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Adhesion of Enteropathogenic *Escherichia coli* to Human Intestinal Enterocytes and Cultured Human Intestinal Mucosa

STUART KNUTTON,* DAVID R. LLOYD, AND ALEXANDER S. McNEISH

Institute of Child Health, University of Birmingham, Edgbaston, Birmingham B16 8ET, United Kingdom

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The adhesion of classic enteropathogenic *Escherichia coli* (EPEC) strains of human origin to isolated human small intestinal enterocytes and cultured small intestinal mucosa was investigated. An adhesion assay with isolated human enterocytes prepared from duodenal biopsy samples was developed and tested with EPEC strains known to cause diarrhea in healthy adult volunteers. In the assay a mean of 53 and 55% of enterocytes had brush border-adherent *E. coli* E2348 (O127;H6) and E851 (O142:H6), respectively, whereas the value for a nonpathogenic control strain and a plasmid-cured derivative of strain E2348 was 0%. A collection of 17 EPEC strains was also tested for the ability to colonize cultured human duodenal mucosa. Extensive colonization occurred with 13 strains, including serogroups O55, O86, O111, O114, O119, O127, O128, and O142; and in each case electron microscopic examination of colonized mucosa revealed the characteristic histopathological lesion reported by others in natural and experimental EPEC infections. EPEC strains were seen to adhere intimately to the enterocyte surface, causing localized destruction of microvilli. The plasmid-cured derivative of strain E2348, which colonized cultured mucosa much less efficiently than the parent strain, nevertheless produced an identical lesion, indicating that plasmid-encoded factors are not essential for adhesion and the brush border-damaging property of EPEC.

Enteropathogenic *Escherichia coli* (EPEC) was the term used by Neter (24) to refer to serotypes of *E. coli* that were epidemiologically associated with outbreaks of diarrhea in young infants in the 1940s and 1950s. The term is currently used to denote *E. coli* strains that cause diarrhea but that do not produce heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST) and are not invasive by the Serény test (8). The pathogenic mechanism of diarrhea caused by EPEC has yet to be established, although enterotoxins distinct from LT and ST have been identified in some EPEC strains (11, 15, 25).

Results of several recent studies of EPEC infections in infants and animals have suggested that adhesion of bacteria to the intestinal mucosa is important in pathogenesis (21, 26, 27, 32). Electron microscopic examination of infected mucosa revealed that EPEC intimately adhered to and was partially surrounded by cuplike projections (pedestals) of the enterocyte surface, with only 10 to 12 nm separating bacterial and host membranes. In regions of attachment, brush border microvilli were lost, and their associated cytoskeletal elements were destroyed or disrupted. *E. coli* isolates that cause such a characteristic intestinal histopathological lesion have been termed attaching, effacing *E. coli* (21). Identical lesions have been found in biopsy samples from infants with diarrhea caused by EPEC O111, O119, and O125 (27, 29, 32) and in experimental infections in animals caused by EPEC O26, O111, O114, O127, O128, and O142 (21, 26, 31).

To study the adherence properties of diarrheic *E. coli* we developed in vitro systems in which human intestinal cells were used. In previous reports we described adhesion assays in which duodenal enterocytes were used to study enterotoxigenic *E. coli* (ETEC) (13, 14) and colonic enterocytes were used to study *E. coli* pathogens isolated from patients with bloody diarrhea (G. T. Hinson, S. Knutton, M. K. L. Lam-Po-Tang, A. S. McNeish, and P. H. Williams, Infect. Immun., submitted for publication). In each case brush border adhesion of bacteria was promoted by specific adhesion fimbriae. Specific adherence factors are also likely to be essential for the pathogenicity of EPEC, and so we developed in vitro adhesion assays using human small intestinal enterocytes and cultured intestinal mucosa to study this group of human pathogens. In this report we describe our findings with a collection of 17 EPEC strains of human origin covering the major classical EPEC serogroups.

MATERIALS AND METHODS

Bacterial strains. The characteristics and origins of the strains used in this study are shown in Table 1. Strains were classified as EPEC on the basis of serotype and lack of reactivity in tests for the production of LT and ST and in tests for invasive capacity. *E. coli* E2348 and E851 previously have been shown to cause diarrhea in human volunteers (15). The 60-megadalton plasmid in strain E2348 was removed by the plasmid incompatibility technique (1). Nonpathogenic laboratory strain HB101 was used as a negative control. Stock cultures of the strains were subcultured into Mueller-Hinton broth (Oxoid Ltd., Basingstoke, United Kingdom) and incubated aerobically at 37°C for 18 h.

Enterocyte adhesion. Duodenal mucosal biopsy samples were taken with informed consent from adult volunteers and transported to the laboratory in ice-cold HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered modified Eagle medium (MEM). Enterocytes from four or five biopsies were isolated by an EDTA chelation procedure (14) and suspended in 18 ml of MEM containing 0.5% D-mannose. For each adhesion assay 25 μl of a bacterial broth culture (~10^8 bacteria per ml) was added to 3 ml of the enterocyte suspension (~10^5 cells per ml), and the contents were incubated at 37°C for 3 h on a rotary mixer. Enterocytes were sedimented at 100 × *g* for 1 min, and nonadherent bacteria were removed by repeated washing. Brush border adhesion of bacteria was assessed by phase-contrast microscopy. Each assay consisted of the examination of 100 enterocytes selected at random and the determi-

* Corresponding author.

TABLE 1. Characteristics of EPEC strains

Strain	Serotype[a]	Origin	Source[b]	Production of characteristic intestinal histopathological lesion[c]
3605-83	O26:H11	Infant diarrhea, U.S.	CDC	−
660-79	O55:H7	Infant diarrhea, U.S.	CDC	+
177-83	O86:NM	Infant diarrhea, Brazil	CDC[d]	+
135	O86		CVD	−
184-83	O111:NM	Infant diarrhea, Brazil	CDC[d]	+
1367-80	O111:NM	Infant diarrhea, U.S.	CDC	+
BC2-82	O111	Infant diarrhea, U.K.	BCH	+
1923-77	O111:H21	Infant diarrhea, U.S.	CDC	+
E128010	O114:H2	Infant diarrhea, Bangladesh	CVD[e]	+
2036-80	O119:H6	Infant diarrhea, U.S.	CVD	+
E2348	O127:H6	Infant diarrhea outbreak, U.K.	CVD[e]	+
Plasmid-cured E2348	O127:H6	Laboratory-derived variant of E2348	CVD	+
1092-80	O127:NM	Infant diarrhea, U.S.	CDC	+
EB1-82	O127	Infant diarrhea, U.K.	EBH	+
182-83	O128ac:H21	Infant diarrhea, Brazil	CDC[d]	+
2311-77	O128:H2	Infant diarrhea, U.S.	CDC	−
E851	O142:H6	Infant diarrhea outbreak, U.K.	CVD[e]	+

[a] NM, Nonmotile.
[b] CDC, K. Wachsmuth, Centers for Disease Control, Atlanta, Ga.; CVD, M. M. Levine, Center for Vaccine Development, Baltimore, Md.; BCH, M. Penny, Birmingham Children's Hospital, Birmingham, United Kingdom; EBH, M. Penny, East Birmingham Hospital, Birmingham, United Kingdom.
[c] Demonstrated by scanning electron microscopy.
[d] Originally obtained from R. L. Guerrant, University of Virginia, Charlottesville, Va.
[e] Originally obtained from B. Rowe, Central Public Health Laboratory, Colindale, United Kingdom.

nation of the percentage of brush borders with adherent bacteria.

Adhesion to cultured intestinal mucosa. The system used to culture mucosal biopsy samples from the human small intestine was adapted from that of Browning and Trier (2). Samples from duodenal biopsies were placed into the culture system within 20 min and oriented with the villi facing upward on sterile foam sponge supports that had been previously saturated with bicarbonate-buffered culture medium (NCTC 135–Dulbecco MEM [1:1]) containing 10% newborn calf serum. The level of the culture medium was subsequently adjusted so that a thin layer covered the villous surface of the tissue. The petri dishes were covered, placed inside a large container, gassed with 95% O_2–5% CO_2, and sealed. Biopsy samples were maintained on a rocking table at 37°C.

For adhesion studies 25 μl of a bacterial broth culture was immediately placed on the mucosal surface of the biopsy samples, and biopsy samples were cultured in the presence of bacteria for up to 12 h. The culture medium was replaced with fresh medium every 2 to 3 h to maintain the pH and to prevent overgrowth of bacteria. It was not possible to remove all of the medium from the sponge support, and so reinoculation with bacteria following each change of medium was unnecessary. At the end of the culture period biopsy samples were removed and thoroughly washed in several changes of fresh medium prior to fixation and tissue processing.

Tissue processing. Biopsy samples to be examined by light micoscopy were fixed in Formalin and embedded in paraffin, and sections were stained either with hematoxylin-eosin or Giemsa strain. Tissue for electron microscopy was fixed in 3% phosphate-buffered (0.1 M, pH 7.4) glutaraldehyde, postfixed in 1% buffered osmium tetroxide, block stained in aqueous 1% uranyl acetate, and embedded in Epon. Thin sections stained with toluidine blue were examined by light microscopy to select areas for electron microscopy. Ultrathin sections were stained with aqueous uranyl and lead salts and examined in an electron microscope (EM301; Philips). For scanning electron microscopy, glutaraldehyde- and osmium-fixed tissue samples were dehydrated through graded acetone solutions and dried to the critical point. Specimens were mounted on stubs, coated with gold, and examined in a scanning electron microscope (S4 [Cambridge]; 100A [ISI]). For negative staining of bacteria, 10 μl of a washed bacterial suspension was mixed with equal volumes of bacitracin (150 μg/ml) and ammonium molybdate (2%, pH 7.0). A total of 10 μl was applied to carbon-coated grids for 30 s, and the excess liquid was removed with filter paper.

RESULTS

Adhesion of EPEC strains E2348 and E851 to human enterocytes. Strains E2348 and E851 were selected as test

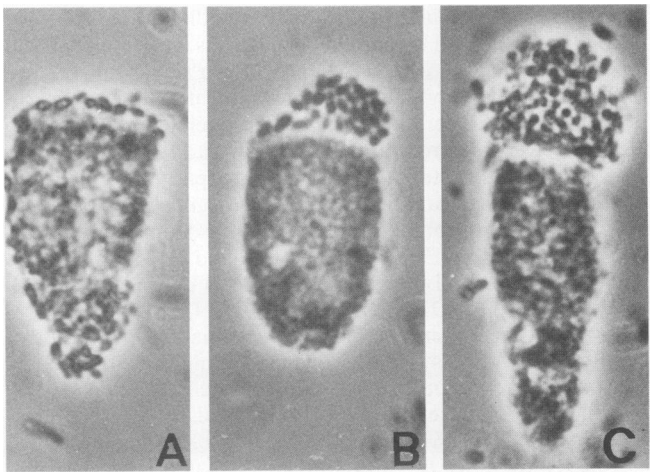

FIG. 1. Phase-contrast micrographs showing isolated human duodenal enterocytes with adherent EPEC. EPEC adhered to both brush border and basolateral enterocyte surfaces in the absence of D-mannose (A); in the presence of D-mannose adhesion of strains E2348 (B) and E851 (C) was specifically localized to the enterocyte brush border. Magnification: ×1,000.

TABLE 2. Adhesion of EPEC to isolated human duodenal enterocyte brush borders

Strain	% Brush borders with adherent bacteria in:		
	Donor 1	Donor 2	Donor 3
E2348		54	56
E851	55	50	
Plasmid-cured E2348		0	0
HB101	0	0	0

organisms because of their known pathogenicity in adult volunteers (15). Incubation of concentrated suspensions of bacteria (10^9 to 10^{10}/ml) with duodenal enterocytes for 30 min at 37°C, which are conditions used to assay ETEC strains (14), did not result in detectable levels of brush border adhesion. Brush border adhesion of EPEC was observed, however, when dilute suspensions of bacteria (10^6 to 10^7/ml) were used and the incubation time was increased to 2 h or longer. These are essentially the conditions used to assay EPEC adhesion to cultured HeLa and HEp-2 cells (7). Assays carried out in the absence of D-mannose resulted in EPEC adhesion to both brush border and basolateral cell surfaces (Fig. 1A). We have previously shown (18) that type 1 fimbriae, which are produced by both E2348 and E851, promote attachment of bacteria to enterocyte basolateral but not brush border surfaces (13). Thus, to prevent type 1 fimbrially mediated adhesion of bacteria to basolateral surfaces, which can interfere with the assessment of brush border adhesion, assays were routinely performed in the presence of 0.5% D-mannose.

In the presence of mannose, adhesion of E2348 and E851 was specifically localized to the enterocyte brush border (Fig. 1B and C). Bacteria were observed to adhere to each other as well as to brush borders (Fig. 1B and C), thus making a quantitative assessment of adhesion by counting the total number of adherent bacteria impossible. Adhesion therefore was quantitated by determining the percentage of cells with adherent bacteria. Following 1-, 2-, and 3-h incubation times, the percentage of cells with adherent E2348 bacteria was approximately 0, 30, and 50%, respectively. We standardized our assay by using a 3-h incubation period. Under these assay conditions and with cells from three different donors, a mean of 52.5 and 55% of the brush borders had adherent E851 and E2348 bacteria, respectively, whereas the value for a plasmid-cured derivative of E2348 and the nonpathogenic control strain HB101 was 0% (Table 2).

Adhesion to cultured intestinal mucosa. Although the isolated enterocytes provided a relatively quick and simple

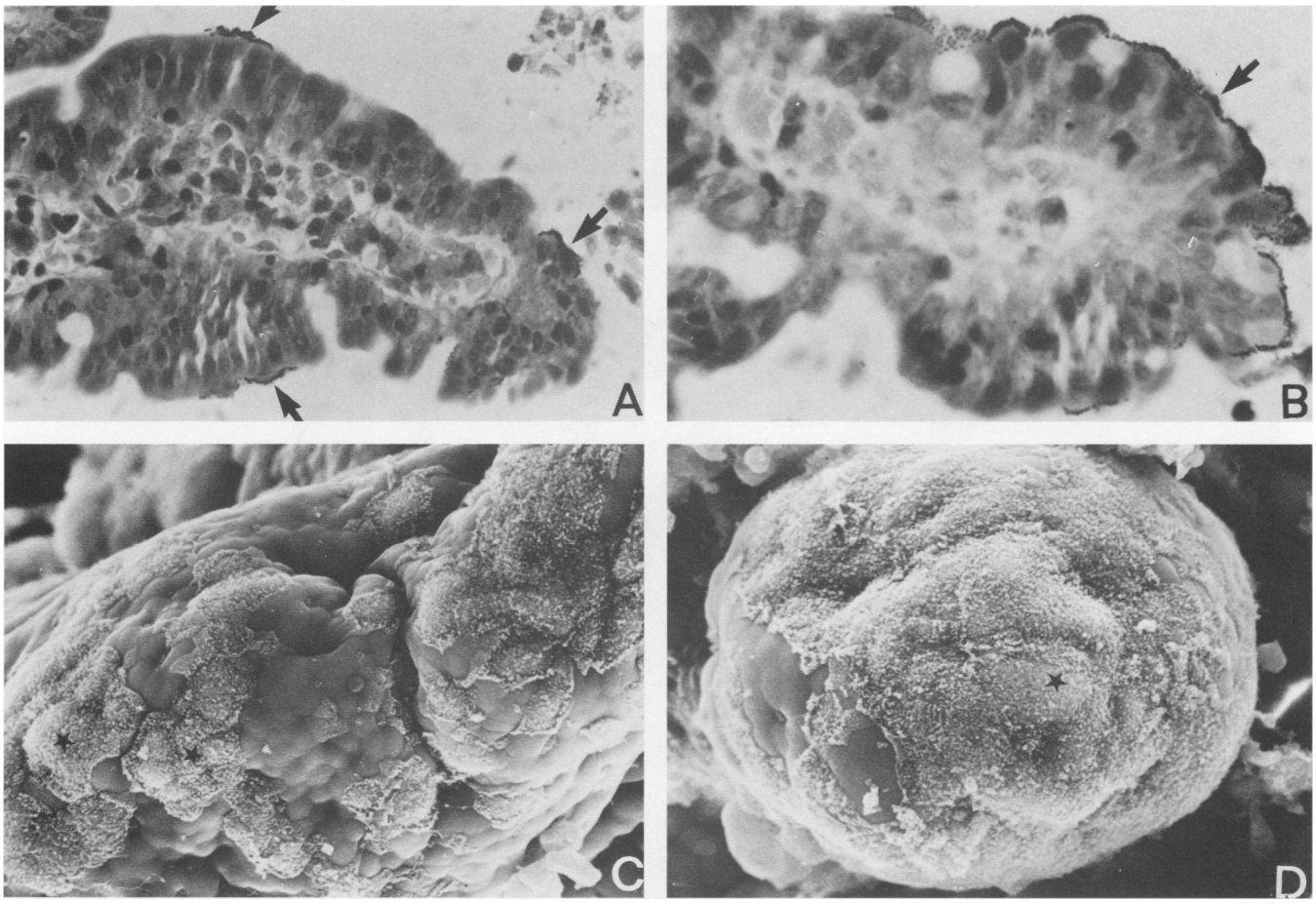

FIG. 2. Histologic (A and B) and scanning electron micrographic (C and D) examination of human duodenal mucosal biopsy samples infected with EPEC strain E2348 for 3 (A), 6 (B), 9 (C), and 12 (D) h. Small colonies of adherent bacteria seen at 3 h (arrows, panel A) increased in size with time (arrows, panel B; stars, panel C), and after 12 h virtually the entire mucosa was colonized by bacteria (stars, panel D). Magnification: A, ×320; B, ×480; C, ×650; D, ×525.

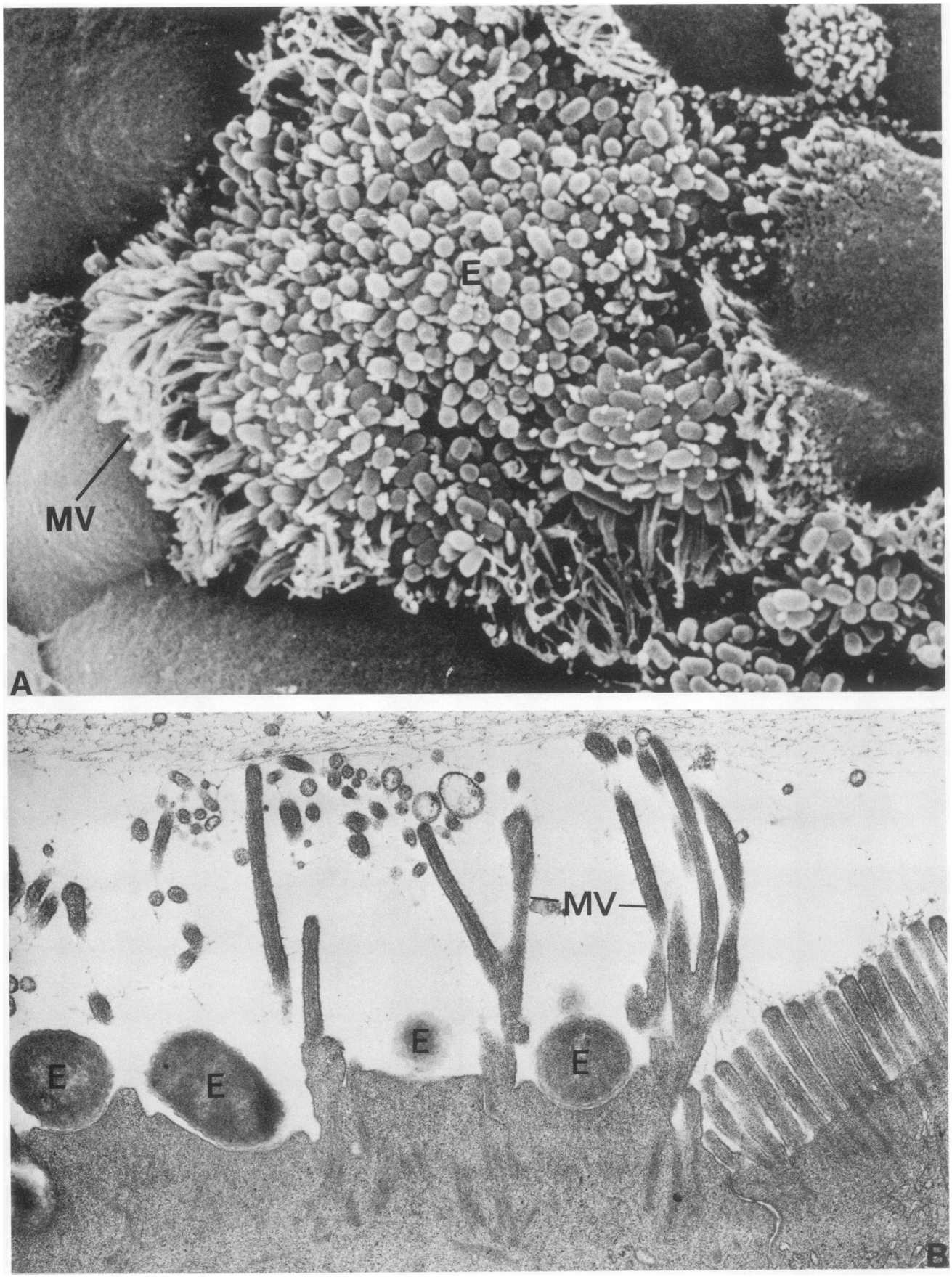

FIG. 3. Scanning (A) and transmission electron (B) micrographs of duodenal biopsy samples infected with E2348 for 6 h. Micovilli (MV) were destroyed at sites of EPEC (E) attachment, but the remaining microvilli, particularly at the edge of a microcolony, were grossly elongated. Note that the mucosal surface of affected enterocytes is raised above the level of adjacent unaffected cells which appear normal. Magnification: A, ×5,000; B, ×30,000.

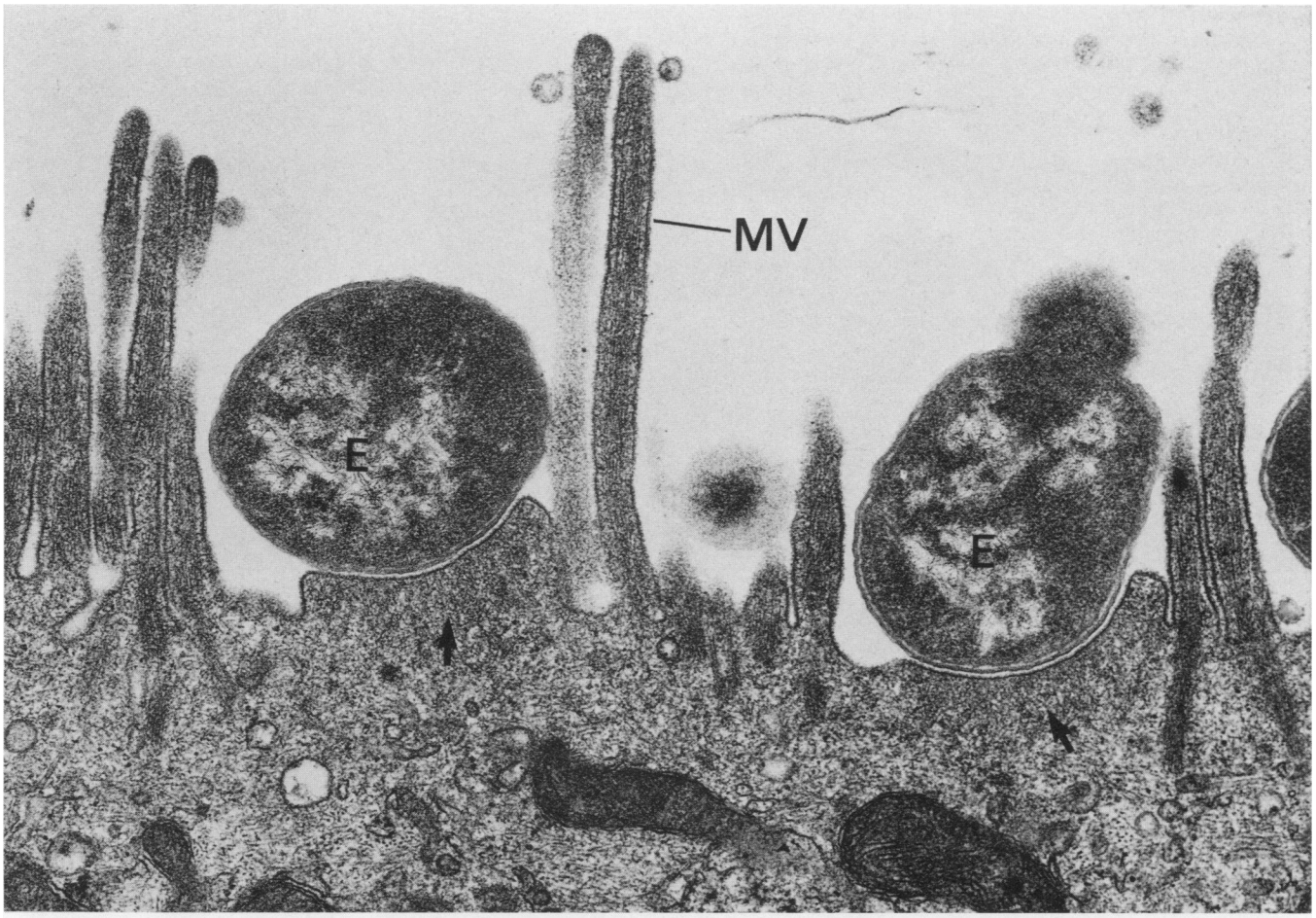

FIG. 4. Electron micrograph of cultured human intestinal mucosa infected with EPEC strain E2348 for 8 h. Bacteria are intimately attached to cuplike projections of the apical enterocyte membrane, with only ~10 nm separating bacterial and enterocyte membranes. At regions of attachment, brush border microvilli (MV) were destroyed and the associated cytoskeletal elements were disrupted. Concentrations of short filaments are present in the apical cytoplasm beneath attached bacteria (arrows). Magnification: ×45,000.

assay for EPEC adhesion, they were found not to be suitable for ultrastructural studies. We therefore investigated the possibility of using cultured human intestinal mucosa as a model of EPEC adhesion. Duodenal biopsy samples maintained in culture for up to 12 h routinely showed good morphological preservation of the mucosa both by light and electron microscopy. To test the ability of EPEC to adhere to cultured mucosa, biopsy samples were cultured with EPEC strain E2348 for 3, 6, 9, and 12 h. Histological and scanning electron microscopic examination of the tissue revealed, after 3 h, small microcolonies of attached bacteria (Fig. 2A); the microcolonies subsequently increased in size with time (Fig. 2B and C), and after 12 h in culture a large percentage of the mucosal surface was colonized by bacteria (Fig. 2D). When colonized mucosa was examined at higher magnification by scanning electron microscopy (Fig. 3B) or by transmission electron microscopy (Fig. 4), the characteristic histopathological lesion reported by others in infants and animals was observed.

Transmission electron microscopy of tissue sections revealed bacteria that were intimately attached to and partially surrounded by cuplike projections of the apical enterocyte membrane, with only 10 nm separating bacterial and enterocyte membranes. In regions of attachment the glycocalyx was absent and brush border microvilli had been destroyed. Instead of an organized brush border cytoskeleton, concentrations of short filaments with a diameter similar to those of microvillous core filaments were seen beneath attached bacteria (Fig. 4). In scanning electron micrographs bacteria were seen to be intimately attached to the mucosal surface, with the few remaining uneffaced microvilli visible between closely adjacent bacteria (Fig. 3B).

Other striking features of EPEC-colonized mucosa were the dramatic elongation of some microvilli (Fig. 3) and vesiculation of the microvillous membrane (Fig. 5). Microvillous elongation was particularly noticeable around the edge of bacterial colonies in scanning electron micrographs (Fig. 4B), suggesting that microvillous elongation precedes microvillous destruction as bacteria divide and colonies increase in size; the few remaining microvilli seen between closely adjacent bacteria within a colony were also elongated. In affected cells microvilli up to 3 μm in length were common, whereas the typical length of human duodenal brush border microvilli was <1 μm. EPEC-induced destruction of brush border microvilli appeared to occur by a process of membrane vesiculation (Fig. 5). Membrane vesicles with a membrane profile identical to that of the microvillous membrane but lacking any internal organized cytoskeletal elements (Fig. 5C) were specifically localized to sites of EPEC attachment and brush border destruction (Fig. 5B).

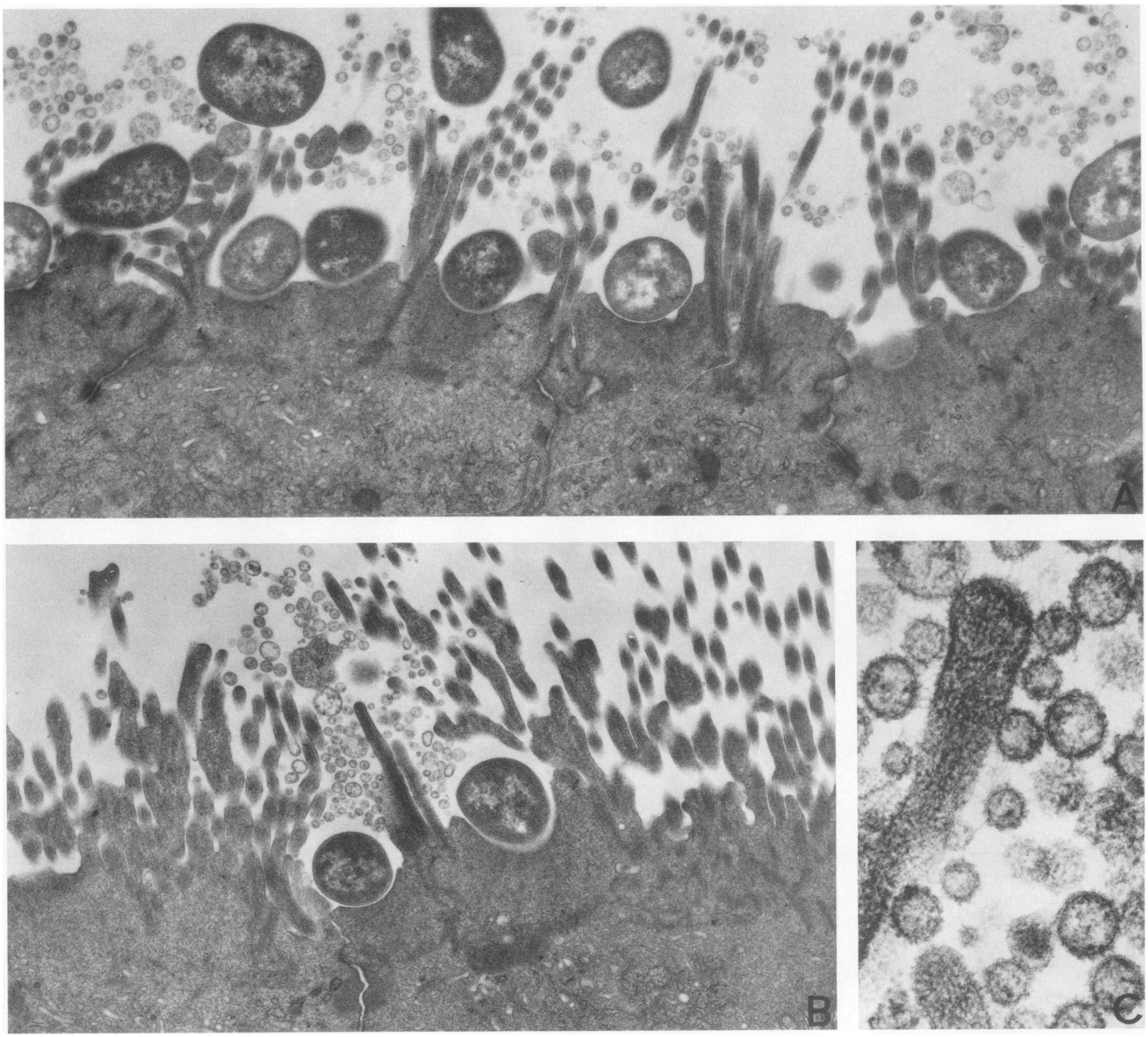

FIG. 5. Electron micrographs of EPEC-colonized mucosa illustrating EPEC-induced vesiculation of brush border microvilli. Vesicles with a membrane profile identical to that of the microvillous membrane (C) were frequently seen at sites of EPEC attachment and microvillous destruction (A). Vesiculation was specifically localized to sites of EPEC attachment (B). Magnification: A, ×18,000; B, ×17,000; C, ×180,000.

Intimate attachment of bacteria to cuplike projections of the apical enterocyte membrane devoid of microvilli represents the final stage of EPEC adhesion. We carefully examined tissue that had been infected for 3 h to see if we could detect earlier stages of the attachment process. In a few instances bacteria were seen at stages prior to intimate attachment. The earliest stage was attachment of bacteria to an apparently intact brush border (Fig. 6A). This appeared to be followed by elongation and vesiculation of brush border microvilli (Fig. 6B). The distinct electron-translucent zone between bacteria and brush border membranes seen at this stage suggests that elongation and vesiculation of microvilli precedes intimate attachment and that, at this stage, attachment is promoted by adhesins that extend from the bacterial surface but that are not visualized in this type of preparation.

EPEC-induced brush border damage was only observed in epithelial cells with adherent bacteria; other enterocytes, including those immediately adjacent to affected cells, appeared to be normal (Fig. 3). The cuplike projections of apical cytoplasm on which the bacteria appeared to sit were invariably raised above the level of the surrounding unaffected mucosa. This was particularly noticeable in scanning electron micrographs (Fig. 3B) and in sectioned material when the mucosa was sectioned through the edge of a bacterial colony (Fig. 3A). No specific cellular damage other than brush border damage was apparent in affected cells, and in none of our preparations did we observe epithelial cell invasion by EPEC.

The characteristic EPEC lesion was readily detected by scanning electron microscopy (Fig. 3B). This technique, rather than the more time-consuming transmission electron microscopy of tissue sections, was therefore used to exam-

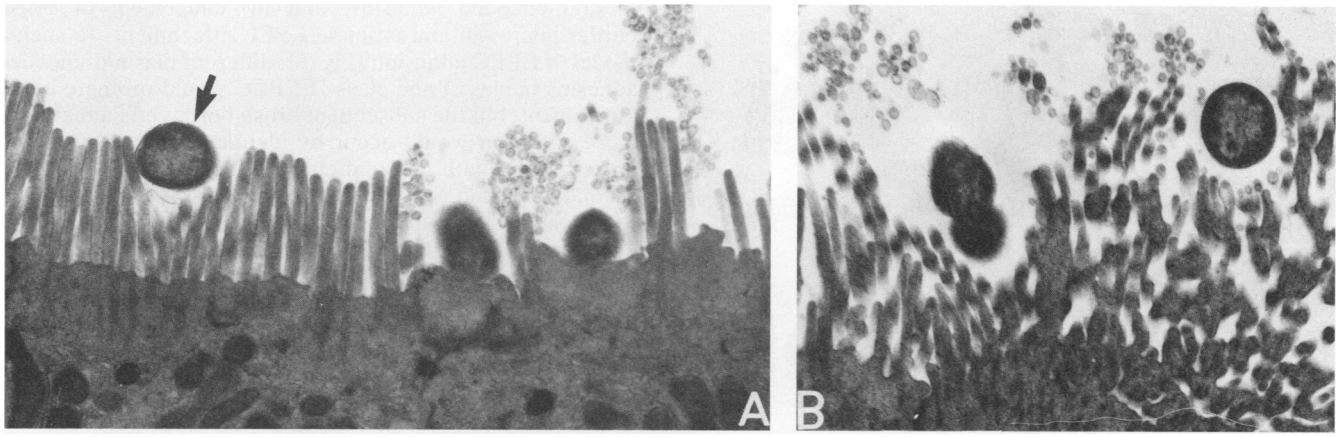

FIG. 6. Electron micrographs of cultured mucosa infected with EPEC for 3 h showing initial attachment of bacteria to an intact brush border (arrow panel A) and brush border vesiculation at a stage prior to intimate EPEC adhesion (B). Note the distinct electron-translucent zone separating bacterial and brush border membranes. Magnification: ×12,000.

ine a collection of human EPEC strains for their ability to colonize cultured human intestinal mucosa. Of 17 strains examined, 13 strains from serogroups O55, O86, O111, O114, O119, O127, O128, and O142 extensively colonized cultured duodenal mucosa and produced the characteristic histopathological lesion; 3 strains, from serogroups O26, O86, and O128, did not adhere (Table 1). A plasmid-cured derivative of strain E2348, which was nonadherent in the isolated enterocyte assay (Table 2), did exhibit some adherence to cultured mucosa after 9 to 12 h (Table 1), although only a few small microcolonies of bacteria were observed (Fig. 7A) compared with the extensive mucosal colonization of the parent strain (cf. Fig. 7A and 4D). The mechanism of adhesion of both strains was identical, however, and the characteristic lesion was observed when mucosa infected with plasmid-cured E2348 was examined by high-resolution scanning and transmission electron microscopy (Fig. 7B).

DISCUSSION

The adhesion of bacteria to host intestinal mucosal surfaces is now recognized as an important event in the pathogenesis of diarrheal disease. The ability of ETEC to colonize the gut and cause disease has been directly correlated with the presence of adhesion fimbriae which allow ETEC to bind to specific receptors on enterocytes of the proximal small intestine (10). To study ETEC adhesion we developed an in vitro assay in which human small intestinal enterocytes were used (13, 14). In this study we showed that the same assay system, with minor modifications, can be used to quantitatively assess EPEC adhesion. Adult human enterocytes offer a better and more routinely available source of intestinal cells than fetal tissue, which previously has been used to assess EPEC adhesion (20). In experimental infections of animals, intestinal colonization by EPEC was found to be most pronounced in the distal ileum and colon (21, 31). Specific brush border receptors for EPEC are clearly present on human duodenal enterocytes; it remains to be seen if cells from other regions of the intestine (e.g., the colon) will prove to be more appropriate for an EPEC adhesion assay.

EPEC achieves mucosal colonization by a mechanism that is strikingly different from that of ETEC. EPEC, unlike ETEC, actually damages the mucosa, and this gives rise to the characteristic intestinal histopathological lesion seen in electron micrographs. The ultrastructural features of muco-

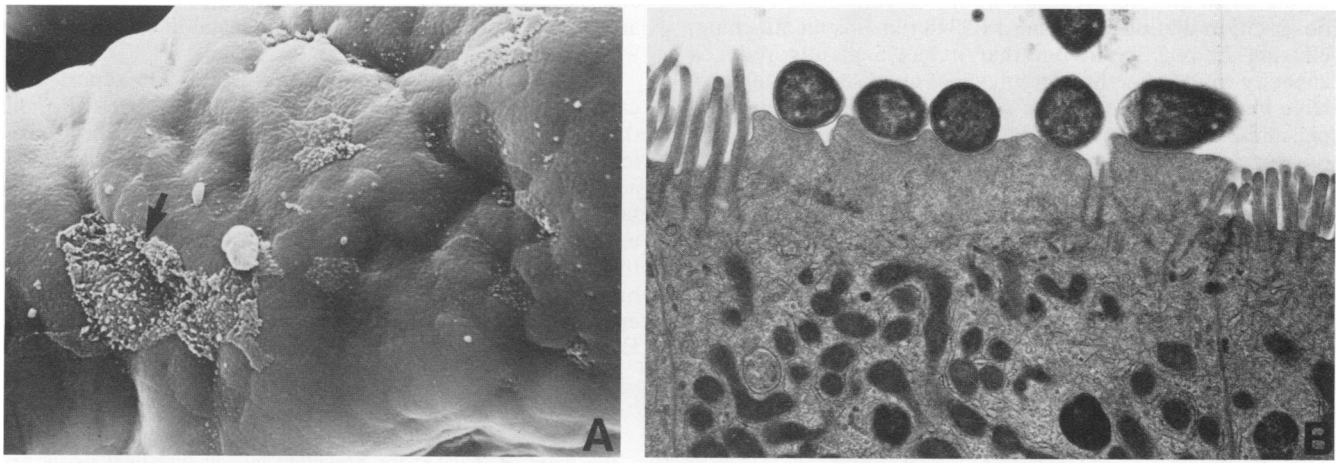

FIG. 7. Scanning (A) and transmission electron (B) micrographs of human duodenal mucosal biopsy samples infected with plasmid-cured E2348. Only a few small isolated colonies of adherent bacteria were seen after 12 h of culture (A). By transmission electron microscopy bacteria were seen to be intimately attached to cuplike projections of the apical enterocyte membrane and to cause localized destruction of microvilli (MV). Magnification: A, ×850; B, ×12,000.

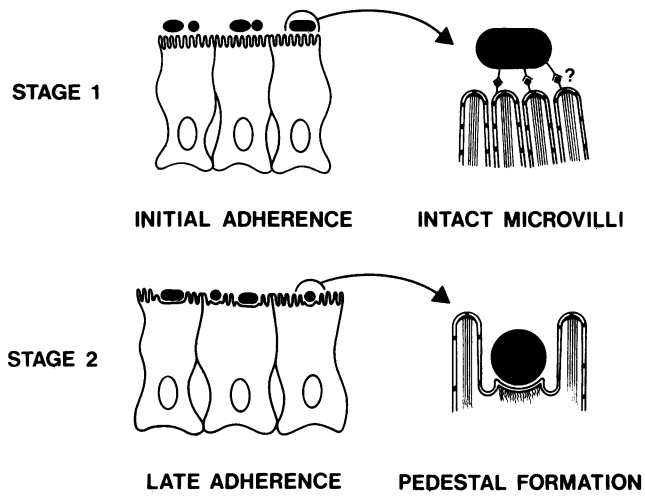

FIG. 8. Representation of the proposed two-stage model of EPEC adhesion.

sal colonization by EPEC seen in this in vitro study closely parallel those previously described for natural EPEC infections in infants (27, 29, 32) and experimental infections in animals (21, 26, 31) and indicate that cultured human intestinal mucosa is a useful model of intestinal colonization by EPEC. The characteristic intestinal lesion was demonstrated with 13 of 17 strains examined, including EPEC from serogroups O55, O86, O111, O114, O119, O127, O128, and O142, thus providing further evidence that most, if not all, EPEC isolates are attaching, effacing *E. coli*.

E. coli virulence determinants, including colonization factors, have been found in many cases to be encoded on plasmids (9), and plasmid-encoded factors have been implicated in intestinal colonization by EPEC (1, 33). The presence of a 60-megadalton plasmid, pMAR2 of EPEC strain E2348, has been shown to correlate with the ability of this strain to cause diarrhea in healthy adults (17). The fact that two of nine volunteers who ingested plasmid-cured E2348 developed mild diarrhea, however, suggests that intestinal colonization may have taken place in these individuals but to a lesser extent than that which occurred with the parent strain. Such an interpretation would be consistent with our observation that plasmid-cured E2348 remains an attaching, effacing *E. coli* strain but that it has a greatly reduced capacity to colonize human intestinal mucosa. Although we have been able to detect the attaching, effacing property of plasmid-cured E2348 in our human in vitro model, the same property was not detected in experimental infections of piglets (16).

A close correlation exists in ETEC between the presence of specific colonization factors and certain O serogroups (30); a similar pattern is now emerging in the case of EPEC. Plasmid genes coding for an adhesin designated EPEC attachment factor have been identified in EPEC serogroups O55, O111, O119, O127, and O142 (class I EPEC), whereas a different adhesin is expressed in the less common serogroups O44, O86, and O114 (class II EPEC) (23). These plasmid-encoded adhesins are important for pathogenicity (17), and yet results of our studies with plasmid-cured E2348 indicate that other nonplasmid factors confer the microvillous-effacing capability of EPEC. These observations can be reconciled if mucosal adhesion by EPEC involves two distinct stages: (i) initial attachment of bacteria promoted by plasmid-encoded adhesins, and (ii) effacement of brush border microvilli and intimate EPEC attachment. In such a model of EPEC adhesion (Fig. 8), different plasmid-encoded adhesins in class I and class II EPEC would promote initial attachment, but the subsequent brush border effacement and close adhesion would occur by identical mechanisms. Our observations with plasmid-cured E2348 indicate that the second stage can occur in the absence of the first stage, but the presence of plasmid-encoded adhesins appears to greatly enhance the ability of EPEC to colonize the mucosa.

A two-stage adhesion mechanism is also supported by ultrastructural observations. Initial attachment to an intact brush border appeared to be of a nonintimate type, and this was promoted by adhesins, possibly fimbrial, which extend from the bacterial surface. Specific fimbriae have been shown to confer on the rabbit EPEC strain RDEC-1 the ability to adhere to rabbit intestinal brush borders (6), and fimbriae that could promote attachment of human EPEC were identified by Rothbaum and co-workers (27) in EPEC serotype O119. In an accompanying paper (12) we present preliminary evidence that the plasmid-encoded adhesin of strain E2348, which promotes initial attachment to cultured cells, is fimbrial in nature. In contrast, the intimate nature of the interaction between EPEC and the mucosal surface at late stages of attachment indicates adherence promoted by nonfimbrial adhesins.

The sequence of events between initial nonintimate attachment and late intimate attachment of EPEC appears to involve a dramatic elongation of brush border microvilli followed by their destruction by a process of membrane vesiculation. Calcium-induced effects on the microvillous structure are a close counterpart of the changes described in this study and previously in *E. coli* RDEC-1 infections in rabbits (5, 28) and suggest one possible mechanism whereby EPEC might cause intestinal epithelial cells to shed their microvilli. At calcium concentrations below 10^{-6} M, the normal situation in the cytosol, the microvillous core cytoskeleton, is a stable rigid structure. Above this threshold the actin filaments of the core are broken into short filaments by the action of the calcium-sensitive microvillous actin-severing protein villin (19, 22). This leaves a thermodynamically unstable microvillous membrane that fragments into vesicles (4). The residual enterocyte plasma membrane, which lacks an organized cytoskeleton, would be deformable and would allow EPEC to bind strongly over a large surface area to produce the characteristic pedestal-like structures on which bacteria appear to sit.

A number of agents other than EPEC are reported to cause intestinal epithelial cells to shed their microvilli, and this has led to the suggestion that it may represent a basic level of protection against pathological and other surface-active agents (3). If this is true, it is a level of protection that fails in the case of EPEC, although just how these enteropathogens prevent themselves from being shed into the intestinal lumen with the vesiculated microvillous membrane is not clear. This could be one function of plasmid-encoded adhesins that promote the initial attachment of EPEC (12).

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