhea and shedding of rotavirus and hemolytic *E. coli*-7 were compared between the piglets on the four dietary regimens by computing the average number of days each piglet had diarrhea and shed rotavirus and hemolytic *E. coli*-7. These averages were analyzed for significance by using a one-way analysis of variance (17).

Microbiology. After the piglets had been fed according to their respective dietary regimens for 7 days, they were infected per os with approximately 10^9 rotaviral particles and 24 h later with approximately 10^9 hemolytic, enteropathogenic *E. coli* (O157:K · :NM) (called *E. coli*-7). These were days 1 and 2, respectively, of the experiment (see figures). To determine whether the piglets developed resistance to the dual infection, they were reinfected 13 days after the initial infection with the same dose of rotavirus and *E. coli*-7 and in the same sequence, except for two groups of five piglets, one group fed high solids three times a day and the other fed low solids three times a day (see Fig. 5). The purpose of not reinfecting these two groups of piglets was to determine the length of persistence of diarrhea and fecal shedding of rotavirus and *E. coli*-7 after an initial infection. Both *E. coli*-7 and the pool of rotavirus were used in a previous study (10).

Fecal shedding patterns of rotavirus and *E. coli*-7 were monitored as before, using an enzyme-linked immunosorbent assay for detecting rotavirus and appropriate bacteriological and serological techniques for isolating and identifying *E. coli*-7 (10). The small intestines of piglets that died or were killed in extremis were examined by immunofluorescence and scanning electron microscopy for adhering *E. coli* (10, 14).

RESULTS

Diarrhea. (i) High solids three times a day. There was a marked and significant difference ($P < 0.01$) in the severity of diarrhea between the piglets fed high solids three times a day and the piglets fed according to the other dietary regimens. All of the piglets fed high solids three times a day had diarrhea from days 3 to 8 (Fig. 1). This was followed by a gradual decline to 18% by day 12. At day 13 they were reinfected with rotavirus, followed by *E. coli*-7 on day 14. The rate of diarrhea rose to 100% on day 15 and declined to 28% by day 20. Four of 15 piglets on this regimen died or were killed in extremis between days 5 and 7. *E. coli*-7 was found adhering to ileal villi in these piglets (Fig. 2).

(ii) High solids 24 times a day. Piglets fed according to this dietary regimen experienced less diarrhea ($P < 0.01$) than the piglets fed high solids 3 times a day but more than the piglets fed low solids 24 or 3 times a day ($P < 0.01$). All of the piglets fed high solids 24 times a day had diarrhea on days 3 and 4 (Fig. 1). This was followed by a rapid decline to zero by day 10. These piglets did not experience diarrhea when reinfected at days 13 and 14 with rotavirus and *E. coli*-7, respectively.

(iii) Low solids three times a day. Seventy percent of the piglets had diarrhea by day 4 (Fig.

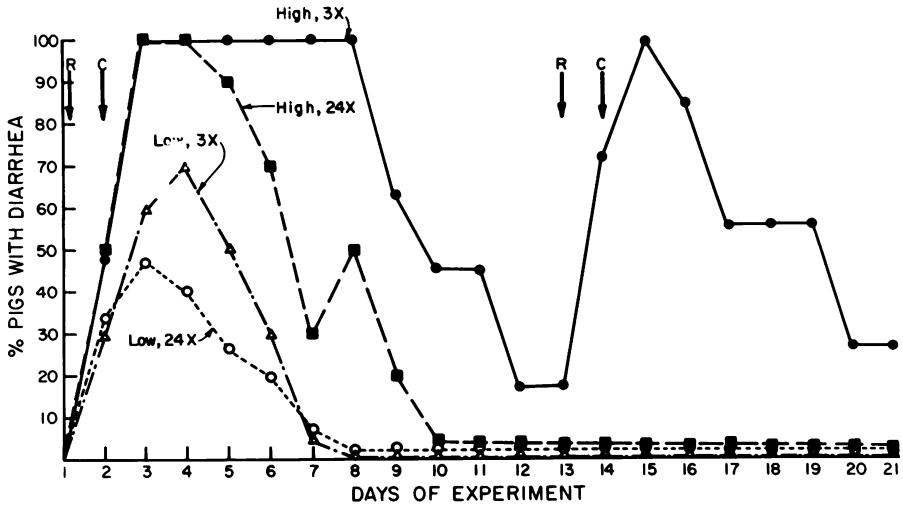

FIG. 1. Percentage of piglets with diarrhea. R, Piglets infected with rotavirus; C, piglets infected with *E. coli*-7. Symbols: ●, high solids 3 times a day; ■, high solids 24 times a day; △, low solids 3 times a day; ○, low solids 24 times a day.

1), followed by a rapid decline to near zero by day 7. The amount of diarrhea in this group was not significantly different from that of the group fed low solids 24 times a day. Piglets fed low solids 3 times a day did not experience diarrhea when reinfected at days 13 and 14 with rotavirus and *E. coli*-7, respectively.

(iv) Low solids 24 times a day. These piglets experienced the least amount of diarrhea, i.e., 50% by day 3, zero by day 7, and no diarrhea

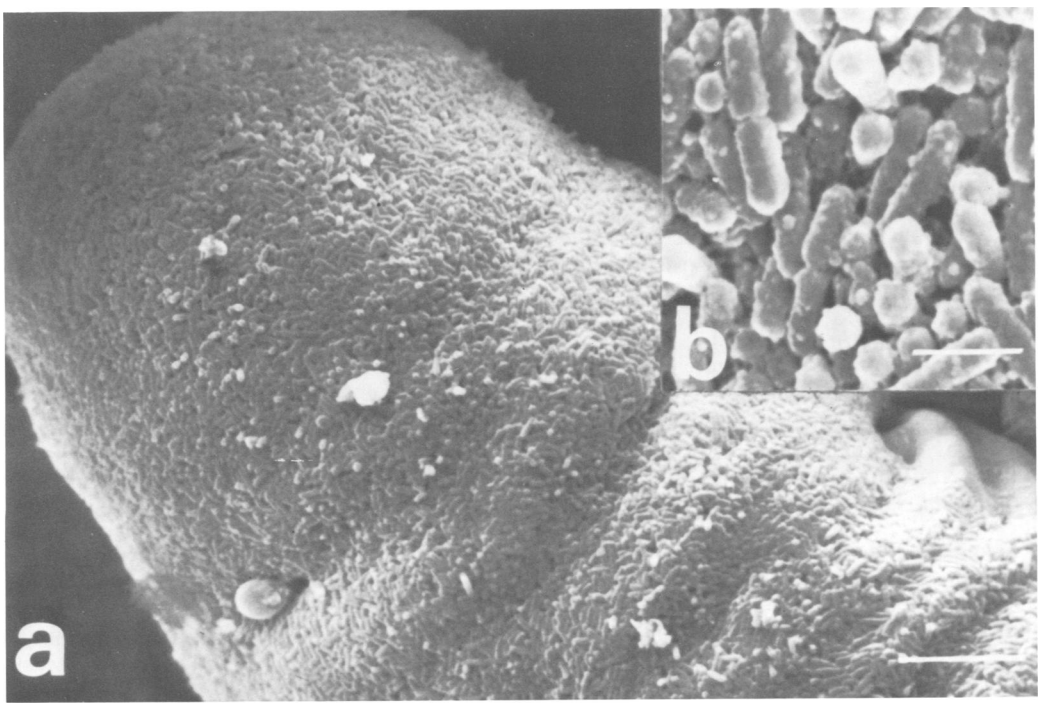

FIG. 2. Scanning electron micrograph showing a section from the ileum of a piglet fed high solids three times a day and dually infected with rotavirus and hemolytic *E. coli*-7. (a) Villus completely covered with palisading *E. coli*-7. Bar, 10 μm. (b) An enlargement of (a) showing the dense packing of adhering *E. coli*-7. Bar, 1 μm.

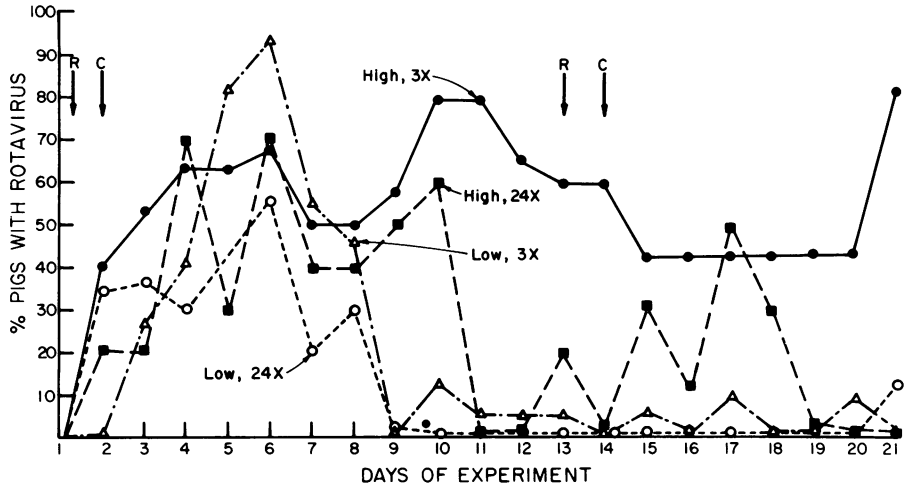

FIG. 3. Percentage of piglets shedding rotavirus. R, Piglets infected with rotavirus; C, piglets infected with *E. coli*-7. Symbols: ●, high solids 3 times a day; ■, high solids 24 times a day; △, low solids 3 times a day; ○, low solids 24 times a day.

when reinfected at days 13 and 14 with rotavirus and *E. coli*-7, respectively.

Rotavirus. (i) High solids three times a day. Again, there was a marked and significant difference ($P < 0.01$) between piglets fed according to this dietary regimen and those fed according to the other three regimens. Forty to 80% of the piglets shed rotavirus for the entire 21 days of observation (Fig. 3).

(ii) High solids 24 times a day. Piglets fed according to this dietary regimen shed significantly more rotavirus ($P < 0.01$) than those in the group fed low solids 24 times a day but not more than the piglets fed the low solids 3 times a day. Forty to 70% of the piglets fed high solids 24 times a day (Fig. 3) shed rotavirus from day 2 to day 11. Twenty to 50% of the piglets shed rotavirus for 4 days after they were reinfected at day 13 with rotavirus.

(iii) Low solids three times a day. About 90% of the piglets shed rotavirus by day 6, a figure which declined rapidly to zero by day 9 (Fig. 3). There was a negligible response to reinfection with rotavirus at day 13 (10% or less). There was a significant difference ($P < 0.05$) in the shedding pattern between this group and those fed low solids 24 times a day.

(iv) Low solids 24 times a day. A peak of 55% of the piglets shed rotavirus by day 6, followed by rapid decline to zero by day 9 (Fig. 3). There was a negligible response to reinfection with rotavirus at day 13.

Hemolytic enteropathogenic *E. coli*-7. (i) High solids three times a day. Again, there was a significant difference ($P < 0.01$) in the response of piglets fed according to this regimen compared with piglets fed according to the other three regimens. About 90% of the piglets in this group shed *E. coli*-7 from day 3 to day 7; the percentage declined to zero by day 11 (Fig. 4). Fifty percent of the piglets shed *E. coli*-7 for 1 day when reinfected on day 14 with *E. coli*-7.

(ii) High solids 24 times a day; low solids 3 times a day; low solids 24 times a day. Piglets fed according to these three regimens (Fig. 4) responded in a similar manner (not significantly different from each other) when infected with *E. coli*-7, i.e., about 40 to 90% of the piglets shed *E. coli*-7 by day 3, declining to zero by day 9. About 20% of the piglets fed high solids and low solids 24 times per day shed hemolytic *E. coli*-7 for 1 day when reinfected with *E. coli*-7 on day 14.

Persistence of rotavirus and diarrhea. Rotavirus and diarrhea (but not *E. coli*-7) persisted for at least 39 days in piglets fed high solids three times a day (Fig. 5). In contrast ($P < 0.01$), no diarrhea and shedding of rotavirus was seen from day 22 to 39 in piglets that were fed low solids three times a day.

DISCUSSION

We noted previously that orally inoculating newly weaned 3-week-old piglets with hemolytic enteropathogenic *E. coli* (O157:K · :NM) was of little consequence unless the piglets were concurrently infected with rotavirus (10). Then the syndrome of weanling diarrhea (colibacillosis), as seen in the field and described by many

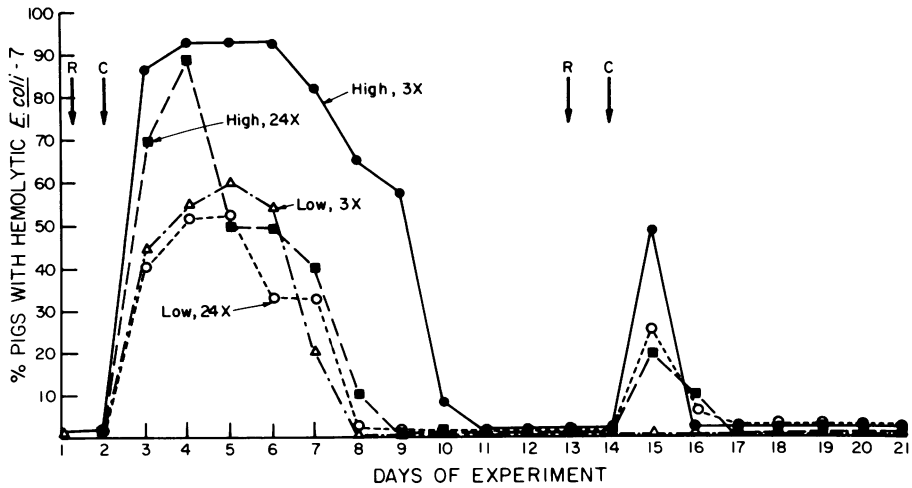

FIG. 4. Percentage of piglets shedding *E. coli*-7. R, Piglets infected with rotavirus; C, piglets infected with *E. coli*-7. Symbols: ●, high solids 3 times a day; ■, high solids 24 times a day; △, low solids 3 times a day; ○, low solids 24 times a day.

investigators in the past, was reproduced (2, 3, 5, 8, 9, 15, 16, 18–21). Dually infected piglets experienced severe diarrhea accompanied by ready colonization of the small intestines by adhering hemolytic enteropathogenic *E. coli*. One possibility considered by us was that an initial infection with rotavirus, a virus that multiplies in and destroys small intestine epithelial cells, produced malabsorption (22) which in turn provided a nutrient milieu that favored the selection and growth of enteropathogenic *E. coli*. Thus, we theorized that the dietary regimen might influence the course and severity of weaning diarrhea. Investigators have long felt that diet plays a role in weanling diarrhea because there is a drastic change in dietary regimen at weaning which coincides with the initiation of diarrhea (8, 9, 18–20).

Experiments reported here were designed to manipulate the luminal nutrient milieu of the gut by using four markedly different liquid dietary regimens. The first dietary regimen was de-

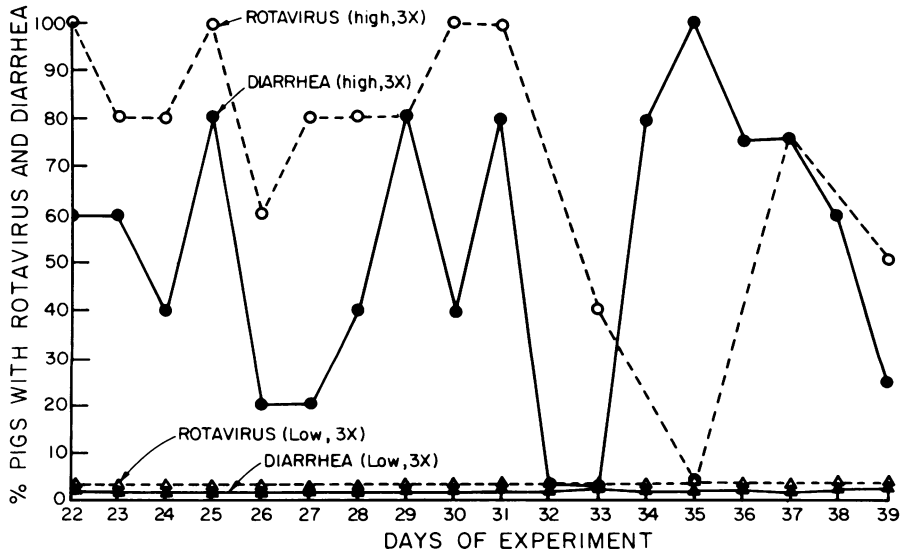

FIG. 5. Percentage of piglets with diarrhea and shedding rotavirus. Symbols: ●, diarrhea, high solids 3 times a day; ○, rotavirus, high solids 3 times a day; ▲, diarrhea, low solids 3 times a day; △, rotavirus, low solids 3 times a day.

signed to tax digestion and absorption by presenting nutrient surges to the gut. This was done by feeding large amounts of a high-caloric, high-protein diet (high solids) in three equal increments within a short interval (9 a.m., 1 p.m., and 7 p.m.). This diet is similar to sow's milk (11). The second dietary regimen was more natural to the piglets in that it mimicked the dietary regimen of a sow nursing her piglets. In this case, piglets were fed the same total daily nutrient intake as those fed by the first regimen except that it was presented in 24 equal increments throughout the day. This dietary regimen produces (as it does in nursing piglets) rapid, efficient rates of weight gain (11). Presumably, the rapid gain is sustained by a continuous flow of nutrients presented in a manner that maximizes assimilation of nutrients. The other two dietary regimens (low solids) served as controls. These piglets were fed the same volume and on the same schedules as the piglets fed on the high-solids regimens except that the total nutrient intake was one-third that of the high-solids regimens.

Results of the experiments reported here show that the dietary regimen designed to tax the digestive and absorptive capacities of the piglets (high solids three times a day) produced the most prolonged diarrhea, colonization of the gut by hemolytic *E. coli*, and persistent shedding of rotavirus in the feces of dually infected piglets ($P < 0.01$). The same daily nutrient intake divided into 24 equal increments and fed hourly produced a less severe response ($P < 0.01$), and the least severe response ($P < 0.01$) was seen in piglets fed one-third the nutrient intake either hourly or three times a day. Thus, in terms of the intent of these experiments and consistent with the above results, we offer the explanation that feeding high solids three times a day produced a nutrient surge which in concert with the rotavirus-damaged epithelium overwhelmed the digestive and absorptive capacities of the piglets. Malabsorption and concomitant diarrhea ensued. This resulted in an enteroenvironment which favored for a limited time (ca. 9 days) the colonization of the gut by hemolytic *E. coli* and for an unlimited time the persistent fecal shedding of rotavirus (at least 39 days).

Fecal shedding of hemolytic *E. coli* ceased about 9 days post-inoculation regardless of dietary regimen. Also, piglets resisted dual infection with hemolytic *E. coli* and rotavirus 13 days after the initial infection (Fig. 3 and 4), except for those fed high solids three times a day. Piglets fed high solids three times a day continued to have diarrhea and to shed rotavirus whether or not they were reinfected, for as long as they were observed (39 days after the initial rotavirus infection) (Fig. 3 and 5). Perhaps the rotavirus persisted because the altered enteroenvironment increased the efficiency of infection of enterocytes by rotavirus. If this were so, then a cycle of rotavirus shedding, fueled by malabsorption, would be generated for as long as the dietary regimen contributed to the malabsorption. Conversely, as was observed, feeding piglets by a less burdensome dietary regimen (low solids) would limit the cycle.

We reported previously on a persistent rotaviral infection associated with multiple episodes of diarrhea in piglets reared in isolation (13). At the time, we could not account for the chronicity, but in hindsight the dietary regimen used for those piglets was similar to that used here for the piglets fed high solids.

We feel that diet plays an important role in the provocative events leading to weanling diarrhea. We envision the sequence from normal to abnormal as follows. (i) Piglets are weaned into an environment contaminated with enteropathogens; (ii) the gut becomes susceptible to enteropathogens at weaning because it is no longer bathed by protective antibody coming from sow's milk (12); (iii) the ubiquitous enteropathogen (rotavirus) infects and destroys absorptive epithelium, thereby producing a malabsorption of nutrients; (iv) this malabsorption creates an enteroenvironment which not only favors the colonization of the small intestines by enteropathogenic *E. coli* but also increases the efficiency of infection of enterocytes by rotavirus; and (v) the more burdensome the dietary regimen, the more efficient the reinfection by rotavirus and the more persistent the diarrhea and shedding of rotavirus. Why rotavirus persisted and hemolytic *E. coli*-7 did not is currently under study. To this end, we are investigating the effect of malabsorption produced by noninfectious agents on an infection by rotavirus and enteropathogenic *E. coli*, along with the immune response of the gut to these two enteropathogens.

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LITERATURE CITED

1. **Barnum, D. A., P. J. Glantz, and H. W. Moon.** 1967. Colibacillosis. CIBA Veterinary Monograph Series no. 2. CIBA Pharmaceutical Co.
2. **Buxton, A., and J. R. Thomlinson.** 1961. The detection of tissue-sensitizing antibodies to *Escherichia coli* in oedema disease, haemorrhagic gastro-enteritis and in normal pigs. Res. Vet. Sci. **2**:73–88.
3. **Chopra, S. L., A. C. Blackwood, and D. G. Dale.** 1964. Enteritis of early weaned pigs. I. Enteropathogenic *Esch-*

erichia coli. Can. J. Comp. Med. Vet. Sci. **28**:239–247.
4. **Coalson, J. A., and J. G. Lecce.** 1973. Herd differences in the expression of fatal diarrhea in artificially reared piglets weaned after 12 hours vs. 36 hours of nursing. J. Anim. Sci. **36**:1114–1121.
5. **Ellis, R. P.** 1978. Serologic and epidemiologic investigations of colibacillosis in pigs, p. 161–165. Proceedings of the Second International Symposium on Neonatal Diarrhea. Veterinary Infectious Disease Organization. University of Saskatchewan, Saskatoon, Canada.
6. **Gaastra, W., and F. K. de Graaf.** 1982. Host-specific fimbrial adhesions of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. **46**:129–161.
7. **Gordon, J. E., I. D. Chitkara, and J. G. Wyon.** 1963. Weanling diarrhea. Am. J. Med. Sci. **245**:345–377.
8. **Kenworthy, R., and W. D. Allen.** 1966. The significance of *Escherichia coli* to the young pig. J. Comp. Pathol. **76**:31–44.
9. **Kenworthy, R., and W. E. Crabb.** 1963. Intestinal flora of young pigs, with reference to early weaning, *Escherichia coli*, and scours. J. Comp. Pathol. Ther. **73**:215–228.
10. **Lecce, J. G., R. K. Balsbaugh, D. A. Clare, and M. W. King.** 1982. Rotavirus and hemolytic enteropathogenic *Escherichia coli* in weanling diarrhea of pigs. J. Clin. Microbiol. **16**:715–723.
11. **Lecce, J. G., and J. A. Coalson.** 1976. Diets for rearing colostrum-free piglets with an automatic feeding device. J. Anim. Sci. **42**:622–629.
12. **Lecce, J. G., and M. W. King.** 1978. Role of rotavirus (reo-like) in weanling diarrhea of pigs. J. Clin. Microbiol. **8**:454–458.
13. **Lecce, J. G., and M. W. King.** 1980. Persistent rotaviral infection producing multiple episodes of diarrhea in weanling pigs reared in isolation, p. 21–26. Proceedings of the Third International Symposium on Neonatal Diarrhea. Veterinary Infectious Disease Organization. University of Saskatchewan, Saskatoon, Canada.
14. **Lecce, J. G., M. W. King, and R. Mock.** 1976. Reovirus-like agent associated with fatal diarrhea in neonatal pigs. Infect. Immun. **14**:816–825.
15. **Nielsen, N. O., H. W. Moon, and W. E. Roe.** 1968. Enteric colibacillosis in swine. J. Am. Vet. Med. Assoc. **153**:1590–1606.
16. **Neilsen, N. O., and J. H. Sautter.** 1968. Infection of ligated intestinal loops with hemolytic *Escherichia coli* in the pig. Can. Vet. J. **9**:90–97.
17. **Steel, R., and J. Torrie.** 1960. Principles and procedures of statistics. McGraw-Hill Book Co., New York.
18. **Stevens, A. J.** 1963. Symposium: enteritis in pigs. I. Coliform infections in the young pig and a practical approach to the control of enteritis. Vet. Rec. **75**:1241–1245.
19. **Stevens, A. J.** 1963. Enteritis in pigs—a working hypothesis. Br. Vet. J. **119**:520–526.
20. **Tzipori, S., D. Chandler, M. Smith, T. Makin, and D. Hennessy.** 1980. Factors contributing to the postweaning diarrhea in a large intensive piggery. Aust. Vet. J. **56**:274–278.
21. **Wilson, M. R.** 1981. Enteric colibacillosis diseases of swine, p. 471–477. *In* A. D. Leman, R. D. Glock, W. L. Mengeling, R. H. C. Penny, E. Scholl, and B. Straw (ed.), Diseases of swine, 5th ed. Iowa State University Press, Ames.
22. **Woode, G. N., C. Smith, and M. J. Dennis.** 1978. Intestinal damage in rotavirus infected calves assessed by d-xylose malabsorption. Vet. Rec. **102**:340–341.