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Association of *Escherichia coli* with the Small Intestinal Epithelium

I. Comparison of Enteropathogenic and Nonenteropathogenic Porcine Strains in Pigs

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Two enteropathogenic strains of *Escherichia coli* (EEC) differed from a nonenteropathogenic strain of *E. coli* (NEEC) in their association with porcine small intestinal epithelium. The EEC characteristically were found along villi from tip to base and contiguous to the brush border. They were not in crypts. In contrast, the NEEC characteristically remained in the central lumen near the tips of villi and was only occasionally contiguous to the brush border. No organisms were detected within epithelial cells. The difference in distribution between EEC and NEEC was apparent in ligated jejunal loops 45 min postexposure. The association between host and bacterial cells was most consistently demonstrated on frozen sections of intestine, as other histological techniques removed many bacteria. However, cellular details of the association were best demonstrated in chemically fixed tissues.

The term "enteropathogenic *Escherichia coli*" (EEC) was used by Neter (16) to designate strains of *E. coli* with the "particular potential" to cause diarrheal disease. Human EEC can be divided into two groups. One group penetrates intestinal epithelial cells and does not produce enterotoxin (7). A second group remains in the intestinal lumen and does produce enterotoxin (7, 20). All swine and ruminant EEC recognized to date belong to this latter enterotoxigenic group (14). For enterotoxigenic EEC to cause diarrhea in swine and ruminants, they must be present in the toxin-sensitive jejunum in numbers which approximate those commonly found (colonization) in the large intestine (19). The characteristics that enable EEC to colonize the jejunum are largely unknown; however, they do so more intensely than nonenteropathogenic *E. coli* (NEEC) (14). Jejunal colonization requires that EEC multiply in this area and not be removed by peristalsis and villous motility. Adhesion of EEC to intestinal epithelium is one way by which such effects could be avoided. There is evidence that some EEC of porcine (1, 2, 11, 21) and human (4, 24) origin either adhere to or become intimately associated with intestinal epithelial cells.

The objectives of this investigation were (i) to test additional porcine EEC strains for the ability to associate with pig small intestinal epithelium (ii) to compare their ability to do so with that of a porcine NEEC strain, and (iii) to compare different methods of demonstrating and quantitating this association in the pig intestine.

MATERIALS AND METHODS

Bacteria. The EEC strains used were 263 [serotype 08:K87(B)88a, b(L):H19] and 431 [serotype 0101:KU460(A):NM] originally isolated from piglets with diarrhea. The NEEC strain 123 (serotype 043:K-:H28) originated from a healthy piglet. All three strains were used in the nonpiliated phase as determined by electron microscopic examination. Cultures were maintained on Trypticase soy agar (BBL; agar) in sealed tubes, subcultured on agar with 5% sheep blood (blood-agar), and grown overnight in Trypticase soy broth (BBL; broth) at 37 C. Dilutions were made in broth immediately before the pigs were exposed.

Pigs. In experiment 1 (intragastric exposure), 28 pigs from eight naturally farrowed litters were separated from the sow immediately after birth prior to nursing, kept in individual cardboard boxes under heat lamps, not fed, and exposed to *E. coli* 12 hr after birth. Pigs in the first four litters (experiment

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TABLE 1. *Design of experiment I involving 28 colostrum-deprived newborn pigs exposed to Escherichia coli intragastrically*

Expt	No. of		*E. coli* inoculated	
	Litters	Pigs per litter	Strain	Viable count
1A	4	2	263	10^6
		1	123	10^6
1B	4	2	431	10^6
		2	123	10^9

1-A) were exposed to either strain 263 or 123 and in the second four litters (experiment 1-B) to either 431 or 123 (Table 1).

In experiments 2 and 3 (intestinal loop exposure), three and eight pigs, respectively, were taken from sows by hysterectomy. The pigs were placed in sterile cloth sacks and kept at 37 C without feed or water until they were exposed to *E. coli* when 2.5 to 12 hr old. Suckling pigs were also used for part of experiment 2 as indicated below.

Exposure. In experiment 1, 10^6 or 10^9 *E. coli* (Table 1) were suspended in 20 ml of broth plus 1% glucose and given to the pigs intragastrically via stomach tube. Pigs exposed to different strains of *E. coli* were held in different isolation rooms, and all were killed 12 hr postexposure.

In experiments 2 and 3, several ligated jejunal loops of 10-cm length (12) in each pig were exposed to 3×10^8 *E. coli* cells in 1 ml of fresh broth. Pigs were killed 3 hr postexposure in experiment 2, and two pigs were killed at each of the following times postexposure in experiment 3: 0.75, 1.5, 3, and 6 hr.

Necropsy. In experiment 1, segments of jejunum and ileum were ligated and removed. Some segments from each area were frozen, some were fixed in 10% Formalin, and others were used for bacterial counts. In experiments 2 and 3, ligated jejunal loops were treated as the segments in experiment 1 except that glutaraldehyde fixative was also used in experiment 3. Segments were frozen by immersion in small beakers of 2% methylcellulose in 0.15 M saline (17) held in a dry ice-ethanol bath. From these, frozen sections (−20 C) were cut in a cryostat (set to cut sections 8-μm thick), mounted on glass slides held at room temperature, dried, and fixed in methanol for 60 sec; alternate sections were stained with either fluorescent-antibody (FA) or Toluidine Blue (TB). Formalin-fixed segments were embedded in paraffin; sections (5-μm thick) were cut and stained with Giemsa solution. Glutaraldehyde-fixed tissues were prepared for electron and light microscopy (13).

FA. Antisera to the O and K antigens of the three *E. coli* strains were prepared in rabbits and conjugated to fluorescein isothiocyanate (25). FA-stained sections were counterstained with Evan's blue (9) and examined in a fluorescence microscope under blue light. Duplicate controls of most FA slides were stained with FA directed against a strain of *E. coli* other than that expected in the section, and nonspecific bacterial fluorescence was not observed.

Bacterial counts. For total counts, 10-cm segments of intestine with their contents were placed in a jar, the total volume of the sample was adjusted to 30 ml by adding cold 0.3% peptone water (peptone water), and the sample was held in an ice bath for 1 to 3 hr. To determine the percentage of the viable *E. coli* associated with the epithelium, separate bacterial counts of luminal washings and intestinal wall were also conducted on 10-cm segments of intestine. These segments were injected, according to their capacity, with 1 to 2 ml of peptone water and inverted gently four times; the luminal washings were aspirated with a syringe. The volume of each luminal washing and intestinal wall sample was also adjusted to 30 ml with peptone water, and the samples were held in an ice bath. After all samples for the day were prepared as above, they were homogenized in a Virtis-45 homogenizer at full speed for 10 sec. Then 10-fold dilutions were made in peptone water, plated on blood-agar, and incubated overnight at 37 C. Colonies were identified on the basis of their morphology and counted. Their identity was confirmed by agglutination of at least four colonies per sample in homologous OK antiserum.

Bacteria with colonial and cellular morphology similar to *E. coli*, which did not belong to any of the three strains inoculated, were designated as indigenous coliform bacteria (ICB). The ICB were enumerated the same way as the other strains; however, ICB did not agglutinate in any of the OK antisera.

RESULTS

Intragastric exposure (experiment I). At necropsy, 12 hr postexposure, diarrhea was observed in four of eight pigs exposed to strain 263, one of eight exposed to 431, and none of 12 exposed to strain 123. The total counts of the

TABLE 2. *Number of newborn pigs listed according to number of viable Escherichia coli in 10-cm segments of intestine 12 hr after intragastric exposure to E. coli*

Strain of *E. coli*	*E. coli* count[a]						
	10^9	10^8	10^7	10^6	10^5	10^4	10^3
263	2	3	2	0	1	0	0
431	0	0	0	6	2	0	0
123[b]	0	0	0	2	4	1	1
ICB[c]	0	3	7	6	4	6	1

[a] Median count from three or four loops examined per pig.

[b] Exposed to 10^9 cells of strain 123. Strain 123 was not recovered from pigs exposed to 10^6 cells of strain 123. Pigs from which strain 263 or 431 was recovered were exposed to 10^6 bacteria.

[c] Indigenous coliform bacteria (ICB) were enumerated in all but 1 of the 28 pigs.

TABLE 3. *Percentage of the total viable Escherichia coli remaining with the intestinal wall after washing from pigs 12 hr after intragastric exposure*

Strain	No. of		*E. coli* remaining with wall (%)	
	Pigs	Segments	Mean	Range
263	8	30	70.7	3.8–97.6
431	8	24	66.9	25.1–92.8
123	8[a]	20	47.0	3.5–67.5
ICB[b]	27	75	42.0	1.5–99.9

[a] Exposed to 10^9 bacteria of strain 123. Strain 123 was not recovered from pigs exposed to 10^6 cells of strain 123. Pigs from which strain 263 or 431 was recovered were exposed to 10^6 bacteria.

[b] Indigenous coliform bacteria (ICB) were enumerated in all but 1 of the 28 pigs.

inoculated strain were highest in intestinal segments from pigs exposed to strain 263, intermediate in those exposed to strain 431, and lowest in those exposed to strain 123 (Table 2). In contrast to strains 263 and 431, strain 123 was not recovered from the pigs exposed to 10^6 bacteria. It was recovered in experiment 1-B where the strain 123 inoculum was increased to 10^9 bacteria (Tables 1 and 2). In addition to the strain inoculated, there were also numerous ICB in 27 of these 28 naturally farrowed pigs (Table 2).

In preliminary tests on the effect of washing, 21 pairs of adjacent intestinal segments in intragastrically exposed pigs were compared as follows: one segment was washed once, the other three times. Despite the marked variation between members of individual pairs, the difference between the mean percentages of bacteria

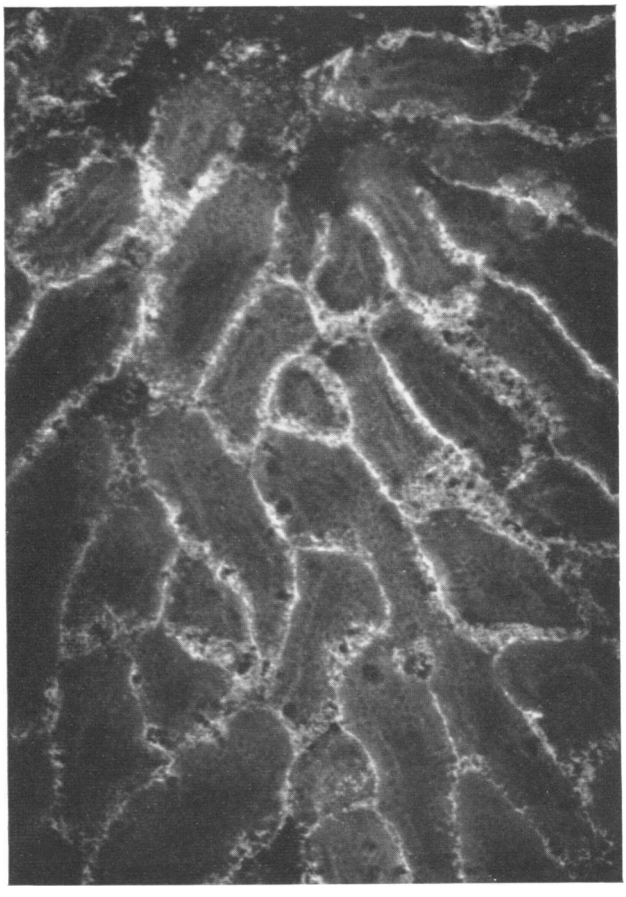

FIG. 1. *Uniform distribution of the EEC strain 263 along the entire length of intestinal villi; central lumen is at the top. Fluorescent antibody-stained, frozen section of jejunum of newborn pig 12 hr after intragastric exposure to strain 263.*

remaining with the wall after one and three washings was only 1.3%. For this reason, segments were washed three times, and the bacteria which remained with the intestinal wall after washing were considered to be associated with the intestinal epithelium. The range of the percentage remaining varied greatly between segments exposed to the same strain (Table 3).

It was difficult to find more than an occasional bacterium in frozen sections prepared from segments which contained 10^6 or less total bacteria. For this reason, only sections from some seg-

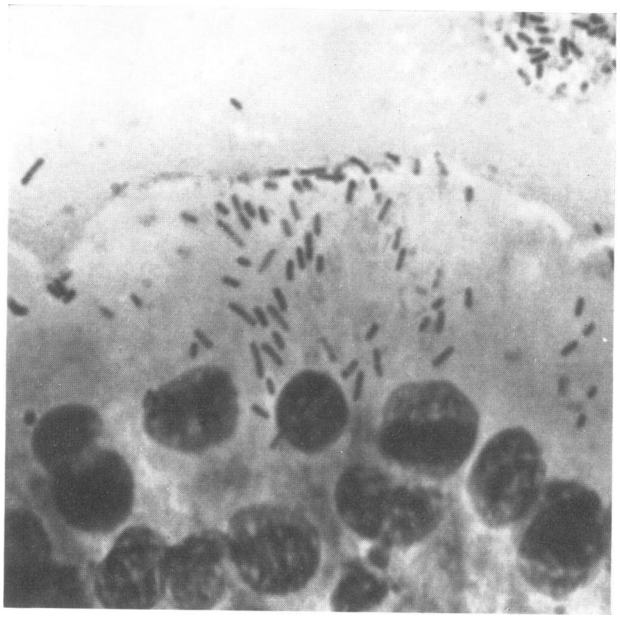

Fig. 2. E. coli strain 263 contiguous to the apical portions of obliquely cut epithelial cells. Toluidine Blue-stained, frozen section from ligated jejunal loop in newborn pig 3 hr postexposure.

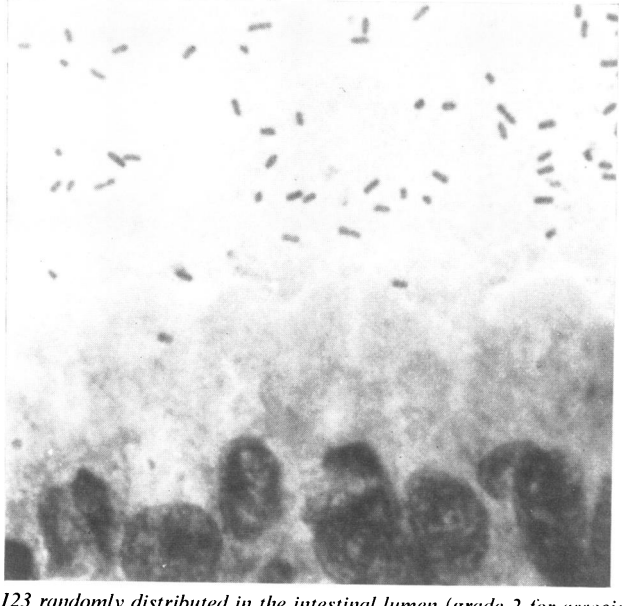

FIG. 3. *E. coli strain 123 randomly distributed in the intestinal lumen (grade 2 for association index). Toluidine Blue-stained, frozen section from ligated jejunal loop in newborn pig 3 hr postexposure.*

ments (Table 2) were used to determine the microscopic distribution of bacteria. Segments with sufficient bacteria for evaluation contained bacteria in the central lumen, intervillus spaces, and occasionally (apparently artifactually) over epithelium, lamina propria, submucosa, and muscularis. Bacteria were not observed in crypts from any of the pigs exposed by either method to any of the strains, regardless of the method of examination.

In FA-stained sections from segments which contained strain 263, there were numerous fluorescing bacteria along most villi from tip to base (Fig. 1). In TB-stained sections from these segments, many bacteria were contiguous to the apical portions of epithelial cells where they were concentrated at the extreme apex and diminished toward the nucleus (Fig. 2). The distribution of ICB was studied in three TB-stained sections of segments from which only ICB had been cultured. Most ICB were in the central lumen, and they decreased markedly in number from villus tip towards the base. They were contiguous to epithelial cells only randomly and when there were numerous bacteria present in adjacent luminal content.

The differences in distribution between strains 263 and ICB in experiment 1 also occurred with other strains in experiments 2 and 3. For this reason, the microscopic distribution of all strains in this study was evaluated according to two criteria, and these criteria combined to make an "association index." The first criterion was taken from FA-stained frozen sections examined at low power in which the intensity and extent of bacterial fluorescence at the villus base was graded from 1 for none to 5 for maximum. Since ICB did not stain with FA, this portion of the association index for ICB was taken from TB-stained sections, and the number of bacteria

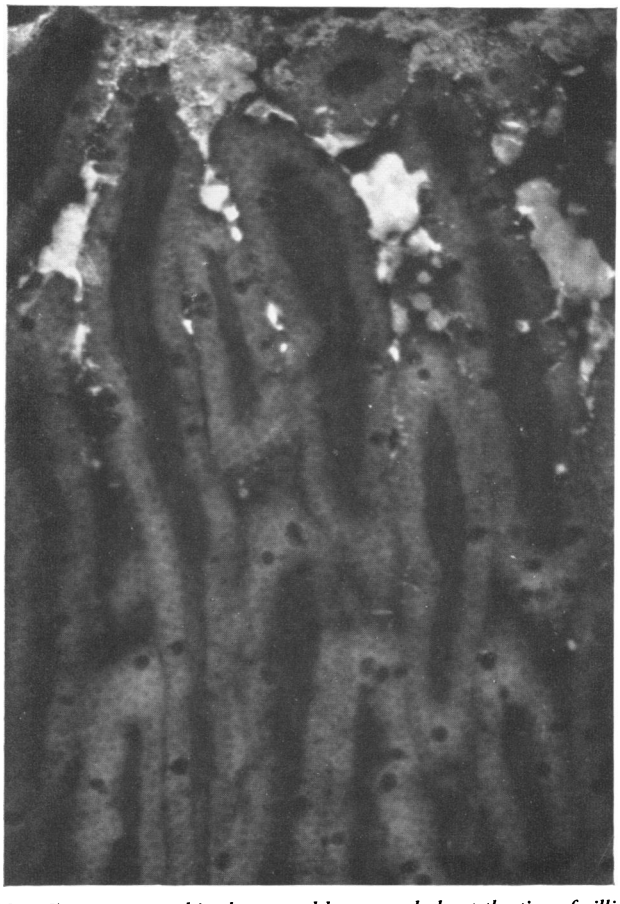

FIG. 4. *Strain 123 (NEEC) concentrated in the central lumen and about the tips of villi, markedly decreasing in concentration toward the villus base. Fluorescent antibody-stained, frozen section of ligated jejunal loop in newborn pig 3 hr postexposure.*

at the villus base was graded. The second criterion for the association index was determined in frozen sections stained with TB and examined with the oil immersion objective. Areas on the villi where bacteria occurred in proximity to the epithelium were found, and the tendency of these bacteria to be contiguous to epithelial cells was graded from 1 for none to 5 for maximum (Fig. 2 and 3).

The association index for a segment was obobtained by multiplying the values obtained in evaluating the two criteria. Thus, an index of 25 indicated numerous bacteria at the villus base with a marked tendency to be contiguous to the apex of the epithelial cells (Fig. 1 and 2); whereas, an index of 1 indicated there were no bacteria at the villus base, and bacteria in that segment were not contiguous to the epithelium where they occurred (Fig. 3 and 4).

TABLE 4. *Comparison of frozen sections of unwashed intestine and viable counts of washed intestine as aids in studying the tendency of Escherichia coli to be associated with the epithelium of ligated intestinal loops in newborn pigs*

Strain of *E. coli*	Viable counts			Frozen sections		
	No. of loops	*E. coli* remaining with wall (%)		No. of loops	Association[a] index	
		Mean	Range		Mean	Range
431	9	30.6	13.4–46.6	6	22.0	16–25
263	9	37.9	5.7–84.1	6	16.5	9–25
123	9	63.8	32.7–89.3	6	12.5	3–16

[a] Maximum possible association with epithelial cells is 25; minimum is 1.

In experiment 1, the mean association index of strain 263 in 9 suitable segments of 16 examined was 20.1 and that for ICB in 3 suitable segments of 22 examined was 3.7. Sixteen segments exposed to strain 431 and 22 exposed to strain 123 were examined, but none contained sufficient bacteria for evaluation.

The majority of the intestinal content was lost during the processing of paraffin sections; the number of visible bacteria was much lower than in frozen sections from the same or adjacent segments, and those that remained were distributed irregularly. In contrast to frozen sections, bacteria over the tissue were rare in paraffin sections. Apparently, the bacteria were less frequently moved by the knife when cut from parffin blocks. Most bacteria which remained were in the intervillus space, preferably where two villi were in close apposition. Here, characteristically, strain 263 occurred in a layer contiguous to the brush border. Where present, ICB were distributed randomly in the remaining luminal mucus.

Intestinal loop exposure (experiment II). The total number of viable *E. coli* in loops 3 hr after exposure varied from 1.2×10^9 to 6.0×10^9 per loop. Mean total counts for all loops exposed to each strain were similar (4.1×10^9 for 263, 3.1×10^9 for 431, and 3.1×10^9 for 123). Bacteria other than the strain inoculated were not detected in cultures or sections from any pig in

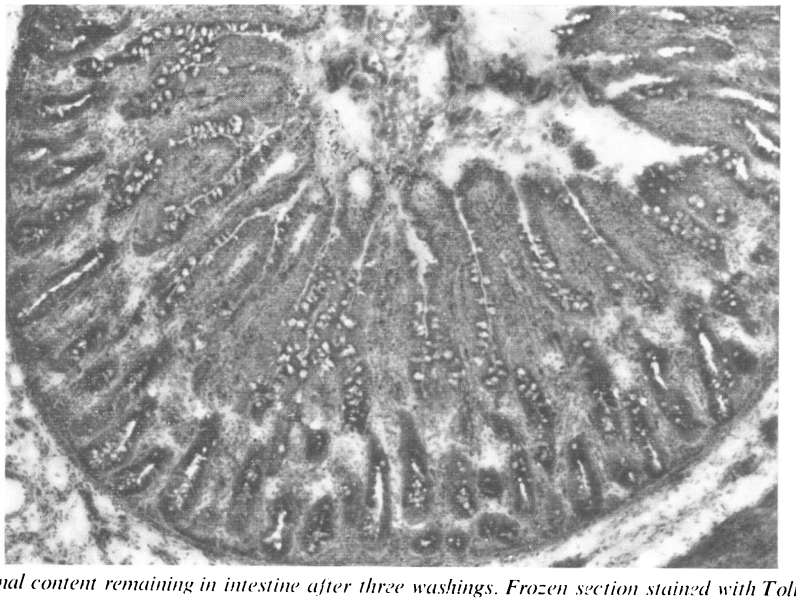

FIG. 5. *Luminal content remaining in intestine after three washings. Frozen section stained with Toluidine Blue.*

experiments 2 and 3. As with pigs exposed intragastrically, the percentage of the total bacteria of each strain remaining with the intestinal wall after washing varied from loop to loop within the same pig and from pig to pig (Table 4). In contrast to the intragastrically exposed pigs, the mean percentage of NEEC (strain 123) remaining with the wall after washing was higher than that for EEC (strains 263 and 431).

In contrast to intragastrically exposed pigs, all ligated loops contained sufficient bacteria that their microscopic distribution could be determined readily. The distribution of strains 263 and 431 in frozen sections was generally similar to that of strain 263 in the intragastrically exposed pigs; i.e., they tended to be distributed along the entire villus, contiguous to the apical portions of epithelial cells. In loops exposed to strain 123, intense fluorescence was usually confined to the lumen near the tip of the villus (Fig. 4) and, where present near the epithelium, its distribution was random (Fig. 3). The association index was highest for strain 431, intermediate for strain 263, and lowest for strain 123 (Table 4). In paraffin sections, the intestinal lumen was generally empty and the number of bacteria present in all areas was less than in frozen sections. However, where they remained, strains 263 and 431 were distributed like strain 263, and strain 123 was distributed like ICB in paraffin sections in experiment 1. Thus, the comparative distribution of the three strains determined by microscopic examination was different from that indicated by the percentages of viable bacteria remaining with the wall after washing (Table 4). For this reason, an additional experiment on the effect of washing was conducted. Eighteen ligated jejunal loops were created in suckling pigs, and six were exposed to each strain 263, 431, or 123. Three hours later, the loops were removed and washed three times as usual. An abundance of bacteria-laden mucus remained in the central lumen in seven of these washed loops (Fig. 5) when frozen sections from all 18 were examined.

Intestinal loop exposure (III) experiment. The rate of multiplication of strains 263 and 123 in ligated loops was similar (Fig. 6). Fluid accumulated only in loops exposed to strain 263 and was recognizable by 3 hr postexposure (Fig. 6). The different distributions of the two strains in frozen sections was apparent in experiment 2 by 45 min postexposure. The number of bacteria increased, but the comparative distributions were unchanged after prolonged exposure.

In contrast to frozen sections taken from loops exposed to either strain 263 or 123, TB-stained sections (1-μm thick) prepared from the same

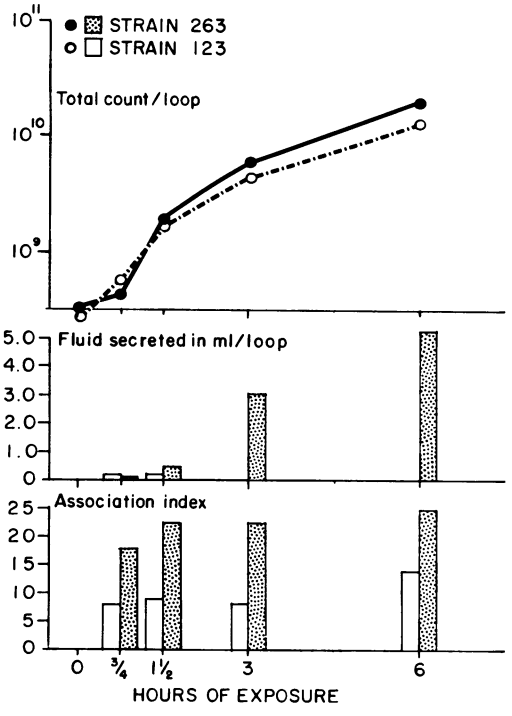

FIG. 6. *Comparison of bacterial growth rate, rate of fluid secretion, and association index of the EEC strain 263 and the NEEC strain 123 in ligated jejunal loops of newborn pigs at different times after exposure.*

tissues, but fixed in glutaraldehyde and embedded in plastic, contained only a few widely scattered bacteria; there was no difference in distribution between the two strains. Most bacteria were removed during preparation of the latter sections. There were occasional bacteria in contact with the brush border or free in the lumen near the bases of the villi and in contraction folds. They were never found within epithelial cells or in the lamina propria. Blocks from the loops exposed for 3 and 6 hr and which contained bacteria visible in 1-μm sections were selected for electron microscopy.

In electron microscopic examination, bacteria in the lumen, three or four bacterial cell widths from the brush border, were disregarded. Bacteria occurred in three locations relative to the villus absorptive cells: (i) single bacteria between microvilli some of which were bent aside; (ii) the bacterial cell wall just in contact with the tips of several microvilli or the visible glycocalyx (Fig. 7); and (iii) bacteria in the lumen one-half to one bacterial cell width from the tips of the microvilli (Fig. 8). Both strains occurred in all three locations; however, bacteria of either strain were rare in location i. Strain 123 usually

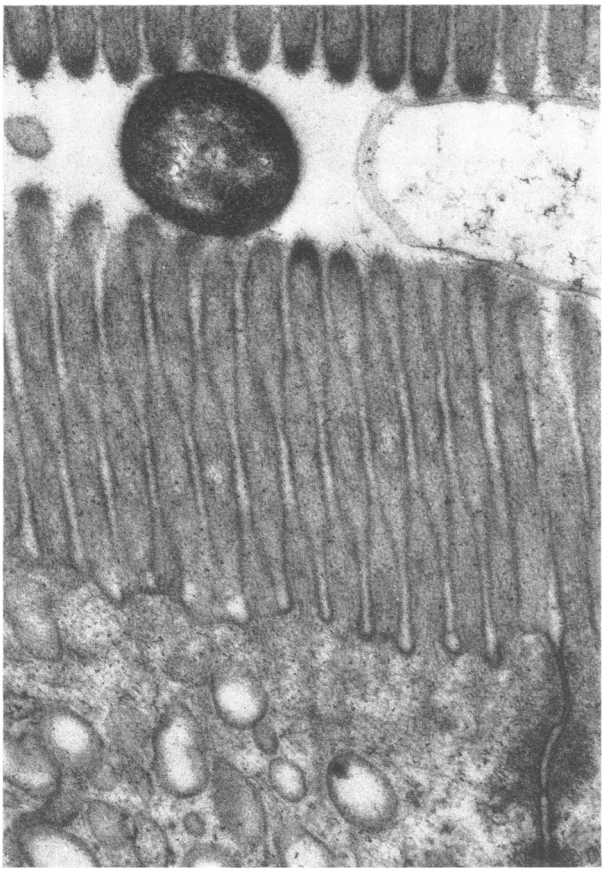

FIG. 7. *E. coli strain 123 in contact with microvilli from two epithelial cells in ligated jejunal loop of newborn pig 6 hr postexposure. Epithelial cell glycocalyx, microvilli, and apical cytoplasmic tubules are intact.* × *41,000.*

occurred in location ii and strain 263 usually in location iii. In one section, there were areas in the lumen which contained some debris as well as strain 263. The debris was arranged around the bacteria, like a negative stain, at the distance which characteristically separated 263 cell wall and microvilli (Fig. 8).

No abnormal epithelial cells were detected by ultrastructural examination of areas adjacent to bacteria of either strain nor along the majority of the epithelium, which was seen in frozen sections to be covered by strain 263. Bacteria were never observed within epithelial cells or in lamina propria.

DISCUSSION

Initially, it was assumed that washing would consistently remove the luminal bacteria. Consequently, it was anticipated that bacterial counts to determine the percentage of bacteria which remained with the wall after washing would yield more precise data than microscopy observations (6). Repeated washing of ligated intestinal loops did not consistently remove the luminal content (Fig. 5) which contributed to the variations observed (Table 4). Thus, our assumption was not valid, and our comments will be based on results obtained from microscopy observations and total bacterial counts only.

The EEC strains 263 and 431 had a greater tendency to be associated with epithelium than did the NEEC strain 123 or ICB. We assume the latter to be *E. coli* and, as none of the pigs from which ICB only were recovered developed diarrhea, that they were nonenteropathogenic. These results agree with others (1, 2, 4, 11, 21, 22, 24) and support the hypothesis that EEC have a greater tendency to associate with the epithelium than NEEC. The distribution of EEC and NEEC differed in two respects: (i) EEC tended to be distributed along the entire villus whereas NEEC remained in the central lumen; and (ii) wherever EEC occurred near epithelial cells, they were

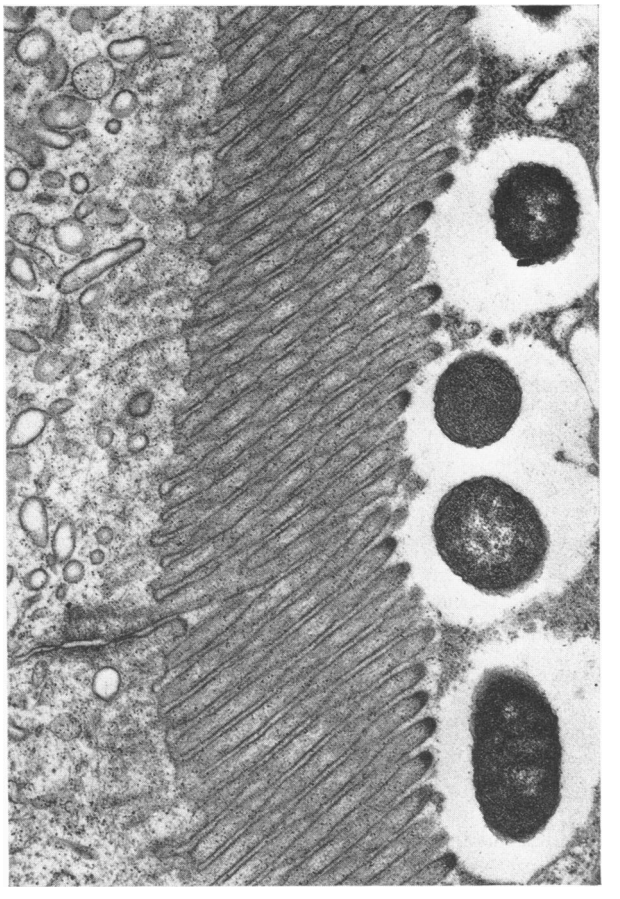

FIG. 8. *E. coli strain 263 applied to microvilli of epithelial cells in ligated jejunal loop of newborn pig 6 hr post-exposure. The bacterial cell walls are separated from microvilli by an electron lucent region which has been outlined by debris in the intestinal lumen. Epithelial cell microvilli, apical intercellular junctional complex, and cytoplasmic tubules are intact.* × *24,000.*

preferentially contiguous to the brush border whereas NEEC were randomly distributed in luminal mucus.

The possibility that the distribution shown in Fig. 2 was caused by movement of bacteria from the lumen over onto the epithelium, by the knife as the sections were cut, was considered. However, this pattern was characteristic for EEC not NEEC, and artifacts would probably effect both categories of bacterial equally. Furthermore, this distribution of EEC occurred along epithelium all around the villi and on villi all around the sections without regard to the direction in which the knife moved through the tissue. Thus, the pattern shown in Fig. 2 is not an artifact. The impression was gained that the frozen sections were more than 8 μm thick (the nominal section thickness set on the cryostat during cutting) and that the thick sections cut obliquely through the irregularly protruding epithelial cells resulted in bacteria contiguous to epithelial cells as appearing in Fig. 2.

Penetration of epithelial cells by *E. coli* has been reported in porcine small intestine after longer periods of exposure than those used in this study (3, 23). It was often impossible to determine whether EEC were within epithelial cells or extracellular and adjacent to the irregular brush border in the relatively thick frozen sections examined here (Fig. 2). We were unable to demonstrate bacteria in epithelial cells of thinner sections prepared from chemically fixed tissues, embedded in paraffin or plastic, and examined by light and electron microscropy. Most bacteria were removed by chemical fixation and subsequent processing; however, some remained in the lumen contiguous to the brush border. Intracellular bacteria would not have been removed. Therefore, we conclude that the EEC did not penetrate epithelial cells during the exposure

times used in this study. Diarrhea occurred in intragastrically exposed pigs and fluid accumulated in ligated loops without penetration of epithelial cells by *E. coli*. This is additional evidence that such penetration is not required for the production of small intestinal fluid loss by the enterotoxigenic *E. coli* used in this study.

The mechanisms whereby the association between EEC and host cells is established are unknown; however, association was apparent in ligated intestinal loops 45 min postexposure to strain 263. The interaction of actively motile individual villi repeatedly thrust into a central viscous mass of luminal mucus and the resultant removal of adherent graphite particles from these villi was described by Florey (5). A variety of bacteria are normally present in layers applied to epithelium in avillous areas of the alimentary tract such as stomach and large intestine of several mammals (15, 18). The interaction between motile villi and luminal mucus may explain why this phenomenon has not been observed in the small intestine of normal mammals, except the ileum of rats (18). The effects of EEC on villus motility are unknown; however, if inhibitory, as concluded by Drees and Waxler (2), they would alter this interaction and partially explain the characteristic distribution of EEC.

If one assumes that EEC reduce villus motility, thereby permitting them to be distributed along the entire villus, additional factors would more readily explain their preferential application to the brush border of epithelial cells. For example, streptococci vary greatly from strain to strain in their tendency to adhere to isolated oral epithelial cells in vitro (8). Cell surfaces may contain specialized anatomic structures, steric molecular configurations, or physical forces determined by chemical charge which promote adhesion of bacterial cells. The latter have been shown to influence the adhesion of *E. coli* to clay particles (10). The electron-lucent regions which usually separated the cell walls of strain 263 from microvilli in ultrathin sections (Fig. 8) could represent extracellular bacterial products which are the usual site of contact between host and this particular strain. One of the several possible explanations would be that this space is occupied by the antigen K 88, which is present in strain 263, but not in 123. However, this region is unlikely to contain a common organelle of attachment for all EEC as it was not present around all cells of this strain and apparently did not occur in pigs exposed to a different pig EEC (3).

EEC strains 263 and 431, which were both subsequently shown to associate with epithelium, colonized the small intestine of intragastrically exposed pigs more effectively than the NEEC strain 123 (Table 2), and the latter was shown to have little tendency to associate with epithelium. This difference in colonization by EEC and NEEC was apparent even in experiment 1-B where the inoculum of strain 123 was increased to 10^3 times that for strain 431 (Table 1 and 2). These observations are consistent with the hypothesis that the ability of EEC to colonize the small intestine more readily than NEEC is due to the ability of the former to associate with the epithelium. However, strain 263 also reached greater numbers in the small intestine of intragastrically exposed pigs than did strain 431 (Table 2). When these two strains were compared in the same pigs by using ligated intestinal loops, they had similar growth rates and the association index of strain 431 exceeded that for strain 263. Thus, it is unlikely that strain 263 colonized the orally exposed pigs more effectively than strain 431 because of an ability to associate more readily with epithelium or to multiply more rapidly in the intestine than strain 431. The pigs exposed to strain 263 in experiment 1-A and those exposed to strain 431 in experiment 1-B (Table 1) were from different litters. Thus, the differences between strains 263 and 431 (Table 2) may reflect differences between pigs from different litters rather than between strains of bacteria. The ICB which were apparently NEEC also colonized the small intestine of some pigs (Table 2) and were not associated with the epithelium. However, in contrast to the three strains inoculated experimentally, ICB were presumably continuously ingested from the environment. The high gastric *p*H at this age would permit ingested ICB to continually flood, and possibly multiply, in the small intestine while passing through (19).

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