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Intestinal Colonization of Neonatal Animals by *Campylobacter fetus* subsp. *jejuni*

LEANNE H. FIELD, JUDY L. UNDERWOOD, LEODOCIA M. POPE, AND L. JOE BERRY*

Department of Microbiology, The University of Texas, Austin, Texas 78712

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Neonatal mice (2.3 to 2.8 g) were inoculated intragastrically with different human isolates of *Campylobacter fetus* subsp. *jejuni*. At weekly intervals thereafter, mice were sacrificed and dilution plate counts were performed on segments of the gastrointestinal tract. Mice were uniformly colonized by some strains for 2 weeks, whereas other strains were being cleared at that time. One strain (BO216) persisted in some mice for 3 weeks. The greatest number of organisms (10^7) was recovered from the cecum and large intestine. The small intestine had from 10^2 to 10^5 colony-forming units. Colonization of the stomach was not found consistently. One strain killed 13% of the infected mice. Deaths occurred between 1 and 5 days postinfection. Two other strains killed a smaller percentage of challenged animals, and two additional strains killed none. Retarded weight gain was noticed in some, but not all, of the infected mice. The intestines of neonatal rats and rabbits were colonized much the same as those of mice, whereas hamsters were resistant to colonization. Preweanling mice, up to about 6.5 to 7.0 g, could be colonized with *C. fetus* subsp. *jejuni* after intragastric challenge, but weanling mice of larger weight (9.8 g) and young adult mice (18.3 g) could not. Scanning electron photomicrographs of the lower ileum showed campylobacters in and below the dried mucous gel that lines the intestines. The use of this model for additional studies is discussed.

Within the last 5 years, a new enteric pathogen for humans has been identified, even though the organism itself has been known for decades (9). *Campylobacter fetus* subsp. *jejuni*, formerly known as *Vibrio fetus* and later as "related vibrios," has been identified as a cause of enteritis (gastroenteritis or gastroenterocolitis). The linkage of the bacterium to diarrhea in human patients was strongly suggested in 1973 when Butzler et al. (8) used a selective filtration technique to examine the stools of 800 children and 100 adults with diarrhea and isolated campylobacters in about 5% of the cases. In 1977, Skirrow (38) isolated campylobacters from stools by inoculating them directly onto a newly developed selective culture medium that contained antibiotics. He examined stools in about 800 cases of sporadic diarrhea and found *C. fetus* subsp. *jejuni* more often than any other enteric pathogen. Because of the difficulty in isolating this etiological agent, including its need for a microaerophilic environment, its role in diarrheic disease in humans has been overlooked. At the present time, *C. fetus* subsp. *jejuni* is being isolated from patients with enteritis more frequently in some areas than *Salmonella* sp., *Shigella* sp., *Escherichia coli*, or parasites (9, 21).

Campylobacter enteritis has an international distribution (2, 9, 20, 21, 34, 44, 47). It is frequently found in children (4, 21) and is believed to cause infantile diarrhea in third world countries (12). A significant number of outbreaks has been described (11, 31, 35), the largest of which affected 2,000 people in a Vermont town of about 13,000 inhabitants (10). It was thought to be transmitted in the water supply.

The symptoms associated with campylobacter enteritis vary widely in severity. Those symptoms most often seen include fever, abdominal pain that is often severe, and diarrhea that is usually bloody (2, 9, 20, 21, 38). In some patients the disease is mild, self-limiting, and over within 2 weeks or less without treatment (9, 20). In its severest form, there is an acute gastroenteritis, with as many as 20 stools per day (6, 46), mimicking ulcerative colitis or Crohn's disease (26, 27, 46). Abdominal pain may be severe enough to prompt attending physicians to carry out exploratory laparotomies (19, 27, 38), appendectomies (19, 41), and, in one case, a Cesarian section (23). Some deaths have been reported (9, 20, 29).

Much remains to be done before the epidemiology of campylobacter enteritis is understood. There appear to be a number of animal reservoirs of the organism, since it has been

isolated from feces or carcasses of chickens (some commercially processed) (16) and from feces of dogs (3, 45), cats (7, 39), cattle (11, 35), wild ducks (24), and other animals (1, 7, 9, 43). Puppies and kittens with diarrhea have been linked to human disease (3, 19, 26, 39), and person-to-person spread has been documented (9, 20, 28). Moreover, in two cases, humans have acquired campylobacter enteritis under controlled conditions. In one, a human volunteer became ill after ingesting a culture of *C. fetus* subsp. *jejuni*, and in the other, an individual acquired the disease as a result of a laboratory accident (33, 40). In the latter case, the illness occurred in a scientist who was experimentally inoculating puppies with the microorganism. (The man and not the puppy was successfully infected.)

Because there is no good animal model, little is known about the pathogenesis of campylobacter enteritis. Only a few animals have been found susceptible to experimental infection, but even when success has been reported by one group, others have failed to confirm it (9). Monkeys (5, 9, 42), puppies (33), kittens (33), gnotobiotic puppies (32), mice (25), and chicks (9) have been tested without uniform results. Our results with intragastrically challenged neonatal mice show that persistent colonization of the large bowel for periods up to 3 weeks can be achieved. This paper presents these and related data with neonatal mice, rats, and rabbits as models for campylobacter enteritis.

MATERIALS AND METHODS

Animals. Crl:CFW(SW)BR mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were used to establish a breeding colony in the University of Texas at Austin departmental animal facilities. The offspring were infected when only a few days old (2.3 to 2.8 g). Infant golden hamsters and infant rats (Sprague-Dawley, Madison, Wis.) were obtained from breeding stock acquired from the Animal Resources Center, The University of Texas at Austin, and brought to the departmental facilities. Pregnant white New Zealand rabbits were obtained from David Cunningham, Dripping Springs, Tex. All of these infants were tested when less than 1 week of age.

All animals except rabbits were fed Purina Mouse Chow until the last few weeks of the study when Wayne Lab Blocs were substituted. Rabbits were given Purina Rabbit Chow, Complete Blend. Food and water were available at all times, unless otherwise stated.

Organisms. *C. fetus* subsp. *jejuni* strain BO216, a blood isolate from a 46-year-old male, was provided by Susan Gibson, Texas State Health Department, Austin. Strains AC1 and AC127 were isolated in Atlanta from pediatric patients with diarrhea, and strain A2999 was isolated from an outbreak of campylobacter enteritis in Connecticut. These three strains were sent to us by Joy Wells, Enteric Diseases Laboratory Section, Epidemiologic Investigations Branch, Centers for Disease Control, Atlanta, Ga. Strains 517 and 9588 were obtained as recent stool isolates from patients with diarrhea and were provided by laboratories of local physicians. After having been received in our laboratory, strains were passaged a minimal number of times on brucella agar (Difco Laboratories, Detroit, Mich.) containing 10% defibrinated sheep blood and were frozen in brucella broth (Difco) containing 10% glycerol for use as stock cultures.

Cultures medium and growth conditions. Cultures used for inoculation of infant animals were grown on brucella agar containing 5% defibrinated sheep blood. The same medium containing the following antibiotics was used to recover the organisms from gastrointestinal tissues: cephalothin, 7.5 μg/ml (Keflin, Eli Lilly & Co., Indianapolis, Ind.); amphotericin B, 1 μg/ml (E. R. Squibb & Sons, Inc., Princeton, N.J.); trimethoprim, 2.5 μg/ml (Sigma Chemical Co., St. Louis, Mo.); and vancomycin, 5 μg/ml (Sigma). At the above dosage levels of antibiotics, the efficiency of plating was no different from that of brucella blood agar without antibiotics. A microaerophilic atmosphere was achieved by partially evacuating the air from a modified milk can to about 0.50 atm (50.65 kPa) (two times) and refilling it with a mixture of 90% nitrogen and 10% carbon dioxide. This yielded an atmosphere of about 5% oxygen. All plates were incubated at 42°C and counted at the end of 48 to 72 h.

Preparation of inocula for intragastric infection. Strains of *C. fetus* subsp. *jejuni* were opened from the freezer onto brucella agar containing 5% blood and incubated for 24 to 48 h. After an additional transfer and incubation for 18 to 24 h, the growth from the plates was suspended to a standard turbidity. The colony-forming units (CFU) per milliliter of inoculum were obtained by diluting the suspension of brucella broth and plating on brucella agar containing 5% blood.

Inoculation of animals. Infant mice were inoculated as previously described (13, 17, 30). Briefly, mice weighing between 2.3 and 2.8 g were fasted 3 to 5 h at 35°C before inoculation. The inoculum was administered intragastrically in a volume of 0.05 ml with a 1-ml tuberculin syringe equipped with a blunted 21-gauge needle tipped with polyethylene tubing. After inoculation, infants were held for 1 to 2 h at 35°C and then returned to their dams, with whom they remained for the duration of the study, usually for 3 weeks. Other neonatal animals were fasted and inoculated in a similar manner as follows: rats, 3 days old, 10 g, 0.05-ml inoculum volume; hamsters, 4 days old, 4.0 g, 0.10-ml inoculum volume; rabbits, 6 days old, 80 g, 1.0-ml inoculum volume.

Enumeration of organisms in tissues. Infant mice and hamsters were sacrificed by decapitation, and rats and rabbits were sacrificed by injecting an overdose of pentobartital sodium (Nembutal, Abbott Laboratories, North Chicago, Ill.) into the chest cavity. The gastrointestinal tract from each animal was quickly dissected and placed in a petri plate containing sterile brucella broth. The entire tract was stretched into a linear structure. The small intestine was identified as a segment extending from the stomach to the cecum. It was divided into approximate thirds. The

cecum was taken as a separate piece; the colon comprised the remainder. Undoubtedly, some luminal fluid was lost in sectioning, but the neonatal digestive tract is so small that no extensive outflow was apparent to the naked eye. Each of the six segments of the gastrointestinal tract was homogenized in brucella broth and plated on brucella agar containing 5% blood and the antibiotics listed above.

Scanning electron microscopy. Samples of the gastrointestinal tract to be examined by scanning electron microscopy were prepared and processed as previously described (30). Tissues were examined in an AMR 1000 scanning electron microscope and photographed with Polaroid 55 P/N film.

RESULTS

Lethality in infant mice inoculated intragastrically with *C. fetus* subsp. *jejuni*. Eight groups of 9 to 10 neonatal mice, each group from a single litter, were inoculated intragastrically with 6.4×10^7 to 2.7×10^8 CFU of *C. fetus* subsp. *jejuni* (strain AC1). One mouse died in four of the groups, three died in two groups, and none died in two groups (Table 1). The first deaths occurred on day 2 postinfection in four groups, and the last deaths were seen as late as day 5. No deaths were observed in any group beyond this time for as long as 3 weeks. The infection appeared to take some time to develop and was 13% lethal. The strain used for these results was isolated from a pediatric patient in Atlanta, Ga.

Four additional strains have been tested under similar conditions, all of which kill statistically fewer mice (by the chi-square test) than does strain AC1. With strain AC127 (isolated from another pediatric case in Atlanta), 1 of 39 mice died. Strain BO216 (the only blood isolate tested in this study) killed 2 of 99 mice, whereas strain 9588 (isolated from an 18-year-old female by a local laboratory) was nonlethal for 26 mice. Another pediatric strain (from a local laboratory), strain 517, killed 3 of 82 mice. This makes it apparent that death occurs only rarely in neonatal mice infected with recent isolates from human cases. The bacteria are believed to be responsible for deaths, since trauma from the inoculation would likely kill animals sooner than was observed. If the inoculum is injected into the respiratory tree, there is sneezing and foaming of the suspension around the external nares. Such animals are not retained in the group for study. Moreover, entry of the inoculum into the stomach is visible through the translucent body wall of the neonates. None of the infected rats. rabbits, or hamsters died.

TABLE 1. *Lethality of strain AC1 of C. fetus subsp. jejuni in replicate experiments with neonatal mice infected intragastrically*

Days postinfection	No. of mice dead at each time postinfection							
	9[a]	9	9	10	10	10	10	9
1								
2		1		1		1	2	
3	1	1		1		1	3	
4	1	1		1		1	3	
5	1	1		3	1	1	3	

[a] Initial size of experimental group.

Failure to detect systemic spread of *C. fetus* subsp. *jejuni*. The livers of 25 mice were cultured 1 week after intragastric inoculation, but no colonies appeared after 48 h of incubation. Nine of the mice were inoculated with strain 517, and 16 mice were inoculated with strain AC1. Three mice with ruffled fur and an appearance of illness were sacrificed 1 week postchallenge, and livers, spleens, gall bladders, and urinary bladders were cultured. No growth was seen after incubation. These observations suggest that the campylobacters are confined to the digestive tract of the neonatal mice.

Diarrhea in dams of infected neonates. In some of our most recent experiments, we noticed that four different dams of infected neonates developed large, soft, frequent stools, some of which contained mucus and blood. Even though no diarrhea was evident in the offspring, the dams probably removed the fecal discharge from their offspring soon after it appeared, thereby acquiring the infection. The stools from the dams were culture positive as early as 48 h and as late as 26 days after the infants were inoculated, and the fur of the dams was ruffled and unkempt. Three were dissected, and all showed enlarged ceca, one unusually so since she appeared to be pregnant, but was not. When ceca and large intestines from these mice were plated, all were positive for *C. fetus* subsp. *jejuni*. The diarrhea in the dams was produced by three different strains as follows: BO216 caused two, 517 caused one, and A2999 caused one. Why infected dams were not recognized earlier is unknown, but passage through the neonates might enhance virulence of the microorganisms.

Colonization of the gastrointestinal tract. The ability of four strains of *C. fetus* subsp. *jejuni* to colonize segments of the gastrointestinal tract of neonatal mice was determined by dilution counts at weekly intervals for a total of 3 weeks (Table 2). The most successful strain was BO216, and the poorest strain was AC1. The strain that killed 13% of the mice, AC1, did not colonize very well, since no organisms were recovered at 3 weeks. All strains were present at 1 and 2 weeks and were cultured in largest numbers in the cecum and colon. The lower third of the small intestine was usually colonized,

but in smaller numbers. Strain 517 was not followed at 3 weeks.

Effect of infant size on colonization of the gastrointestinal tract. Colonization of the gut of mice of different body weights was compared 1 week post-inoculation (Table 3). The inoculum used in these experiments, 10^9 CFU, was larger than that used for the data of Table 2. The nursing mice (6.8 g) and the mice that were eating and nursing (6.5 g) gave similar levels of colonization with respect to one another (Table 3) and to mice of smaller size (Table 2). The nursing group was about 10 days old, and those that were nursing and eating, though the same size, were about 2 weeks old. The weaned group (9.8 g) was about 3 weeks old and retained a few organisms in only one of the four animals tested. Young adults (18.3 g) were essentially refractory to colonization. The value of 7.5 that appeared in the cecum of one of the adult mice was obtained by averaging the numbers that appeared on replicate plates of each dilution.

Ability of *C. fetus* subsp. *jejuni* to colonize other neonatal animals. Table 4 shows results with infant rats, hamsters, and rabbits obtained in experiments similar to those for mice (as summarized in Table 2). Rats, as judged by their susceptibility to colonization, are at least equivalent to or may be better than mice as an animal model. Hamsters had the fewest organisms in their intestinal tract. The sample size was small, but was sufficient to establish a trend. None of the animals showed overt symptoms of disease.

Scanning electron photomicrographs of *C. fetus* subsp. *jejuni* in the infant mouse ileum. Figure 1 is a ×750 magnification of the lower ileum of an infant mouse 2 h postinoculation with strain AC1. The villi with a covering of dried mucus are apparent. The bacteria cannot be identified in this figure, but Fig. 2 shows the center region of Fig. 1, magnified ×3,250. Here, numerous microorganisms become visible, and from their curved and spiral shapes, they are presumably *C. fetus* subsp. *jejuni*. Organisms with this morphology have not been among the flora of the infant mouse gut at this stage of development in numerous scanning electron photomicrographs prepared by us over a period of years, nor have they been described by Savage (37). When the lower center of this view was magnified to ×8,300 the picture shown in Fig. 3 resulted. Many of the organisms with campylobacter morphology are seen on, in, and below the mucous layer. The segment in the lower

TABLE 2. *Colonization of gastrointestinal tract of neonatal mice by different strains of C. fetus subsp. jejuni*

Time post-inoculation (wk)	Strain inoculated[a]	Avg CFU in organ or organ segment of positive mice (no. positive/total)					
		Stomach	Small intestine			Cecum	Large intestine
			Upper third	Middle third	Lower third		
1	BO216	25 (2/15)	820 (3/15)	1.3×10^4 (6/15)	2.9×10^4 (7/7)	1.3×10^6 (13/13)	2.0×10^6 (15/15)
	AC127	4,200 (3/10)	1,600 (3/10)	4,800 (7/10)	3.6×10^5 (8/10)	5.1×10^5 (8/10)	2.1×10^6 (8/10)
	AC1	524 (8/14)	1,600 (6/14)	2,240 (10/14)	4.1×10^4 (14/14)	1.8×10^5 (13/14)	1.6×10^6 (14/14)
	517	0 (0/11)	0 (0/11)	0 (0/11)	9.4×10^5 (9/11)	7.0×10^5 (11/11)	5.0×10^6 (11/11)
2	BO216	2,900 (5/22)	2,400 (7/22)	4,000 (7/22)	3.5×10^4 (9/21)	3.3×10^7 (11/22)	2.9×10^7 (21/22)
	AC127	0 (0/11)	2,800 (1/11)	1,100 (2/11)	2,250 (4/11)	1.4×10^6 (6/11)	1.6×10^5 (6/11)
	AC1	10 (1/6)	0 (0/6)	18 (1/6)	500 (1/6)	260 (2/6)	280 (1/6)
	517	0 (0/8)	0 (0/8)	0 (0/8)	65 (1/8)	1,295 (4/8)	1.1×10^4 (4/8)
3	BO216	1.0×10^4 (3/10)	1,400 (3/10)	140 (4/10)	1.1×10^4 (6/10)	5.0×10^6 (3/10)	1.7×10^6 (5/9)
	AC127	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)
	AC1	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/3)

[a] Inoculum (CFU per mouse): BO216, 5.6×10^8; AC127, 5.2×10^7; AC1, 1.1×10^8; 517, 2.0×10^8.

TABLE 3. *Effect of neonatal mouse weight on colonization of the gastrointestinal tract by strain AC1 of C. fetus subsp. jejuni 1 week post-inoculation*[a]

Avg wt (g) of mice at time of inoculation	No. of CFU per organ or organ segment (No. positive/total counted)		
	Small intestine (lower third)	Cecum	Large intestine
6.8 (nursing)	1.3×10^6 (4/4)	7.6×10^6 (4/4)	3.6×10^6 (4/4)
6.5 (eating and nursing)	1×10^6 (4/4)	2.4×10^6 (4/4)	2.2×10^5 (4/4)
9.8 (weaned)	0 (0/4)	5 (1/4)	10 (1/4)
18.3 (adults)	0 (0/4)	7.5 (1/4)	0 (0/4)

[a] Inoculum, 10^9 CFU per mouse.

TABLE 4. *Colonization of the gastrointestinal tract of infant rats, hamsters, and rabbits by C. fetus subsp. jejuni strain BO216*[a]

Time post-inoculation (wk)	Animal species	Avg CFU in organ or organ segment of positive infant rats, hamsters, and rabbits (no. positive/total)					
		Stomach	Small intestine			Cecum	Large intestine
			Upper third	Middle third	Lower third		
1	Rat[b]	0 (0.3)	2,900 (3/3)	3,500 (3/3)	7,900 (3/3)	3.4×10^7 (3/3)	3.1×10^7 (3/3)
	Hamster[c]	1,300 (2/2)	3,800 (2/2)	5,500 (1/2)	2,300 (1/2)	2,000 (2/2)	2.8×10^5 (2/2)
	Rabbit[d]	ND[e]	1,800 (2/2)	5,600 (2/2)	1.7×10^6 (2/2)	1.7×10^6 (2/2)	1.1×10^6 (2/2)
2	Rat	0 (0/3)	0 (0/3)	0 (0/3)	120 (1/3)	3.3×10^6 (1/3)	7.4×10^5 (2/3)
	Hamster	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/2)
	Rabbit	ND	5,200 (2/2)	6,500 (1/2)	6,600 (2/2)	7.3×10^5 (2/2)	7.1×10^6 (2/2)
3	Rat	7,700 (1/2)	1.2×10^5 (1/2)	5.7×10^5 (1/2)	8×10^5 (1/2)	8.5×10^5 (1/2)	4.4×10^5 (1/2)
	Hamster	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)
	Rabbit	ND	ND	ND	ND	ND	ND

[a] BO216 was isolated from the blood of a human patient.
[b] Inoculum (CFU per rat), 2.1×10^8 in 0.05 ml.
[c] Inoculum (CFU per hamster), 6.5×10^8 in 0.1 ml.
[d] Inoculum (CFU per rabbit), 1.5×10^{10} in 1 ml.
[e] ND, Not determined.

right of this figure shows the microvilli, but with no campylobacters attached. This is similar to what is seen in neonatal mice infected intragastrically with *Vibrio cholerae* (N. Guentzel, unpublished data). Figure 4 was taken from a different region of the ileum and represents a magnification of ×14,600. The shape variation of the campylobacters is clearly apparent in this figure, but no flagella can be definitely identified against the fibrous strands of mucus.

DISCUSSION

The results presented in this report make it apparent that neonatal mice, rats, and rabbits are promising models for the study of campylobacter enteritis. Even though the six strains of *C. fetus* subsp. *jejuni* tested in infant mice resulted in a minimum of 0% to a maximum of 13% mortality, the heavy colonization in the cecum and large bowel for a period of 2 to 3 weeks permits a number of useful studies. There is always the hope that a particular mouse virulent strain will emerge. No recent isolates from animals have been tested, and no effort to either compromise the host or enhance the virulence of strains has been made. We have not yet conducted a systematic analysis of large bowel pathology (if any) that accompanies colonization with *C. fetus* subsp. *jejuni*. The recently observed infection of the dams of infected neonates provides another significant lead, since an analysis of the histopathology of the large bowel should be feasible with these animals. Infected dams may contaminate the neonates, but evidence of infection in the adults was not present throughout most of the study. Cultures made of the feces of infected dams during this period were negative. A change in the brand of animal food fed the mice was made shortly before the dams were seen to have soft, mucoid, and, at times, bloody stools. This may be a coincidence or a variable that contributed to the change in susceptibility.

Well-characterized enteric diseases result primarily from toxin production or penetration and ulceration of the gut mucosa or both, even though other factors may contribute to the pathogenic process. All enteric pathogens must attach, and such factors as motility, the presence of pili, the elaboration of mucinase, etc., must be evaluated as possible explanations of why the different strains of *C. fetus* subsp. *jejuni* varied in their ability to colonize. Cholera is primarily a toxigenic disease, even though attachment is enhanced by motility (17, 48). Shigellosis is caused only by organisms that penetrate the mucosa of the colon, but the role played by toxin is unresolved (14, 15). Enteropathogenic *E. coli* strains F release toxin(s), but must also possess attachment organelles (36). The relative importance of one or more of these capabilities has not been established for *C. fetus* subsp. *jejuni*.

Invasiveness by campylobacters is considered by some authors (9) to be a requisite virulence factor for establishing enteritis. This concept is supported by the appearance of bloody diarrhea, bactermia, cholecystitis, and ulcerations of the gut in infected human patients. Moreover, chicken embryo cells in culture can be invaded within 24 h after the addition of campylobacters.

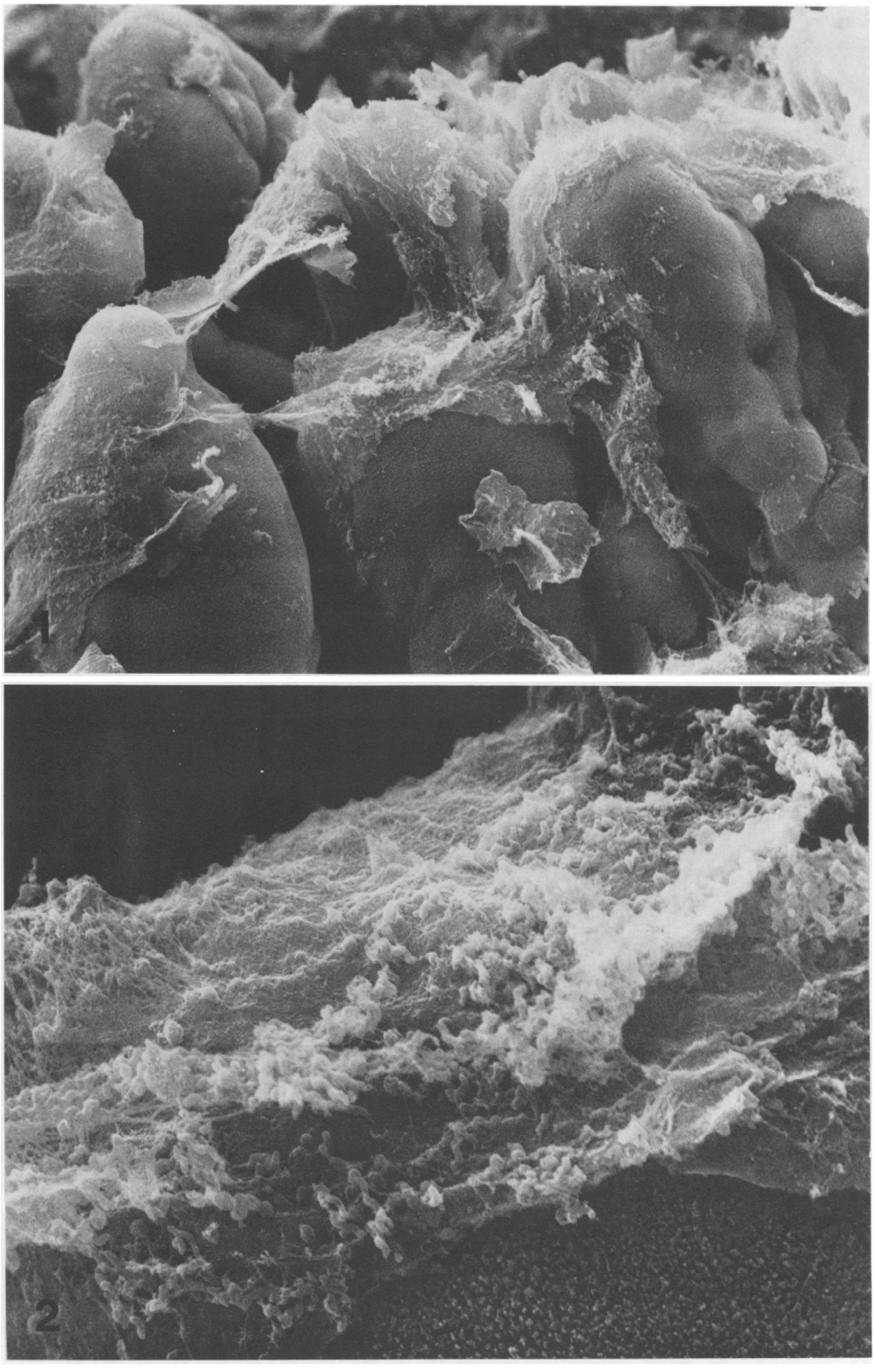
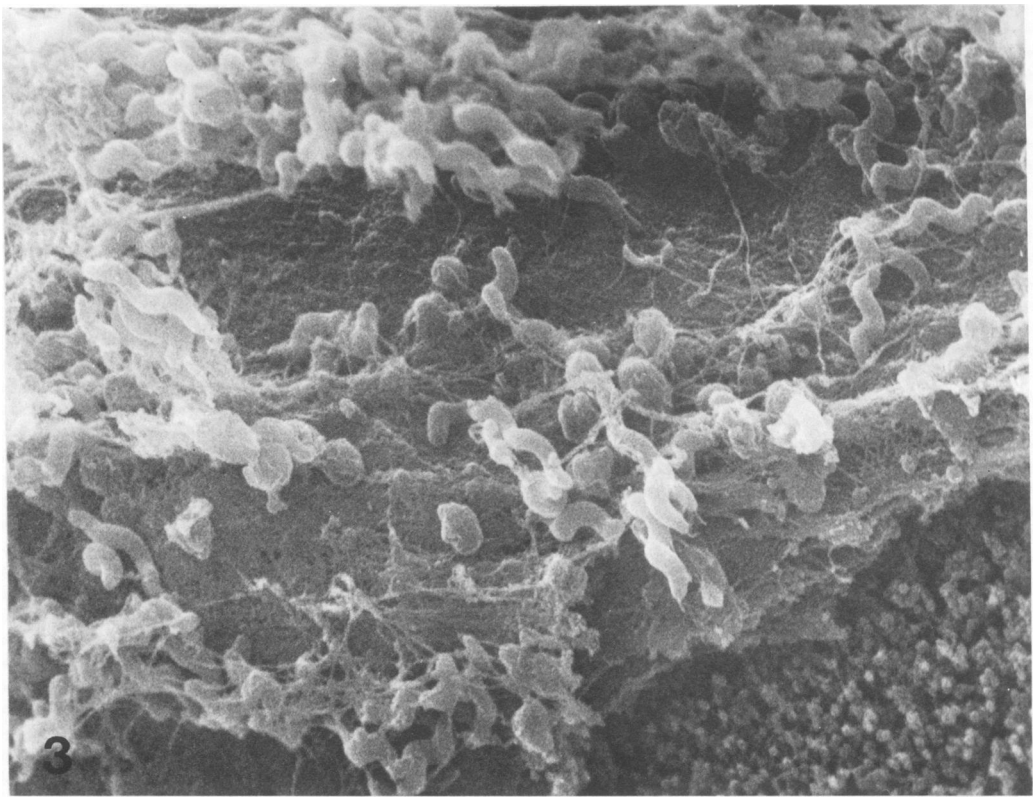


FIG. 1. *Scanning electron photomicrograph (×750) of neonatal mouse ileum 2 h after intragastric infection with C. fetus subsp. jejuni strain AC1. The villi are covered with dried mucus, but no campylobacters are visible.*

FIG. 2. *Same as the central part of Fig. 1, shown at a magnification of ×3,250. Numerous bacteria, believed to be campylobacters because of their shapes, are visible.*



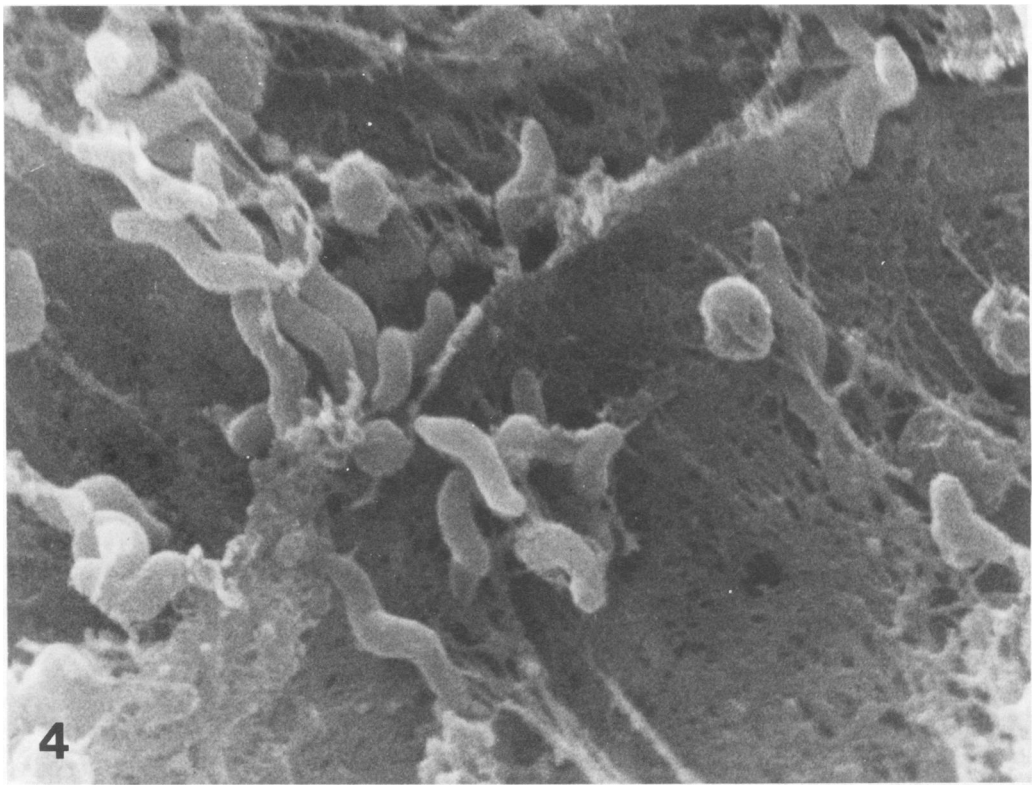


FIG. 3. *Enlargement of the central part of Fig. 2, shown at a magnification of ×8,300. Campylobacters are visible on, in, and below the dried mucous layer.*

FIG. 4. *Photomicrograph made from a different region of the ileum, shown at a magnification of ×14,600.*

Oral infection of 8-day-old chicks with campylobacters resulted in invasion of the intestinal wall by the bacteria (9). Other studies are lacking except for a reported inability to see the organisms in human biopsy specimens (9, 28).

The role of toxigenicity in the pathogenesis of campylobacter enteritis has not been resolved. It has been tested in some preliminary studies in other laboratories by three different techniques. Culture supernatant filtrates failed to stimulate fluid accumulation in ligated ileal loops of rabbits and failed to cause morphological changes of a cholera toxin-like or cytotoxic effect in cultured Chinese hamster ovary cells to which culture supernatants in which the pathogens grew were added (9, 18). Both of these effects are elicited by cholera toxin and the heat-labile toxin of *E. coli*. Only 16 of 100 cultures of *C. fetus* subsp. *jejuni* were positive when tested by Butzler and Skirrow (9) for a heat-stable toxin of the type found in some *E. coli* strains. For this purpose, a culture supernatant was injected directly and intragastrically, and the ratio of intestinal weight to body weight was determined 4 h later. On the basis of these findings, toxin does not seem to make a major contribution to the pathogenesis of campylobacter enteritis.

It is recognized that these studies represent only the beginning of an analysis of the pathogenesis of campylobacter enteritis. Considerable strain variability has already become apparent, and current reports in the literature are revealing wide serological differences in recent isolates (22). Neonatal mice should prove to be a valuable screen for several virulence factors such as lethality, colonizing ability, and transmissibility from infant to dam. Vaccines can be tested by immunizing females at the time of mating to determine whether they possibly protect their offspring, as has been done in this laboratory with *V. cholerae* infections (17). Additional neonatal species can be tested, and neonatal species of rats and rabbits can be studied in greater depth as possible models for campylobacter enteritis. Since no other animal has been consistently infected with *C. fetus* subsp. *jejuni*, much can be learned through experiments with neonatal mice alone.

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