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Attaching and Effacing Activities of Rabbit and Human Enteropathogenic *Escherichia coli* in Pig and Rabbit Intestines

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Three strains of enteropathogenic *Escherichia coli* (EPEC), originally isolated from humans and previously shown to cause diarrhea in human volunteers by unknown mechanisms, and one rabbit EPEC strain were shown to attach intimately to and efface microvilli and cytoplasm from intestinal epithelial cells in both the pig and rabbit intestine. The attaching and effacing activities of these EPEC were demonstrable by light microscopic examination of routine histological sections and by transmission electron microscopy. It was suggested that intact colostrum-deprived newborn pigs and ligated intestinal loops in pigs and rabbits may be useful systems to detect EPEC that have attaching and effacing activities and for studying the pathogenesis of such infections. The lesions (attachment and effacement) produced by EPEC in these systems were multifocal, with considerable animal-to-animal variation in response to the same strain of EPEC. The EPEC strains also varied in the frequency and extent of lesion production. For example, three human EPEC strains usually caused extensive lesions in rabbit intestinal loops, whereas two other human EPEC strains usually did not produce lesions in this system.

The term enteropathogenic *Escherichia coli* (EPEC) was coined to designate *E. coli* of the serotypes shown to contain human pathogens "with the particular potential of causing enteric rather than extraintestinal infection" (12). Since this original definition of EPEC was made, it has been demonstrated that *E. coli* can cause intestinal disease through several different mechanisms. The term EPEC has come to designate strains that cause intestinal disease, do not produce the enterotoxins currently designated as heat labile or heat stable, and are not enteroinvasive as judged by the Sereny test (9, 16, 17). The mechanisms by which EPEC cause disease are not well defined. It is possible that the group currently designated as EPEC includes strains that act through different pathogenic mechanisms.

A strain of *E. coli* serotype O55:H7 colonized, in multiple foci with intimate attachment to absorptive epithelial cells, the ileum (21) and colon (19) of experimentally inoculated newborn pigs. The microvilli of the epithelial cells with the intimately attached *E. coli* 055:H7 were effaced. The strain of *E. coli* used was originally isolated from a human with diarrhea (personal communication, T. E. Staley). The enterotoxigenicity and Sereny test activities of the strain used in these studies were not determined. The studies were carried out before the general application of assays for the enterotoxigenicity and enteroinvasiveness of *E. coli*, but after O55:H7 was a widely recognized human EPEC serotype. We will refer to *E. coli* that attach intimately to and efface microvilli from intestinal epithelial cells in the pattern reported by Staley (21) with the term attaching effacing *E. coli* (AEEC).

The AEEC pattern of epithelial association is notably more intimate (i.e., less distance between the bacterial cell wall and the host plasma membrane) and results in more effacement of microvilli than is characteristic of adherent enterotoxigenic *E. coli* (10). The pattern is also different from the intracytoplasmic epithelial location characteristic of enteroinvasive *E. coli* (5).

Polotsky et al. (14) studied the pathogenicity of human EPEC isolates from infantile enteritis cases. They found that most strains were nonenterotoxigenic and did not cause fluid accumulation in ligated intestinal loops of rabbits. However, light and electron microscopic examination of tissues from the rabbit intestinal loops revealed that the human EPEC were AEEC. Two of the strains studied had both enterotoxigenic *E. coli* and AEEC activities. Non-enterotoxigenic AEEC have been shown to cause a naturally occurring diarrheal disease in

TABLE 1. Characteristics of the *E. coli* strains used

E. coli strain	Serotype	Source	Pathogenicity	Reference
RDEC-1	O15:NM	Rabbit	EPEC-AEEC	4
123	O43:H28	Pig	None	11
HS	O9:H4	Human	None	9
E2348/69	O127:H6	Human	EPEC	9
E128012	O114:H2	Human	EPEC	R. E. Black and M. M. Levine, unpublished data
E851/71	O142:H6	Human	EPEC	9
CHMC 6	O119	Human	EPEC-AEEC	17
CHMC 3	O119	Human	EPEC-AEEC	17

rabbits (4, 15). It has been suggested that the epithelial changes characteristic of AEEC infection in rabbits are caused by a cytotoxin produced by AEEC (22). Human EPEC have been shown to produce a shiga-like cytotoxin (13). The AEEC lesion has recently been demonstrated in the intestinal tracts of humans with naturally occurring diarrhea due to EPEC infections (17, 23). Enteric disease caused by AEEC probably occurs in species other than rabbits and humans. We have occasionally seen the characteristic lesion in calves with naturally occurring diarrheal disease (10). The ability to cause the lesion (attachment and effacement) is not restricted to *E. coli*. The lesion is also characteristically caused by *Citrobacter freundii* in transmissible murine colonic hyperplasia (8).

The above reports warrant a working hypothesis that attaching and effacing activities are necessary and specific virulence attributes of human EPEC. Extensive effacement (loss of absorptive cell microvilli) could theoretically result in sufficient impairment of digestion and absorption to cause diarrhea in affected individuals. The prevalence of AEEC among human EPEC is not known. If confirmed, the reports of Staley et al. (19–21) and Polotsky et al. (14) provide a basis for using animals to test for human AEEC. Such animal systems could also be useful for in vivo studies to determine whether attaching and effacing activities are necessary for the virulence of EPEC.

The objectives of the work reported here were to (i) evaluate intact newborn pigs and ligated intestinal loops in pigs and rabbits as systems to test for AEEC, (ii) determine whether human EPEC strains previously shown to cause diarrhea in human volunteers by unknown mechanisms (9) are AEEC, (iii) determine whether EPEC isolated from humans with naturally occurring AEEC lesions (17) would attach to and efface the intestinal epithelium in pigs and rabbits, and (iv) determine whether a rabbit EPEC-AEEC strain (4) would attach to and efface the intestinal epithelium in pigs. We found that the human EPEC tested were AEEC and that the AEEC activity of rabbit and human EPEC could be demonstrated in all three systems.

MATERIALS AND METHODS

***E. coli* strains and growth conditions.** The *E. coli* strains used and some of their characteristics, known before this study, are listed in Table 1. The rabbit EPEC strain RDEC-1 (4) was used as an AEEC-positive control. Nonpathogenic strains 123 and HS were used as negative controls. The three human EPEC strains in the E series (E2348/69, E128012, and E851/71) have been shown to cause diarrhea in human volunteers (9; R. E. Black and M. M. Levine, unpublished data). The human strains listed as EPEC-AEEC (Table 1) were from the patients with naturally occurring AEEC lesions (17). Bacteria were grown aerobically at 37°C for 24 h in Tripticase soy broth (BBL Microbiology Systems). Ligated ileal loops were inoculated with 1.0 ml per loop of these cultures, and intact pigs were inoculated with 10 ml of culture per pig by feeding (gnotobiotic pigs) or by gavage (caesarian-derived, colostrum-deprived [CDCD] pigs).

Animals. The gnotobiotic and CDCD pigs were reared as reported previously (2, 24). Conventional pigs were obtained from the swine herd of the National Animal Disease Center. New Zealand White rabbits were obtained from Small Stock Industries, Pea Ridge, Ark. Gnotobiotic pigs were 1 to 9 days old and CDCD pigs were 1 to 2 days old when inoculated with *E. coli*. Ligated ileal loop (10-cm long) tests (11) were carried out with 1- and 8-week-old pigs and 9-week-old rabbits (6 to 10 loops per animal). Animals were fasted (12 h for 1-week-old pigs, 24 h for 8-week-old pigs, and 48 h for rabbits) before ileal loop surgery. All ileal loop tests were terminated 24 h after inoculation with *E. coli* (1 loop per *E. coli* strain per animal). Animals were killed with barbiturates given intravenously, and sections of intestine from the loops were immediately placed in 10% formalinized saline or 2.5% glutaraldehyde with sodium cacodylate buffer at pH 7.4.

Tissue processing. Formalin-fixed tissues to be examined by light microscopy were embeded in paraffin; sections 7-mm thick were cut from these and stained with hematoxylin-eosin. The lengths of 10 well-oriented villi per section and the depths of 10 well-oriented crypts per section were measured in some sections with an ocular micrometer. The extent of villous epithelium or of surface epithelium (cecum and colon) affected by AEEC was graded. Grade 0 indicated that no AEEC were seen; grade + indicated that AEEC

TABLE 2. Response of gnotobiotic pigs to *E. coli*

E. coli strain	No. of pigs tested	Incubation (days)[b]	No. of pigs with clinical signs	No. of pigs with AEEC in the following organs[a]:				
				Jejunum		Ileum	Cecum	Colon
				Upper	Lower			
RDEC-1	10	1–30	9	1	3	2[c]	7[c]	4
E851/71	4	1–8	3	0	2	3	4	4
E2348/69	2	1–2	0	0	1	1	1	2
E128012	1	2	0	0	0	0	1	0
HS	3	2–8	0	0	0	0	0	0
123	4	1–30	0	0	0	0	0	0

[a] Foci with bacteria intimately attached to the surfaces of low columnar or cuboidal or sloughing epithelium, detected by light microscopic examination of histological sections.

[b] Time from inoculation until animal was killed.

[c] One negative pig had focal epithelial degeneration and neutrophil infiltration.

were seen but affected less than 10% of the villous or surface epithelium; and grade +++ indicated that at least 50% of the villi had at least one focus (usually several) of AEEC or that 50% of the surface epithelium in the cecum or colon was affected; grade ++ was between grades + and +++. Glutaraldehyde-fixed tissues to be examined by electron microscopy were washed in sodium cacodylate buffer, postfixed in 1% osmium tetraoxide, rinsed in buffer, dehydrated in graded ethanols, and embedded in Epon 812. Thick and ultrathin sections were cut from the Epon blocks on an LKB Ultratome with a diamond knife. Thick sections were stained with toluidine blue and examined by light microscopy to select sections for electron microscopy. Ultrathin sections of selected foci were stained with uranyl acetate and lead citrate and examined with a Philips EM-200 electron microscope at 60 kV.

Intestinal absorption. The ability of the intestine to concentrate the water-soluble, nonabsorbable marker polyethylene glycol 4000 (PEG) in the intestinal lumen was used as an index of water absorption in one group of CDCD pigs. In this experiment, PEG was mixed (5 g/liter) with the milk fed to the pigs. Steady-state marker conditions were established throughout the gastrointestinal tract as described by Hamilton and Roe (6). This involved dividing the daily feed into eight equal portions that were fed to the pigs at intervals during the 5 h immediately before the animal was killed. The concentration of PEG in the contents of jejunum, ileum, cecum, and colon (collected at necropsy) was determined by the method of Hyden (7).

Statistical analyses. Villous lengths and PEG concentrations in one group of CDCD pigs were analyzed with Student's t test; $P < 0.05$ was accepted as significant.

RESULTS

Gnotobiotic pigs. Gnotobiotic pigs raised by the procedures described in this study have, in our experience, consistently excreted unformed, semisolid to viscous feces. The feces of the pigs inoculated with nonpathogenic *E. coli* HS and 123 maintained this characteristic throughout these studies. Feces of pigs inoculated with strains RDEC-1 or E851/71 had this characteristic initially; however, beginning 2 to 4 days after inoculation, the feces of these pigs became more voluminous, less viscous, and contained more mucus than did feces of pigs inoculated with strains HS and 123. Two of the pigs inoculated with strain RDEC-1 were killed when they became moribund during the first week after inoculation. At necropsy, these two pigs were found to have diffuse fibrinopurulent peritonitis and pericarditis. *E. coli* with the same colonial morphology and biotype as RDEC-1 were cultured from the livers of both pigs. With the exception of these two pigs, all pigs remained active and alert throughout the experiments. After 2 weeks of incubation, the three remaining RDEC-1-exposed pigs were less vigorous, slightly smaller, and had much dirtier hair than their three remaining litter mates exposed to strain 123.

The intestinal tracts of pigs exposed to strains HS and 123 were histologically normal (Table 2). In contrast, intestinal tracts from pigs inoculated with human or rabbit EPEC had multifocal epithelial degeneration associated with layers of attached bacteria (AEEC; Table 2). The mucosal border in foci with attached bacteria was frequently irregular, with low columnar to cuboidal or sloughing epithelial cells, in contrast to the uniform tall columnar cells of normal epithelium (Fig. 1). Inflammation with focal neutrophil infiltration of degenerate epithelium, of lamina propria, and of submucosa was commonly associated with the foci of AEEC. Foci of attached bacteria without histologically demonstrable epithelial degeneration or inflammation were also common in these pigs.

The AEEC lesions were seen in all pigs inoculated with the human EPEC strains and in 7 of 10 pigs inoculated with strain RDEC-1 (Table 2). The lesions occurred after as little as 1 day of incubation and apparently persisted without a notable increase in incidence with longer incubation. Lesions were seen in only two of three RDEC-1-inoculated pigs examined after 30 days of incubation. The other two AEEC-negative RDEC-1-inoculated pigs were examined after 2

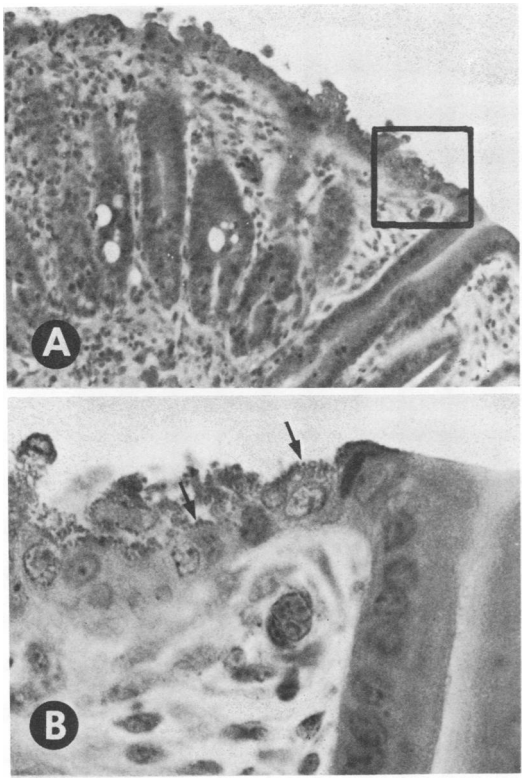

FIG. 1. Histological section of cecum from a gnotobiotic pig infected with the attaching and effacing rabbit *E. coli* strain RDEC-1. (A) Surface epithelium is irregular. Area outlined is shown at higher magnification in (B). (B) Irregular, low columnar to cuboidal, and sloughing epithelial cells have attached bacteria (arrows). Crypt epithelium (right) is not affected.

and 21 days of incubation. In view of the focal distribution of AEEC lesions, they may have occurred at sites and in pigs recorded as negative in Table 2. The lesions were seen more frequently in the large intestine than in the small intestine and more frequently in the ileum than in the upper jejunum. The lesions also tended to be more extensive in the large intestine (usually grade ++ or +++) than in the small intestine (usually grade + or ++). Ileal villi appeared to be somewhat shorter in affected pigs than in those inoculated with strains HS or 123. Surface (large intestine) or villous (small intestine) epithelium was affected more frequently than crypt epithelium.

Tissue sections with AEEC representing each of the three human EPEC strains and RDEC-1 were examined by electron microscopy. The lesions were similar with all four strains (Fig. 2). Affected epithelial cells had effaced microvilli, and bacteria were intimately associated with their apical plasma membranes. The plasma membranes were frequently "cupped" or thrown into "pedestals" beneath the attached bacteria. Electron-dense fibrillar modifications occurred in the epithelial cell terminal web areas most immediately associated with attached bacteria, and there were varying degrees of cytoplasmic degeneration in affected epithelial cells. Bacteria were occasionally seen in the cavities of evacuated goblet cells. Bacteria were sometimes surrounded by cytoplasmic processes as if they had invaded, or had been engulfed by, absorptive epithelial cells.

CDCD pigs. Strains RDEC-1 and E851/71 also produced AEEC lesions in CDCD pigs. The lesions in CDCD pigs developed as quickly as, had a similar distribution to, and were qualitatively and quantitatively similar (usually grade + or ++ in the small intestine and grade ++ or +++ in the large intestine) to those seen in gnotobiotic pigs inoculated with these strains (Table 3, Fig. 3 and 4). There was partial atrophy of villi in the ileum of EPEC-inoculated pigs (Table 3). This occurred whether or not there were AEEC demonstrable in the ileal sections.

There was also apparently some functional impairment associated with infection by strains RDEC-1 and E851/71. Pigs inoculated with the EPEC strains did not concentrate PEG in their large intestines as effectively as did those inoculated with strain 123 (Fig. 5). However, there were no differences between groups in PEG concentration in the small intestine. The trend toward functional impairment in the large intestine, but not in the small intestine, was consistent with the histological distribution of AEEC in these pigs (more frequent and more extensive in the large intestine than in the small intestine).

Strains CHMC 3 and CHMC 6 were also tested in the CDCD pigs to determine whether strains known to have been associated with AEEC lesions in humans would produce the lesion in pigs. CHMC 3 was positive for AEEC in two of four pigs and caused multifocal epithelial degeneration and neutrophil infiltration in all four pigs (Table 3). No lesions were detected in pigs inoculated with strain CHMC 6.

Ligated intestinal loops. None of the strains tested produced histologically detectable lesions in intestinal loops of 8-week-old-pigs (Table 4). However, two of the three human EPEC strains and strain RDEC-1 produced AEEC lesions in intestinal loops of 1-week-old pigs (Table 4). The lesions in pig loops were less extensive than those caused by these strains in the small intestines of orally inoculated intact CDCD and gnotobiotic pigs. Absorptive cells from these lesions were shown by electron microscopy (Fig. 6) to have the microvillus effacement, cupping, pedestal formation, and electron-dense fibrillar modifications of the terminal web area that are characteristic of AEEC in intact animals. Ab-

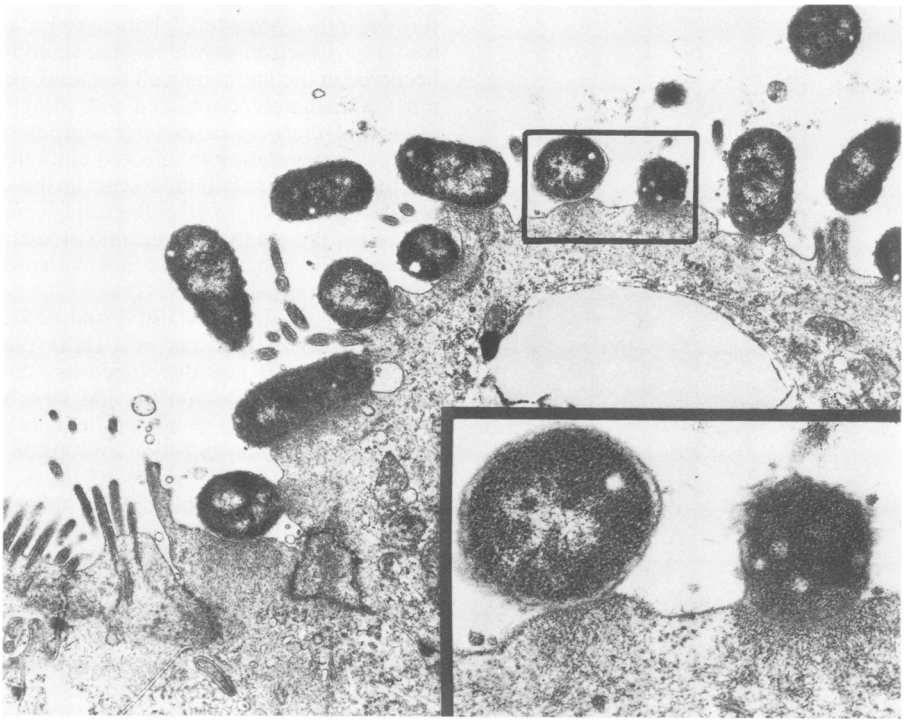

FIG. 2. Electron micrograph of surface epithelium in cecum from a gnotobiotic pig infected with human EPEC strain E128012. Microvilli effaced from epithelial cells with intimately attached *E. coli*. Epithelial cell membranes form cups or pedestals at the base of some of the attached *E. coli*. Inset at lower right shows outlined area at higher magnification. Electron-dense fibrillar modifications are in terminal web areas immediately adjacent to the *E. coli*.

sorptive cells in pigs at this site and age normally have large phagosomes (3). Absorptive cells with AEEC frequently contained bacteria in these large phagosomes (Fig. 6). The cytoplasmic organelles of absorptive cells with AEEC frequently were swollen and had ruptured membranes.

All five of the human EPEC strains and strain RDEC-1 apparently caused AEEC lesions in intestinal loops of rabbits. However, there was considerable variation among these strains in the frequency and extent of lesion production (Table 4). Lesions were detected in only one of three loops exposed to CHMC 3. A single focus with bacteria intimately associated with normal appearing epithelium (not recognized as AEEC) was detected by light microscopy in one section from one of the eight loops exposed to CHMC 6.

TABLE 3. Response of CDCD newborn pigs to *E. coli*

E. coli strain	No. of pigs tested	Incubation (days)[a]	No. of pigs with clinical signs	No. of pigs with AEEC in the following organs[b]:		Dimensions of ileal mucosa (mean ± SD) (μm)	
				Small intestine	Large intestine	Villous length	Crypt depth
RDEC-1	4	2–3	0	1	3[c]	600 ± 189[d]	111 ± 12[d]
E851/71	4	2–3	0	2	4	427 ± 28[d]	153 ± 1[d]
CHMC 3	4	2	0	1	2[c]	ND[e]	ND
CHMC 6	4	2–3	0	0	0	ND	ND
123	4	2–3	0	0	0	768 ± 126	105 ± 10

[a] Time from inoculation until animal was killed.
[b] Foci with bacteria intimately attached to the surfaces of low columnar, or cuboidal, or sloughing epithelium, detected by light microscopic examination of histological sections.
[c] Multifocal epithelial degeneration and neutrophil infiltrations in negative pigs.
[d] Significantly different ($P < 0.05$) from pigs inoculated with strain 123.
[e] ND, Not done.

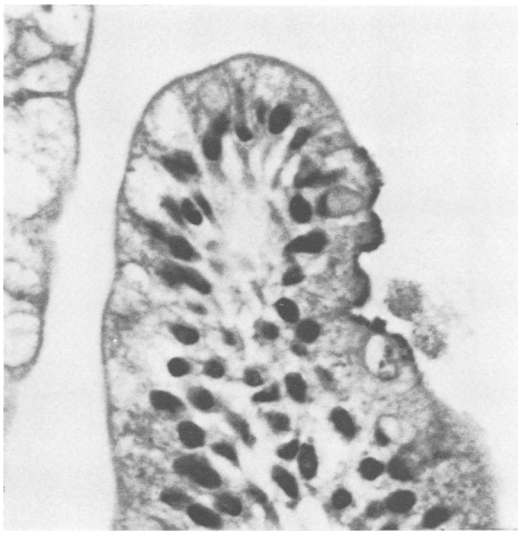

FIG. 3. Histological section of ileum from a CDCD pig infected with human EPEC strain E128012. There is a focus of epithelial cells with intimately attached bacteria (seen at this magnification as an irregular black line to the right) and effaced cytoplasm near the tip of a villus.

Epithelial cells in this (CHMC 6) focus were found by electron microscopy to be mostly, but not exclusively, goblet cells (Fig. 7) and to have the brush border and apical cytoplasmic changes characteristic of AEEC. The AEEC lesion could not be demonstrated anywhere else in this section or in tissues from the other loops exposed to this strain. In contrast to those exposed to CHMC 3 and CHMC 6, loops in the same rabbits exposed to human EPEC strains E128012 or E851/71 had extensive multifocal AEEC lesions demonstrable by light and electron microscopy. Lesions caused by E2348/69, E128012, and E851/71 in rabbit intestinal loops were as extensive as those caused by the rabbit strain RDEC-1. Both absorptive and goblet cells were affected (Fig. 8). *E. coli* was frequently seen in cytoplasmic vacuoles of absorptive cells and in the partially evacuated cavities of goblet cells. In contrast to that reported for strain RDEC-1 in intact rabbits (4), there was no apparent predilection of any of these strains for dome epithelium of Peyer patches in these intestinal loops.

The nonpathogenic strains (HS and 123) did not associate with the epithelium or cause lesions in the intestinal loops of pigs or rabbits (Table 4, Fig. 9). Occasional loops (principals and controls) of some rabbits contained coccidia. Small, slightly curved bacteria thought to be *Campylobacter* sp. were found in the cytoplasm of a few absorptive cells in some loops (principals and controls).

None of the strains tested caused fluid accumulation in any of the intestinal loops of pigs or rabbits.

DISCUSSION

The experiments reported here demonstrated that human EPEC strains E851/71, E2348/69, E1208012, CHMC 3, and probably CHMC 6 are AEEC. These attaching and effacing activities are apparently common among human EPEC (14, 15, 17, 23). It may be that most strains currently designated as EPEC are AEEC. The epithelial damage caused by AEEC (loss of epithelial cells plus loss of microvilli, loss of cytoplasm, and cytoplasmic degeneration in remaining epithelial cells) may be the mechanism by which AEEC causes diarrhea. Such changes would reduce the absorptive capacity of the

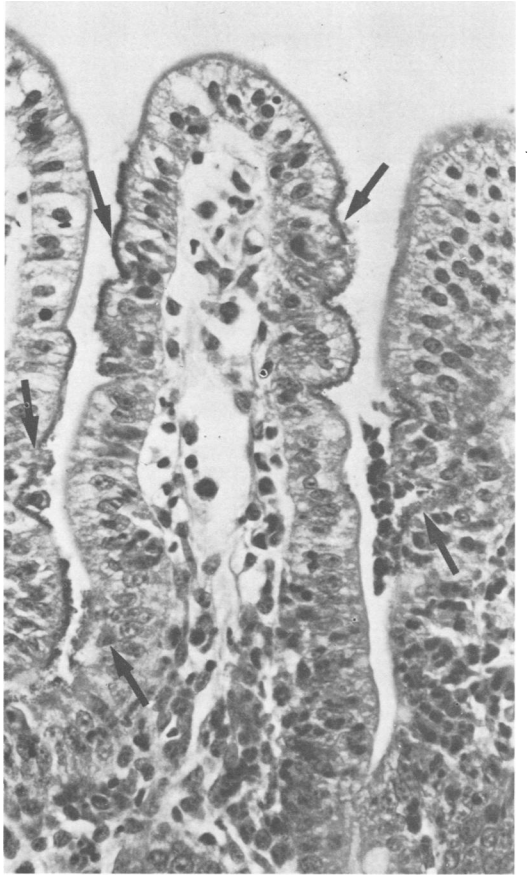

FIG. 4. Histological section of ileum from a CDCD pig infected with human EPEC strain E851/71. There are multiple foci of attached effacing *E. coli* (arrows). The cellular infiltrate in the lamina propria and epithelium, which has exuded into the intestinal lumen over one focus (lower right), was determined at higher magnification to be mostly neutrophils.

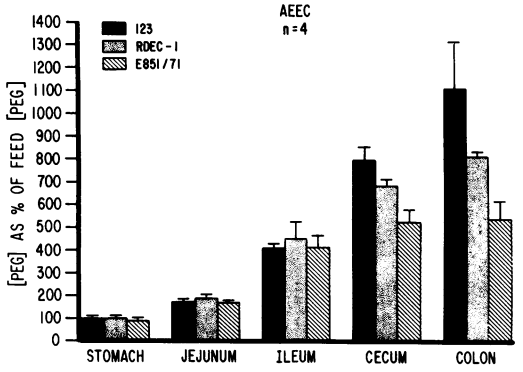

FIG. 5. Concentration (mean + standard error) of PEG in intestinal contents of CDCD pigs infected with different strains of *E. coli*. Pigs infected with AEEC (the rabbit EPEC strain RDEC-1 and the human EPEC strain E851/71) had lower PEG concentrations in the cecum and colon than did pigs infected with the nonpathogenic strain 123. This difference was statistically significant ($P < 0.05$) in the cecum but not in the colon of the group infected with strain E851/71. The group infected with strain RDEC-1 was not significantly different from the group infected with strain 123 at any site.

intestine in the areas affected and, if severe (intensive and extensive), could produce malabsorptive diarrhea.

In contrast to the pigs reported here, severe lesions and signs occur in some humans with AEEC infections (17). The signs in pigs may have been comparatively mild because the lesions were less severe. Intact pig models used in this study may have minimized the clinical impact of malabsorption since most of the damage occurred in the colon, and the diet was milk in restricted quantities. Furthermore, absorption in the large intestine of pigs develops with age, depending in part on the complex flora of the conventional pig (1, 6). Thus, the gnotobiotic and neonatal pigs used probably absorbed most of their food in the proximal intestine before it reached the areas most affected by AEEC. In spite of these limitations, detectable changes in intestinal function apparently are associated with AEEC infections in neonatal pigs. Neonatal pigs normally absorb intact, undigested protein from their intestines. The EPEC-AEEC strain O55:H7 significantly reduced the ability of newborn pigs to absorb protein (20). The lower concentrations of PEG in ceca and colons of AEEC-infected pigs in the study reported here are presumptive evidence of reduced salt and water absorption in the lower part of the intestinal tract (where lesions were most frequent and extensive). However, the (PEG) method as used is not specific for absorption, and the number of animals examined was small. Increased secretion of fluid would also result in a lower concentration of PEG. However, secretion seems unlikely to be the primary mechanism because the AEEC did not cause fluid accumulation in intestinal loops.

The attachment and effacement seen in the lesions reported here were similar to those reported by others (4, 14, 15, 19, 21–23). The multifocal distribution, predilection for villous or surface epithelium, acute inflammation, villous atrophy, degeneration of cytoplasmic organelles, and bacteria in the cytoplasm of some epithelial cells seen in this study are also features of AEEC infections reported previously (4, 14, 15, 19, 21, 22). The apparent predilection of CHMC 6 for goblet cells in the rabbit intestinal loop contrasts with the sparing of goblet cells by AEEC of this serotype in humans (17). Howev-

TABLE 4. Attachment of *E. coli* to and effacement of epithelial cells in ligated intestinal loops of pigs and rabbits examined by light (LM) and electron (EM) microscopy

E. coli strain	Attachment of *E. coli* in the following animals (age) detected under the following microscopic conditions:						
	Pigs (8 wk)	Pigs (1 wk)			Rabbits (9 wk)		
	Light microscopy	Light microscopy		Electron microscopy	Light microscopy		Electron microscopy
	No. of positive loops/no. examined	No. of positive loops/no. examined	Extent[a]	No. of positive loops/no. examined	No. of positive loops/no. examined	Extent[a]	No. of positive loops/no. examined
RDEC-1	0/3	2/3	+	2/3	5/5	+++	3/3
E2348/69	0/3	2/3	+	2/3	5/5	+++	3/3
E128012	0/3	0/3			10/11	+++	3/3
E851/71	0/3	1/3	+	1/3	8/8	+++	3/3
CHMC 3					1/3	+	
CHMC 6					0/8		1/5
HS	0/3	0/3			0/5		0/3
123	0/3	0/3			0/8		0/2

[a] Extent of villous epithelium with AEEC mode of positive reactions: +, Less than 10% of the villi had a focus of AEEC; +++, at least 50% of the villi had at least 1 (usually several) focus of AEEC.

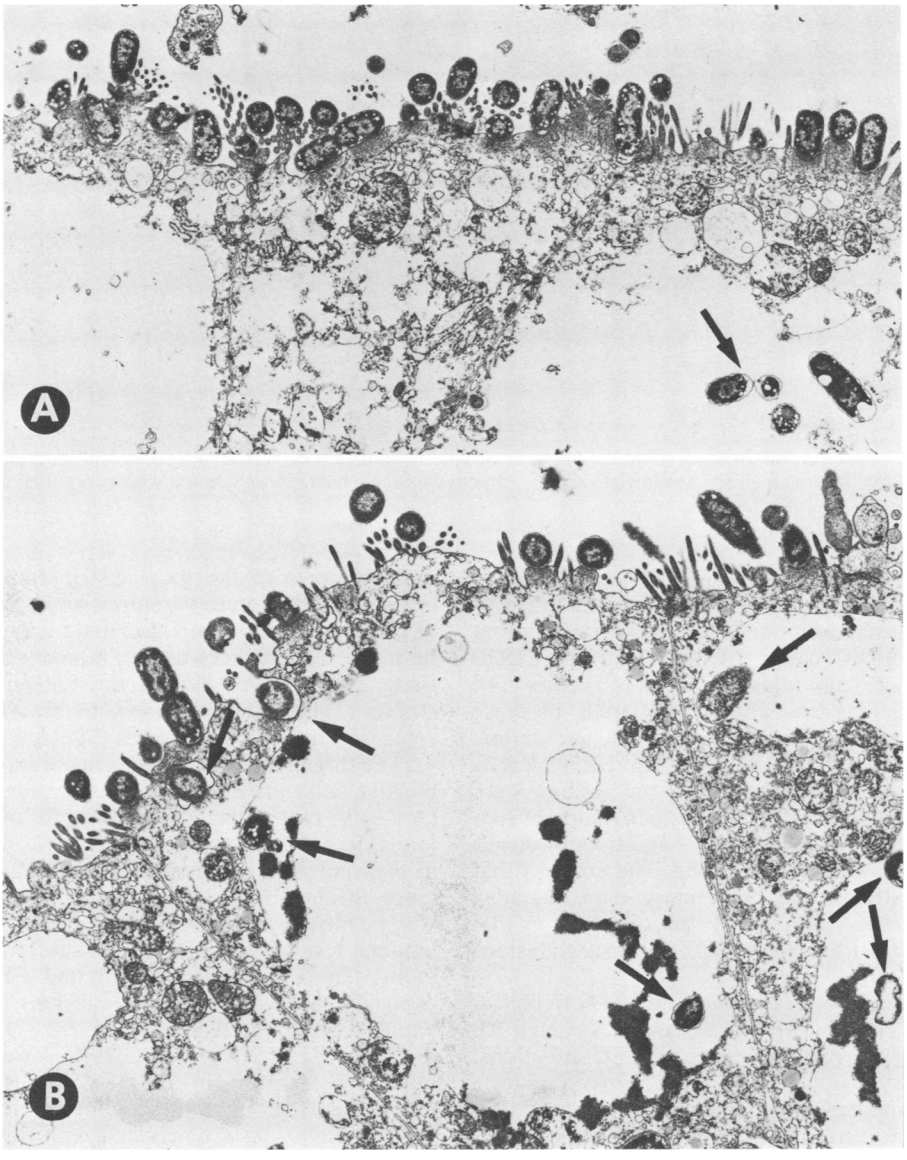

FIG. 6. Electron micrograhs of apical portions of villous absorptive cells from ligated ileal loops of pigs inoculated with human EPEC strain E128012. (A) Effaced microvilli, pedestal formation, cupping, and fibrillar electron-dense modification of terminal web areas of absorptive cells associated with attached *E. coli*. Some *E. coli* (arrow) are in large phagolysosomes. (B) Uptake of *E. coli* (arrows) into large phagolysosomes is clearly seen.

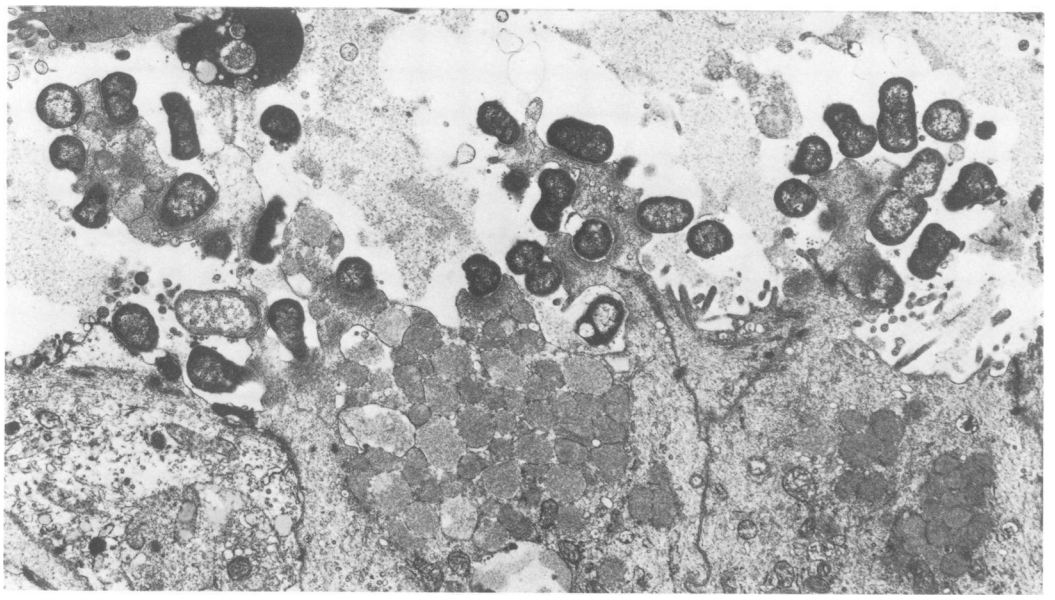

FIG. 7. Electron micrograph of apical portions of villous epithelial cells from a ligated ileal loop of a rabbit inoculated with human EPEC strain CHMC #6. Attached effacing bacteria associated with two goblet cells and two unidentified epithelial cells.

er, Polotsky et al. (14) also observed human EPEC associated with goblet cells in this system. These results confirm earlier reports that human EPEC cause AEEC lesions in CDCD pigs (19, 21) and intestinal loops of rabbits (14). The attaching and effacing activities of these strains were also demonstrable in gnotobiotic pigs and in intestinal loops of conventional newborn pigs. With experience, the lesions were readily recognizable by light microscopy of routine (paraffin-embedded, hematoxylin-eosin-stained) histological sections. The concept that AEEC strains can affect more than one host species was extended by the observation that the rabbit EPEC strain (RDEC-1) caused lesions in pigs.

Intestinal loops of rabbits and newborn pigs or intact CDCD pigs may be useful systems for studying the pathogenesis of AEEC infections. They may also be useful in vivo screening tests to determine whether isolates (human, rabbit, or other species) of unknown pathogenicity are AEEC. However, results reported here indicate distinct limitations on the use of these systems for such tests because of marked animal-to-animal variation as well as apparent variability in the intensity of attaching and effacing activities among positive strains. Tests to detect AEEC by using these systems should include several animals per isolate because there was considerable animal-to-animal variation in response to AEEC (Tables 2 through 4). This variation may have been due in part to sampling error because of the focal distribution of the lesions. In addition to variations among animals of the same species and age, there appeared to be marked differences among human AEEC in these systems. All five of the human EPEC strains (Table 1) were shown to be AEEC at some time during these studies (Tables 2 through 4). The three strains in the E series were usually positive and sometimes caused extensive lesions (+++) in both intact pigs and rabbit intestinal loops. In contrast, strain CHMC 6 was negative in pigs and rabbits (except for one focus in one loop). Strain CHMC 3 was intermediate in that it was positive in CDCD pigs but produced only minimal lesions in one of three rabbit intestinal loops. These same (CHMC 3 and CHMC 6 exposed) rabbits had extensive lesions in loops exposed to strains E128012 and E851/71 (Table 4). The AEEC activity of CHMC 3 was more readily recognized in the large intestine of CDCD pigs than in rabbit ileal loops. However, the number of tests conducted with this strain was too small to determine whether this difference was the result of chance or true differences in the species or organ specificity of CHMC 3. In contrast to the results in animals reported here, CHMC 3 and CHMC 6 apparently caused intensive and extensive lesions in humans (17). These strains may have lost some AEEC activity since their original isolation. Alternatively, human intestine (or the intestines of some humans) may be more susceptible to the AEEC activities of these strains than is the pig or rabbit intestine.

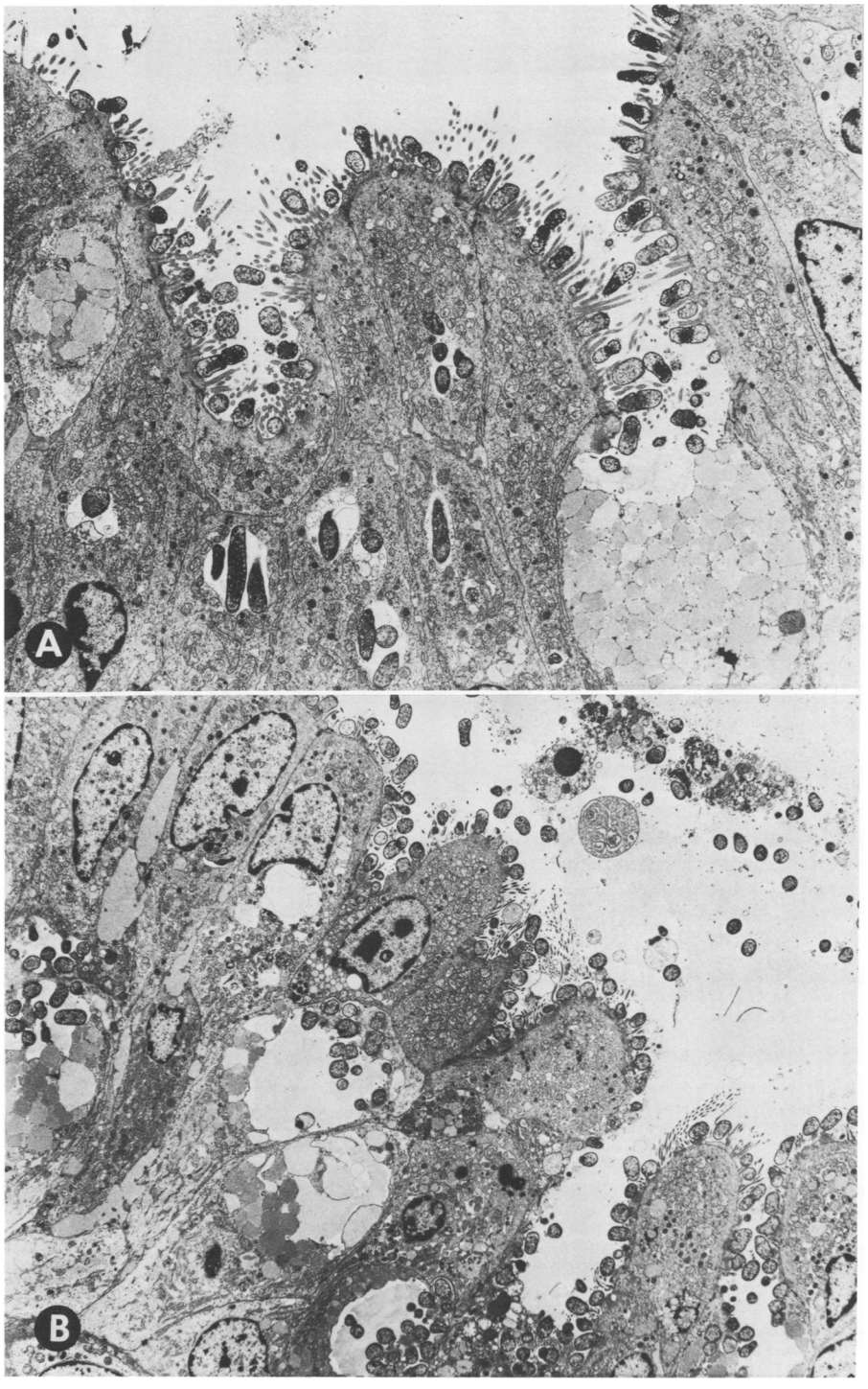

FIG. 8. Electron micrograph of villous epithelial cells from ligated ileal loops of rabbits inoculated with human EPEC strain E2348/69 (A) or rabbit EPEC strain RDEC-1 (B). Both strains attached to the epithelium, effaced microvilli, entered into partially evacuated goblet cells, and occasionally appeared to be in phagolysosomes.

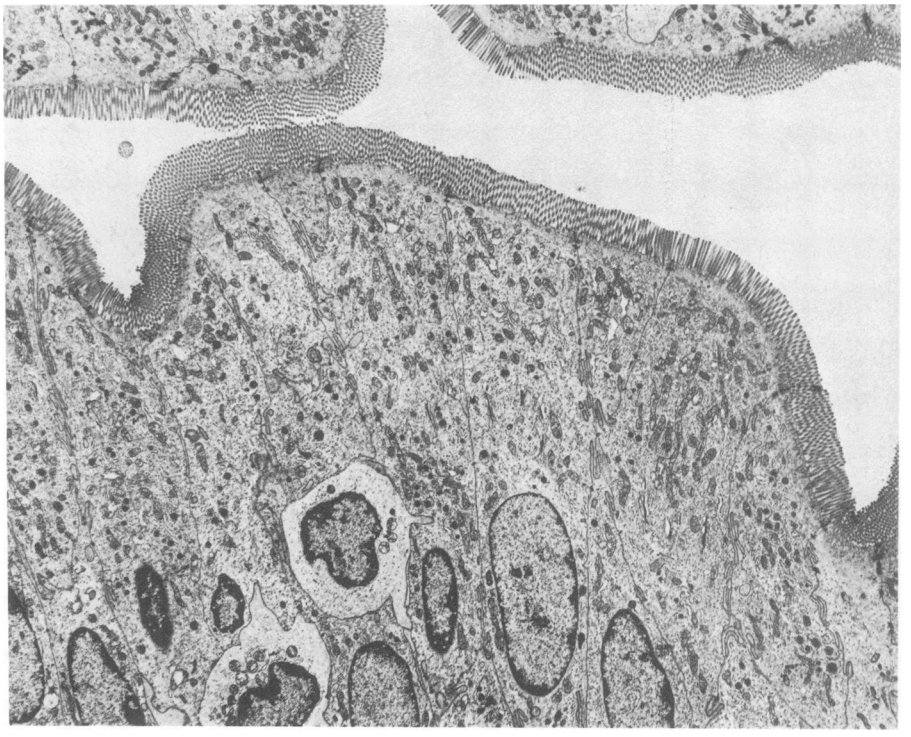

FIG. 9. Electron micrograph of normal villous epithelial cells from a ligated ileal loop of a rabbit inoculated with the nonpathogenic human *E. coli* strain HS.

Whatever the reason(s), rabbit and pig intestines appear to be better for detecting some strains of human AEEC than for others. Thus, there may be human AEEC which are completely negative in the pig and rabbit intestine.

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