TYPE I *ESCHERICHIA COLI* PILI: CHARACTERIZATION
OF BINDING TO MONKEY KIDNEY CELLS*

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The ability of prokaryotic cells to interact with the surface of eukaryotic membranes in a specific manner is likely the initial event in most infections. This interaction probably involves adherence of microorganisms via specific receptors but the mechanism of such binding remains poorly defined.

The adherence of *Streptococcus mutans* is dependent on glucans synthesized by its glucosyltransferases (1) and lipoteichoic acids may be important in binding of gram-positive bacteria (2), however, for most gram-negative bacteria, attention has been directed to surface appendages as mediators of attachment. Duguid et al. (3–5) have demonstrated adherence of pilated enteric bacteria to erythrocytes, leukocytes, and epithelial cells and found this attachment to be sensitive to inhibition by mannose. Other carbohydrates were later found to inhibit hemaglutination by *Shigella flexneri* and *Salmonella typhimurium* (6). Although it has been demonstrated that pilated organisms can have adhesive properties sensitive to inhibition by saccharides, it was not shown that isolated pili are responsible for the binding. Purification of type I pili now makes it possible to test the binding to tissue culture cells of a purified bacterial protein long suspected to be important in bacterial adherence. Such studies can also lead to the determination of whether bacterial binding occurs to specific mammalian binding sites. The analysis of such binding sites is of major importance in understanding individual susceptibility to infections, localization of infections and the sites of colonization by bacterial flora. In the following studies, we examine the interaction of *Escherichia coli* K12 and pili isolated from this organism with monolayers of African green monkey kidney (Vero). Binding of whole pilated bacteria and of purified pili to the Vero cells occurs rapidly and is inhibited or reversed by low concentrations of D-mannose. Concanavalin A, and *Lens culinaris* lectin, when bound to the Vero cell, interfere with bacterial attachment. It is probable then that these bacteria adhere to Vero cells via pili which bind to specific mannose-containing receptors on the cell surface.

Materials and Methods

Preparation of Bacteria. A strain of *E. coli* K12 (kindly supplied by Dr. C. C. Brinton, Jr., University of Pittsburgh, Pittsburgh, Pa., was used which does not contain sex pili or flagellae.

As previously described (7) two colonial variants were isolated: P+[1] contained only pilated organisms and P− only nonpilated organisms.

Care was taken to select colonies of the appropriate colonial morphology until agar plates contained 99% P− or P+. For bacterial attachment studies, a single colony was selected, spread on nutrient agar plates, and grown for 16–18 h at 41°C to ensure stability of colonial types. Only after colonial appearance, gram staining and slide hemagglutination [using guinea pig cells as previously described (7)] confirmed the identity as P+, were the organisms used for subsequent studies.

Radiolabeling. Bacteria were grown in 100 ml minimal glucose medium (7) at 41°C on a gyratory shaker until early log phase at which time 100 μCi of a tritiated L-amino acid mixture (^{3}H-aa) (New England Nuclear, Boston, Mass.) was added. Growth was allowed to continue until mid log phase (approximately 3.5×10^8 colony-forming units [cfu]/ml) and bacteria were collected by centrifugation. They were washed four times in modified Earles basic salt solution without glucose (modified Earles) or until supernatant counts equalled background counts. Bacteria were suspended in modified Earles and the concentration adjusted to 2.8×10^8 cfu/ml (OD 0.2 at 540 nm wavelength with tube diameter of 15 mm). Before incubation with tissue cells, a 20- to 30-min period of preincubation at 37°C was required for reacquisition of pili lost in centrifugation.

Pili, purified from *E. coli* as previously described (7) were labeled with ^{125}I in the presence of 50 μg of chloramine-T for 30 s (8). Sp act was usually 2×10^4 cpm/μg of protein. All preparations used gave a single protein band as determined by Coomasie Blue staining of polyacrylamide gels using 40 μg of protein. A sharp peak of radioactivity was also found at mol wt of 17,500 when samples were run on 10% polyacrylamide gels (9): radiolabeled samples were mixed with 10 μl of bromphenol blue in 40% sucrose, 10 μl of 2% SDS, 2% 2-mercaptoethanol, and 10 μl each of bovine serum albumin, ovalbumin, carbonic anhydrase, and myoglobin which had been previously dansylated according to the method of Inouye (10). This mixture was applied to the gel and, after electrophoresis, migration of the dansylated protein markers was measured under ultraviolet illumination. The gel was sliced and counted in a gamma scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) (Fig. 1).

Incubation with Tissue Culture Cells. Because of the known propensity of *E. coli* to infect kidney tissue in vivo, a standard kidney cell line (Vero) derived from the African green monkey was used to test bacterial and pili binding. Vero cells were grown as a monolayer in Dulbecco's medium with 10% heat-inactivated fetal calf serum, penicillin, streptomycin, and amphotericin in 75-cm² tissue culture flasks. When confluent they were incubated with 0.1% trypsin 0.6 mM in EDTA, washed in Dulbecco's medium, and distributed in 35 × 10 mm plastic Petri dishes in which they were again incubated at 37°C in air containing 5% CO_2 until about 80% confluent. The dishes then contained 5.1×10^5 ($\pm 0.3 \times 10^5$) cells. Monolayers were washed with three volumes of antibiotic-free modified Earles before adherence studies, and a sample of cells from each experiment was tested for viability by determining exclusion of trypan blue.

Unlabeled bacteria were removed from agar plates by sterile swabs, suspended in modified Earles and the optical density adjusted to 0.2. Bacteria radiolabeled as described above were adjusted to the same concentration. ^{125}I-labeled pili were suspended in modified Earles to a concentration of approximately 10 μg/ml. 2 ml of each of these solutions was placed in Petri dishes of washed Vero cells and incubated on a gyratory shaker at 50 rpm at 4°C, 24°C, or 37°C. After the appropriate time interval, the monolayer was washed with at least 10 volumes of 0.01 M sodium phosphate 0.15 M NaCl (PBS) to remove nonadherent pili or bacteria.

For direct visual examination, the monolayer was fixed in 2% glutaraldehyde at 4°C overnight and stained in 1% Giemsa solution for 3–4 h. The monolayers were again washed, covered with 22 x 22 mm glass cover slips and viewed at 900 magnification using a Zeiss photomicroscope (Carl Zeiss Inc., New York). The number of bacteria adherent to 25 tissue culture cells was then counted. Each experiment was repeated at least three times. Counts were done without prior knowledge of experimental conditions and were expressed as average number of adherent microorganisms per tissue cell. The results of experiments were expressed as a percent change from the control for that experiment.

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[1] *Abbreviations used in this paper*: AMM, α-CH_3-D-mannopyranoside; cfu, colony-forming units, Con A, concanavalin A; ^{3}H-aa, tritiated L-amino acid mixture; P+, pilated bacteria; P−, nonpilated bacteria; PBS, 0.01 M sodium phosphate 0.15 M NaCl pH 7.4; PHA, phytohemagglutinin.
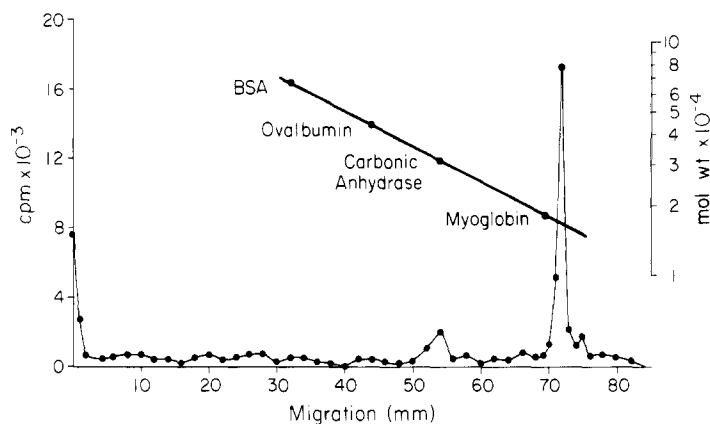
FIG. 1. Radiolabeled pili were boiled for 2 min in 2% sodium dodecyl sulfate and 2% 2-mercaptoethanol and applied to a 10% polyacrylamide gel using the method of Laemmli (9).

To determine the amount of adherent ^{125}I-labeled pili, the washed monolayer was removed by digestion with 1.0 ml of 0.1% trypsin and the entire digest was counted for 1 min in a gamma scintillation spectrometer. The adherence of ^{3}H-aa-labeled bacteria was also determined by trypsinizing the washed monolayer. This digest was then mixed with 12 ml of Aquasol (New England Nuclear, Boston, Mass.) and counted in a liquid scintillation counter (Packard Instrument Co., Inc.). The results of experiments were expressed as a percent change from the control for that experiment. Furthermore, since loss of cells from the monolayer could artificially depress binding using radiolabeled ligands, experiments were always done to enumerate the tissue culture cells remaining as a monolayer after incubations.

Lectins. Concanavalin A (Con A) (three times crystallized) (Miles Laboratories Inc., Miles Research Products, Elkhart, Ind.) and phytohemagglutinin (PHA) (The Wellcome Research Laboratories, Beckenham, England) were used without further purification. Succinylated Con A was made as previously described (11). *Lens culinaris* lectin was prepared from lentil beans by the method of Howard et al. (12) and concentrations determined using $E_{280}^{1\%}$ of 13 and 12.6 for Con A (13) and *Lens culinaris* (12), respectively.

In order to determine that the lectins bound to the tissue cells they were labeled by coupling to horseradish peroxidase and stained with diaminobenzidine in the presence of H_2O_2 (14). The role of lectins bound to Vero cells in inhibiting bacterial binding was determined as follows: Vero cell monolayers were washed and incubated on a gyratory shaker at 50 rpm for 30 min at 37°C with variable concentrations of the lectins in modified Earles. The monolayer was again washed with modified Earles to remove unbound lectins and 2.8 × 10^{8} cfu P+ bacteria labeled with ^{3}H-aa in the same buffer were then similarly incubated for 60 min with the monolayer which had the adherent lectins. The monolayer was again washed, digested, and counted. At each lectin concentration the number of attached bacteria was expressed as a percent of the control and a bacterial-binding curve was determined. The concentration of lectins required to reduce bacterial binding to 50% of the control was measured from these binding curves.

Hapten Inhibition. In order to determine the effect of a variety of saccharides (7) on the binding of ^{3}H-aa-labeled organisms, bacteria prepared as noted above were suspended in Earles to which varying concentrations of these saccharides were added. The final concentration of bacteria was 2.8 × 10^{8} cfu/ml and incubations were done at 37°C for 30 min.

Enzyme Treatment of Tissue Culture. β-Galactosidase (EC 3.3.1.23), β-glucosidase (EC 3.2.1.21), and trypsin (EC 3.4.4.4) (Worthington Biochemical Corp., Freehold, N. J.); pancreatic protease and α-mannosidase (EC 3.2.1.24) Sigma Chemical Co., St. Louis, Mo.); and α-galactosidase (EC 3.2.1.22) (Boehringer Mannheim Corp., New York) were dissolved in modified Earles basic salt solution (pH 7.6) and incubated at 37°C for 30 min with the monolayer. The supernate from this digestion was centrifuged at 30,000 g for 20 min and the amount of released carbohydrate measured by the phenol sulfuric acid method (15). Neuraminidase (EC 3.2.1.18; Worthington Biochemical Corp.) digestion was carried out at pH 6.8 for 30 min in 0.1 M acetate buffer 0.125 M in

NaCl. To determine the necessity for active Vero cell metabolism in adherence, the metabolic inhibitors sodium malonate, 2,4-dinitrophenol grade II (Sigma Chemical Co.), and sodium azide (Matheson, Coleman and Bell, Norwood, Ohio) were dissolved in Earles and incubated with tissue cultures for 60 min. Test incubations were then done for 30–60 min at 37°C using purified pili or organisms diluted in the above solutions.

Vaccination. For the production of anti-pili antibodies, New Zealand red rabbits were injected intravenously with increasing doses of pili (25–300 μg) in PBS every 2–3 days for 10 days, at which time they were bled. Booster doses were given 3–4 days before further bleedings; a similar schedule was used for vaccination with formalin-killed P+ and P− organisms. Sera were heat inactivated (56°C for 30 min), sterile filtered, and kept at 4°C.

Electron Microscopy. Carbon-strengthened, Formvar-coated copper grids of no. 400 mesh were used. For replica production pilated cells or isolated pili were incubated in Earles solution with tissue culture cells grown to confluency on 22 x 22 mm cover slips. These specimens were then vigorously washed in Earles, and dehydrated in graded ethanol and acetone solutions. They were then subjected to critical point drying with CO_2 in a Sorvall CPAS no. 49300 apparatus (Dupont Instruments, Sorvall Operations, Newtown, Conn.). Specimens were stabilized with carbon, platinum shadowed, and floated off cover slips with hydrofluoric acid. Replicas were immersed in Clorox for 30 min to dissolve adherent tissue, washed in distilled water, and placed on copper grids. Transmission electron microscopy was done with a Siemens 101 microscope (Siemens Corp., Medical/Industrial Groups, Iselin, N. J.) at 60 kV.

Results

Experimental Binding System. After incubation of P+ bacteria with Vero cells there was a striking and highly reproducible association of bacteria with the monolayer. For most studies 2.8×10^8 cfu/ml (a total of 5.6×10^8 cfu) were incubated for 60 min at 37°C on a gyratory shaker with a monolayer containing 5 $\times 10^5$ tissue culture cells. By microscopy this results in the adherence of 14.2 (± 4.6) microorganisms per cell (Fig. 2). Nonpilated *E. coli* bind 100-fold less avidly (0.17 ± 0.13 bacteria per Vero cell). During the brief periods of incubations used in our studies there was no significant transformation of P+ to P− colonial types and the total number of viable bacteria (cell associated plus non cell associated) was the same at 60 min (6.6×10^8 cfu/ml) indicating no growth advantage for either variant. Results of the same magnitude were observed using bacteria intrinsically labeled with ³H-aa or by determining numbers of viable organisms associated with the monolayer.

The time-course of cell association using radiolabeled bacteria indicated that 50% of maximal binding occurred at 17 min and 96% by 60 min (Fig. 3a). Attachment of P− increased over time but was still over 100-fold less than P+ at 100 min.

Pili purified from these bacteria showed marked adherence to the tissue culture. The time-course of this association using 10 μg of pili per Petri dish is shown in Fig. 3b. Pili binding continued at virtually the same rate up to 60 min and approximately 50% maximal binding was seen at 40 min. For all further studies only pilated organisms or purified pili were used and test incubations were done for 60 min.

Effect of pH. ¹²⁵I-labeled pili were suspended to a concentration of 20 μg/ml in PBS ranging in pH from 2 to 10 with the ionic strengths kept constant. Maximum pili adherence occurred at pH 4–5; however, even at the extremes of pH where attachment was least, there was 25% maximal attachment (Fig. 4). These results could not be accounted for by differential loss of tissue cells at the highest and lowest hydrogen ion concentrations.
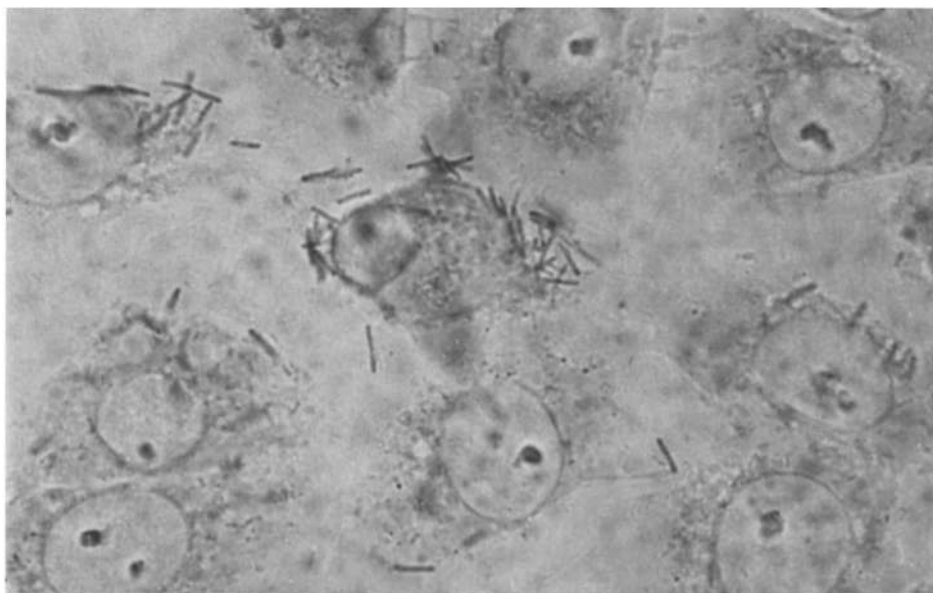
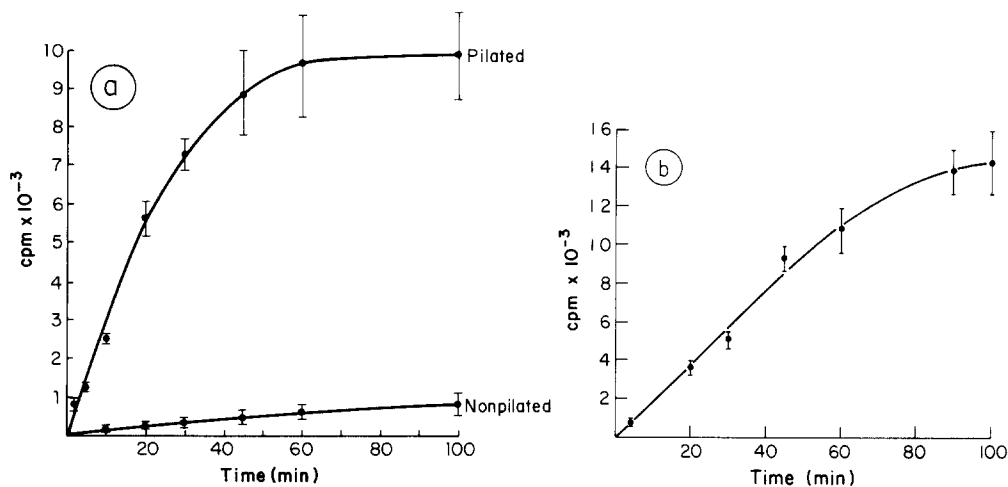FIG. 2.   *E. coli* attached to Vero cells after 60 min at 37°C Giemsa stained. (× 1,800).

FIG. 3.   The ordinates represent bacteria labeled with tritiated amino acids (a) and pili labeled with [125]I (b), which remained associated with washed Vero cells. Incubation was at 37°C. Each point is the mean of 5 determinations ± 1 SD.

In some experiments PBS was used at pH 6, 7, 8, 9, and 10 and acetate-buffered saline (0.01 M sodium acetate, 0.15 M NaCl) was used at pH 2, 3, 4, and 5. Results were identical to those shown in Fig. 4. Similar binding curves at 4°C and 24°C also demonstrated best attachment at pH 4–5. At these temperatures however, the differences in binding over the range of pH tested was less striking.

Effect of Temperature.   To determine the role of membrane fluidity in pili binding, adherence of ^{125}I-labeled pili was measured at two temperatures. After
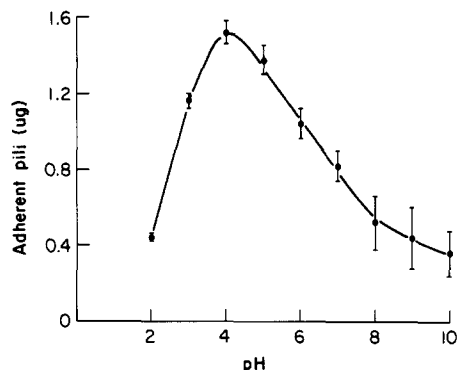
FIG. 4. ^{125}I-labeled pili were incubated with Vero monolayer at various pH's for 60 min. Incubation was at 37°C. In these experiments, only phosphate buffer was used. Each point is the mean of 6 determinations ± 1 SD.

60 min incubation, the amount of bound pili as determined from the specific activity $(1.9 \times 10^4$ cpm/μg) was nearly identical at 4°C (820 ± 42 ng) and 37°C (781 ± 114 ng, means of 8 determinations ± 1 SD, $P = >0.5$).

Hapten Inhibition. Mannose is a potent inhibitor of pili hemagglutination (7) but the role of saccharides in inhibiting *E. coli* adherence to tissue monolayers is unknown. To determine the most potent saccharide inhibitor of bacterial binding, suspensions of ^{3}H-aa-labeled *E. coli* were made in modified Earles containing serial twofold dilutions of the saccharide to be tested. None of the compounds used was toxic to either the Vero cells, as determined by trypan blue exclusion or to the bacteria as determined by growth curves. Maximal inhibition of binding occurs with D-mannose, α-CH$_3$-D-mannopyranoside (AMM), and yeast mannan but L-mannose was ineffective at 100 mM (Table I).

The elution of bound bacteria was attempted to determine the reversibility of the adherence. Binding could be reversed by the addition of those saccharides which were the most potent inhibitors: for example, the addition of 30 mM AMM (a concentration causing 99% inhibition of adherence) for 30 min released from the Vero cells 98% of the bacteria associated at 4°C (to minimize bacterial ingestion). A correlation existed with the results of inhibition experiments (Table I) in that noninhibitory saccharides failed to reverse binding at concentrations of 100 mM.

Lectins. Because bacterial attachment was inhibited by saccharides, the appropriate plant lectins when bound to the Vero cell might block bacterial binding through competition for receptors. The lectins which were preincubated with Vero cells for 30 min at 37°C were bound to the cell surface as shown by diaminobenzidine staining. The amount of ^{3}H-aa-labeled bacteria bound to the Vero cell coated with PHA, *Lens culinaris*, or Con A is shown in Fig. 5. Con A, the most potent inhibitor of bacterial attachment, reduced bacterial attachment by 90% at 20 μg/ml. *Lens culinaris* also reduced attachment by over 80%, but 60 μg/ml was required. Both Con A and *Lens culinaris* binding to the Vero cells could be reversed by washing with AMM. After washing the monolayer with modified Earles solution to remove the AMM, bacterial adherence was again restored.

TABLE I

Inhibition of P+ Binding by Saccharides

Saccharide	Concentration required for 50% inhibition
	μM
α-CH$_3$-D-mannoside	0.5
Yeast mannan	10
D-Mannose	50
D-Fructose	3,000
α-CH$_3$-D-glucoside	18,750
D-Mannitol	37,500
D-Glucose	37,500
L-Mannose	>100,000*
D-Fucose	>100,000
L-Fucose	>100,000
Sucrose	>100,000
D-Galactose	>100,000
Lactose	>100,000
Maltose	>100,000

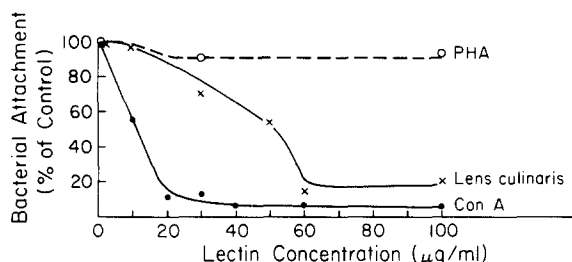* No inhibition detectable at 100,000 μM.

FIG. 5. Lectins were first bound to Vero cells at 37°C for 30 min. ^{3}H-aa-labeled bacteria were then incubated with the monolayer for 60 min. After washing, adherent bacteria were counted in a scintillation spectrometer.

Native Con A when succinylated has a reduced valence and loses the ability to cap receptors (11). Succinylated Con A also inhibited bacterial binding to Vero cells although to a somewhat lesser degree (50% inhibition at 20 μg/ml of lectin). This implies that other cellular effects of Con A requiring the tetrameric form were less important than the blocking of binding sites.

Metabolic Inhibition.   The role of active Vero cell metabolism in binding ^{125}I-labeled pili was determined by preincubating the cell cultures for 60 min in one of the following inhibitors: malonate (10 mM), 2,4-dinitrophenol (0.2 mM), or sodium azide (1.5 mM). As might be expected from the results on temperature dependence of binding, there was no significant difference from control cultures using any of the inhibitors under these conditions (Fig. 6).

Enzyme-Treated Tissue Cultures.   The ability of a variety of proteases and glycosidases to release the putative pili-binding molecules was tested by incubating Vero cells with these enzymes. After washing, binding of ^{125}I-labeled pili was tested. Neuraminidase pretreatment caused an increase in pili binding (125% of control). Other glycosidases used individually including α-mannosidase had no significant effect compared to preincubated controls (Fig. 6), and there
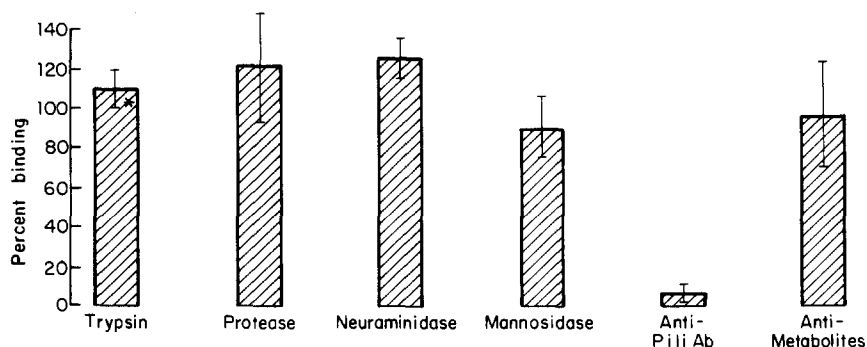
*Arithmetic mean ± S D

FIG. 6. ^{125}I-labeled pili were incubated with the treated or untreated monolayer for 60 min at 37°C. The control for each experiment represents 100% binding. Each mean is the average of 6 determinations ± 1 SD.

was no detectable release of carbohydrate. Trypsin and protease treatment slightly enhanced binding (Fig. 6).

Antibody Studies.   Antibodies to purified type I pili isolated from P+ bacteria formed a single precipitin line against purified pili or sonicated P+ by immunodiffusion. No such precipitin lines were seen using anti-pili antiserum and sonicated P− bacteria.

Such antisera almost completely inhibited binding to Vero cells of P+ organisms or purified pili (Fig. 5). Antisera against whole P+ (which include low titers of anti-pili antibodies) or P− resulted in marked clumping of bacteria but many clumps remained Vero cell associated. These attached bacterial aggregates could be removed by washing the cultures with AMM indicating persistence of attachment in spite of high titers of outer membrane protein antibodies and low titers of anti-pili antibodies.

Electron Micrographs.   The replica technique was used in order to examine the pilus-Vero cell membrane interaction at high resolution while avoiding sectioning the preparations. In Fig. 7, an adherent P+ bacterium is shown; the apparent thickness of the pili is increased because of the platinum shadowing. Purified pili do not appear to attach to the Vero cells by their tips only (Fig. 8) since contact with the cell membrane often extends over a considerable length of a pilus. Those portions of pili which are not in contact with the cell membrane can be differentiated from those on the membrane by their heavier platinum coating. The attachment seen by electron microscopy occurred after washing with PBS but was reversed by washing with AMM (30 mM). No preferential sites of attachment to particular areas of the cell were seen except that pili tended to extend between ridges on the plasma membrane and were usually not seen in the intervening valleys.

Discussion

Type I pili are defined on the basis of microscopy (peritrichous pili, 7.0 nm in diameter) and the ability of bacteria bearing such pili to agglutinate erythrocytes from certain species. They exist on some *E. coli*, Salmonella, Shigella, and
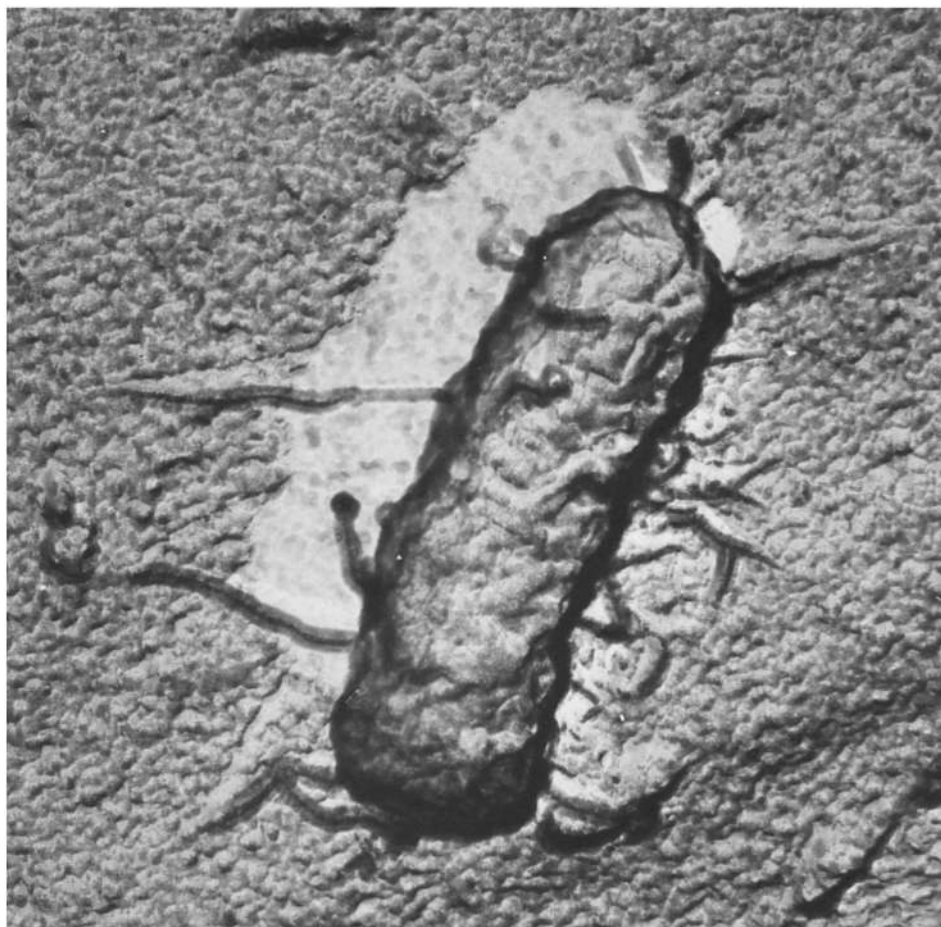
FIG. 7.   Pilated K12 bacterium attached to Vero cell membrane. Note increased thickness of pili due to platinum shadowing. Replica technique (× 65,000).

other gram-negative organisms. These appendages do not appear to be artifacts of prolonged laboratory cultivation since organisms freshly cultured from urinary tract infections are frequently pilated (16, 17).

The type I pili of *E. coli* have previously been purified and their physicochemical properties carefully characterized by Brinton (18). There have however been no published observations on the behavior of these purified pili in biological systems. Since we encountered difficulties in purifying pili using Brinton's procedure a modified technique is employed which easily enables the production of large amounts of partially purified pili (7). To further purify pili by removing outer membrane protein contaminants, isopycnic centrifugation was required.

Duguid has studied pilated bacteria and noted attachment to erythrocytes and epithelial cells (4). Little study has been given to this bacterial-host cell interaction particularly insofar as the host cell is concerned. One well-studied system is the gonococcal-mammalian cell interaction where pili have been shown to mediate adherence to tissue culture cells (19), erythrocytes (20), and sperm (21).
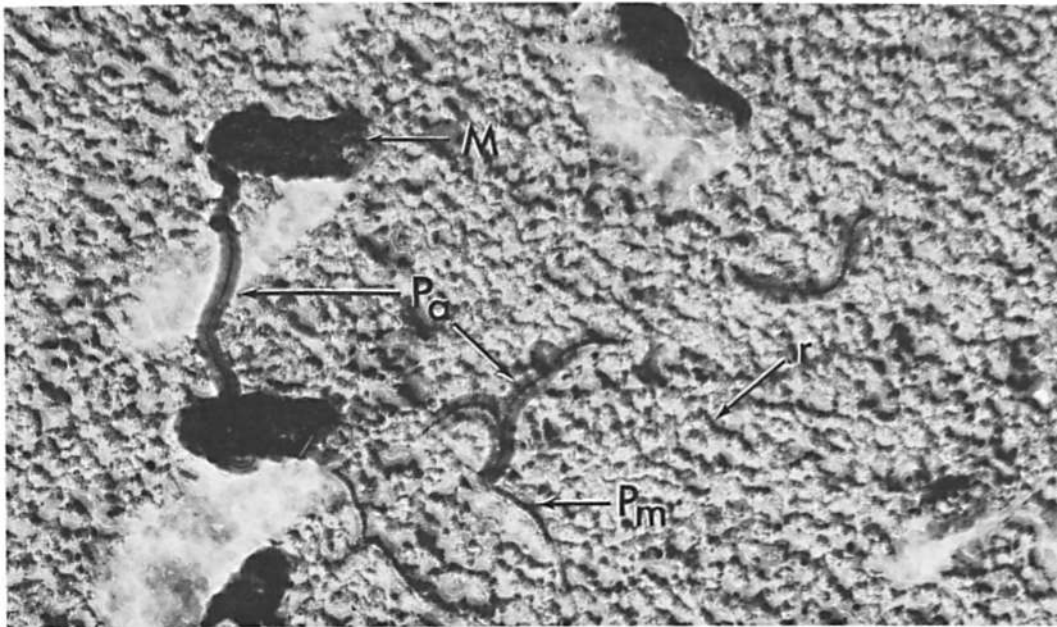
FIG. 8. Purified pili attached to Vero cell membrane. Portions of pili which are not in contact with the membrane are thicker than those on the membrane. Note apparent attachment of pili to cell ridges (r, cell membrane ridges; M, microvillus; P_m, pilus on the membrane; P_a, pilus above the membrane). Replica technique ($\times$ 65,000).

The role of pili in natural infections is less clear but they seem to be of importance in some experimental infections: pilation in Proteus may be important in the pathogenesis of pyelonephritis (22) and seems to be a factor determining site of localization of renal infections (23); pilation has been correlated with virulence in the gonococcus (24), and pilated Salmonella are more virulent for orally infected mice than nonpilated variants (25). Study of such bacteria and their relation to mammalian cells may thus have both practical and theoretical importance and the type I pili binding is an excellent model for such interactions.

Purified pili from K12 *E. coli* bound to Vero cell monolayers. The attachment was rapid and specific, and could be reversed or inhibited by specific analogues of D-mannose. Pili attachment to these cells was independent of temperature or metabolic state and so binding does not require enzymatic reactions.

Attachment of purified pili to Vero cells was optimal at pH 4–5. In this pH range pili have their lowest net charge and may more readily approach the mammalian cell surfaces. However, pili also tend to aggregate at pH 4 and these results may reflect the production of pili-pili aggregates adherent to the monolayer. Both mechanisms could be important in allowing a greater number of bacteria to attach to mucosal surfaces and so may be relevant in pathogenesis of infections particularly in the human urinary tract. Plant lectins also have pH optima for binding: ricin binds to HeLa cells best at pH 6–7 (26) and Con A binds mannose best at pH 6–7 (27).

The lectins Con A and *Lens culinaris* bind to mannose- and glucose-like residues on cell surfaces. We have shown that these lectins when bound to the monolayer inhibited attachment of pilated *E. coli* presumably by blocking the pili-binding site on the Vero cell membrane. Con A however, can have distant effects such as fixation or "anchorage modulation" of membrane proteins (28). This hypothesis was tested by using succinylated Con A which has the same carbohydrate-binding specificity as Con A but is not able to induce anchorage modulation (11). The marked inhibition of bacterial binding by the succinylated Con A makes the possibility of distant membrane effects less likely and competition for binding sites more likely. These results are important in studies relating to isolation of type I pili binding sites.

Hapten inhibition studies using a variety of saccharides confirmed the findings of others on hemagglutination by pilated bacteria (6). Yeast mannan, D-mannose, and alpha methyl mannose were potent inhibitors of binding but other saccharides tested, including L-mannose, were at least 60-fold less effective. The optimal inhibitors of binding to tissue culture cells were different from those which inhibited hemagglutination; notably mannitol, glucose, and alpha methyl glucose were more potent in inhibiting attachment to Vero cells than to guinea pig erythrocytes (7). It is possible that the binding sites are not the same on these two membranes.

Attempts were made to remove the pili-binding site by the use of enzymes in order to determine the nature of the molecules involved. Proteolytic digestion of Vero cells and erythrocytes (7) with trypsin or a protease mixture did not remove the type I pili-binding site but glycoproteins are often more resistent to digestion when present in the cell membrane than when isolated. Neuraminidase treatment significantly increased pili binding; this phenomenon ("stripping") is widely known in the attachment of other substances such as viruses to erythrocytes and other membranes. The mode of action of these enzyme treatments may be related to a reduction in the net negative charge of the cell surface (29), uncovering of cryptic binding sites, changes in mobility of membrane proteins or other as yet unknown mechanisms. Alpha mannosidase treatment of Vero cells did not significantly alter pili binding. If mannose is the receptor, the bulk of these molecules are subterminal or otherwise shielded from cleavage.

Electron microscopy was used to further examine the nature of the bacterial and pili binding to these tissue culture cells. Purified pili were shown to attach to the Vero cells confirming that it is these structures which are the adhesin. Such binding was not confined to specialized portions of the membrane but occurred equally over all parts of the cell surface including microvilli. Pili in many cases extended between ridges on the cell membrane but these structures may have been artifacts of fixation and drying. Attachment of pili over a considerable length along their structure indicates specificity of binding may not reside only in the tips of pili as suggested by Buchanan for gonococcal pili (30).

Antibodies raised against purified pili markedly inhibited P+ or pili binding to Vero cell membranes. Antisera raised against pilated bacteria tended to have lower titers of anti-pili antibodies and such antisera were less effective in inhibiting attachment. Anti-P− antisera were poor inhibitors of P+ binding and

did not inhibit pili binding. Low titer anti-pili antisera or antisera raised against other bacterial surface proteins may alone not be adequate in preventing colonization of mucosal surfaces.

Summary

We have demonstrated binding of purified pili from a strain of *Escherichia coli* to Vero cell monolayers as a model of prokaryotic-eukaryotic cell adherence. Pili bound to the tissue culture in a rapid reaction that did not require enzymatic activation. Attachment occurred optimally at pH 4–5 and could be inhibited by analogues of D-mannose, anti-pili antibodies, or by preincubation of tissue cells with mannose-specific plant lectins. Binding remained after treatment of the monolayer with glycosidases, trypsin, or a protease mixture but was enhanced after neuraminidase treatment. These results indicate that bacterial binding can occur via pili which act like lectins and presumably bind to mannose-containing glycoproteins on mammalian cell surfaces.

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