HOST-PARASITE INTERACTION IN THE RAT RENAL PELVIS

A Possible Role for Pili in the Pathogenesis of Pyelonephritis*

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The major route by which bacteria are presumed to enter the kidney is in retrograde fashion from the bladder. This path is facilitated by means of vesicoureteral reflux, a phenomenon that takes place spontaneously in animals and man (1, 2). Hence the opportunity for establishing a nidus of infection must occur with relative frequency. Despite this seemingly penetrable physiologic barrier (i.e., reflux), the kidney is difficult to infect and procedures employing highly virulent organisms (3), trauma to the kidney (4), or obstruction to the urinary outflow (5) are necessary to produce intrarenal infection. This means that reflux per se is not synonymous with infection and bacterial colonization requires the penetration of an epithelial barrier by the organism. Based on this reasoning, whether or not infection takes place will depend upon the ability of the pelvic or tubular epithelium to resist invasion (6, 7).

Little is known about the interaction between bacteria and the pelvic mucosal cells, however. On the epithelial surfaces, for example along the microvillous border of the intestine, adherence of *Shigella* (8), *Salmonella* (9), and *Escherichia coli* (10, 11) to the epithelial cell is an important first step for bacterial invasion, allowing organisms to concentrate in large numbers and to invade specific regions of the gut. In these infections transepithelial spread occurs by a process similar to phagocytosis; adherent organisms are enveloped by portions of the luminal plasma membrane and penetrate into the cytoplasm of the intestinal epithelial cells. One of the mechanisms by which these, as well as other gram-negative organisms adhere to epithelial surfaces is by means of filamentous appendages called pili (12–14). While possession of these structures is not necessarily correlated with virulence, such an association has been demonstrated for gonococci (15).

To study the interaction between bacteria and the renal pelvic epithelium, the early phases of retrograde *Proteus* pyelonephritis were examined by light and

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electron microscopy in the rat. These observations revealed that during the first 24 h after inoculation increasing numbers of bacteria became adherent to the surface of the pelvic epithelial cells. Intracellular penetration of these cells similar to that seen with invasive bowel pathogens was observed, however it was also evident that the bacteria utilized other mechanisms to gain access to renal parenchyma. Finally, evidence was obtained which suggested that pili played a role in these processes.

Materials and Methods

Female Sprague-Dawley rats, weighing 250–275 g were used for all experiments. Animals were given water and standard rat chow ad libitum. *Proteus mirabilis*, obtained from a patient with urinary tract infection was used as the infecting organism. Stock cultures were prepared by emulsifying an overnight growth of the bacteria on nutrient agar in a 1:1 mixture of fetal calf serum and trypticase soy broth. Small samples of the stock culture were held at −90°C until used.

Bladder Inoculation. Portions of the stock culture were cultured in trypticase soy broth and incubated in a shaking water bath at 37°C for 4 h. The bacterial suspension was washed three times with 0.9% saline and the concentration was then adjusted to contain approximately 5×10^8 organisms by nephelometry. 0.5 ml of this suspension was introduced into the bladder of 20 rats which had been lightly anesthetized with ether and pentobarbital. A 1-in segment of a size 3 French disposable ureteral catheter (American Cystoscope Makers, Inc., Pelham Manor, N. Y.) mounted on a 23-gauge needle, was found to have the proper compliance for convenient urethral catherization. 5 min after inoculation, 0.2 ml of heart blood was obtained for culture. Animals with bacteremia, resulting presumably from excessively high intrapelvic pressure, were eliminated from the study. Rats were sacrificed at 1, 4, 8, and 24 h; 3 days; and 1 wk after inoculation as described below. Additional animals were inoculated with 0.5 ml of 0.9% saline to ascertain the effect of the inoculation procedure on renal pelvic ultrastructure.

Tissue Preparation. Fixation was accomplished by perfusing the renal arteries of anesthetized animals with a paraformaldehyde-glutaraldehyde solution as described by Karnovsky (16). Because too few bacteria were present in the kidney to be detected by usual sampling procedures, the following special techniques were employed to select areas for examination by electron microscopy. Hand cut, 1- to 2-mm thick slices of the perfused kidney, which included tissue from the cortex to the papilla, were placed in Karnovsky's fixative for an additional 4 h at 0°C and washed overnight in a 0.1 M sodium cacodylate solution with 7% sucrose. The tissue was subsequently postosmicated in 1% osmic acid buffered with 0.1 M s-collidine at pH 7.3 and stained in block with 0.5% uranyl acetate. Dehydration was accomplished in a graded series of ethanol and propylene oxide, and the tissue was embedded in Epon epoxy resin. In order to accommodate their large size, embedment of the slices was carried out in 6 × 12-mm plastic molds obtained from Ivan Sorvall, Inc., Norwalk, Conn. Initially, sections 1–2 μm in thickness were cut on an American Optical 820 Rotary Microtome (American Optical Corp., Scientific Instrument Div., Buffalo, N. Y.) with a steel knife. However, we found that sections were more readily obtained using a Sorvall JB4 Microtome (Ivan Sorvall, Inc.) and 1-cm thick glass knives. The sections were affixed to glass slides, stained with toluidine blue or Hubner's stain (17), and examined with the light microscope. When an area of interest was identified, the corresponding region was cut out of the block using a needle-like device described by Boatman and Lowe (18). These smaller blocks were then mounted on aluminum chucks and ultrathin sections were cut. The sections were stained sequentially with uranyl acetate and lead tartrate and photographed with an AEI 801B electron microscope (AEI Scientific Apparatus Inc., Elmsford, N. Y.).

Negative Stains. Copper grids coated with parlodion and carbon, were floated on a drop of the bacterial suspension to be stained for about 30 sec, washed briefly on another drop of the distilled water, and then placed on a drop of freshly made 0.5% uranyl acetate solution for 30 sec. The excess was blotted off and the grid allowed to dry before being examined by electron microscopy.

Results

Light and Electron Microscope Studies

RATS INOCULATED WITH SALINE. The ultrastructure of the rat renal pelvic epithelium has been recently described (19) so that only pertinent features will be noted here. The papilla is lined with a single layer of relatively simple cuboidal cells whose luminal plasma membrane is 75-Å thick and projects into the pelvic space as short microvilli. The contrapapillary surface is invested with transitional epithelium, one to three cells thick. Like transitional cells in the bladder and ureter, these cells are characterized by an unusual luminal plasma membrane measuring 125 Å in thickness. The latter is termed the asymmetric unit membrane by virtue of the fact that the luminal leaflet is thicker than the cytoplasmic leaflet. The contours of the luminal surface are angular and a similar membrane lines fusiform vesicles in the cytoplasm. Some of the latter are filled with smaller vesicles. In the fornical region, epithelial cells have characteristics of both papillary and transitional epithelium.

The renal pelvis of three out of the four control rats inoculated intravesically with saline was normal by electron microscopy. In a fourth rat, however, some fornical cells appeared to have pulled apart from each other which suggest that excessive intrapelvic pressure developed during inoculation in this animal.

INFECTED ANIMALS (1–8 h). Only an occasional bacterium was encountered in animals sacrificed 1 or 4 h after reflux. In one rat, a bacterium was seen in the intercellular space between two fornical cells (Fig. 1). Normally, this compartment is sealed off from the pelvis by the tight junction, a region at the luminal end of the intercellular space where the plasma membranes of contiguous cells are fused. In this instance, however, the intercellular space communicated freely with the pelvis.

By 8 h bacteria were more numerous and several clumps could be seen in the fornix (Fig. 2). Polymorphonuclear leukocytes were present in the pelvis and in the interstitial spaces and capillaries adjacent to these regions where bacteria were seen. Whereas at these earliest times the bacterial surface appeared devoid of appendages (Fig. 1), by 8 h occasional filamentous projections were observed which resembled pili.

INFECTED ANIMALS (24 h). Large numbers of bacilli were present within the pelvis. Particularly striking were arrays of bacteria closely applied to the epithelial cells (Fig. 3). At higher magnification, many pili, measuring about 70 Å in thickness and up to 0.25 μm in length projected outward from these bacteria. For the most part these structures were unbent and appeared to serve as organelles of attachment between bacteria and the kidney cell surface (Fig. 4). A 2-μm space usually separated the cell wall of the bacteria and the plasma membrane of the epithelial cell. Although these bacterial arrays were most commonly encountered along the lateral aspect of the papilla, bacteria seemed as capable of sticking to the asymmetric unit membrane of transitional cells (Fig. 4) as to the more typical membrane of the papillary epithelium (Fig. 5). The ultrastructure of the epithelial cells was not altered in the region of attachment.

Bacteria were also observed within pelvic epithelial cells, always enclosed in a membrane-bound vacuole. Such intracellular bacteria were seen in both the

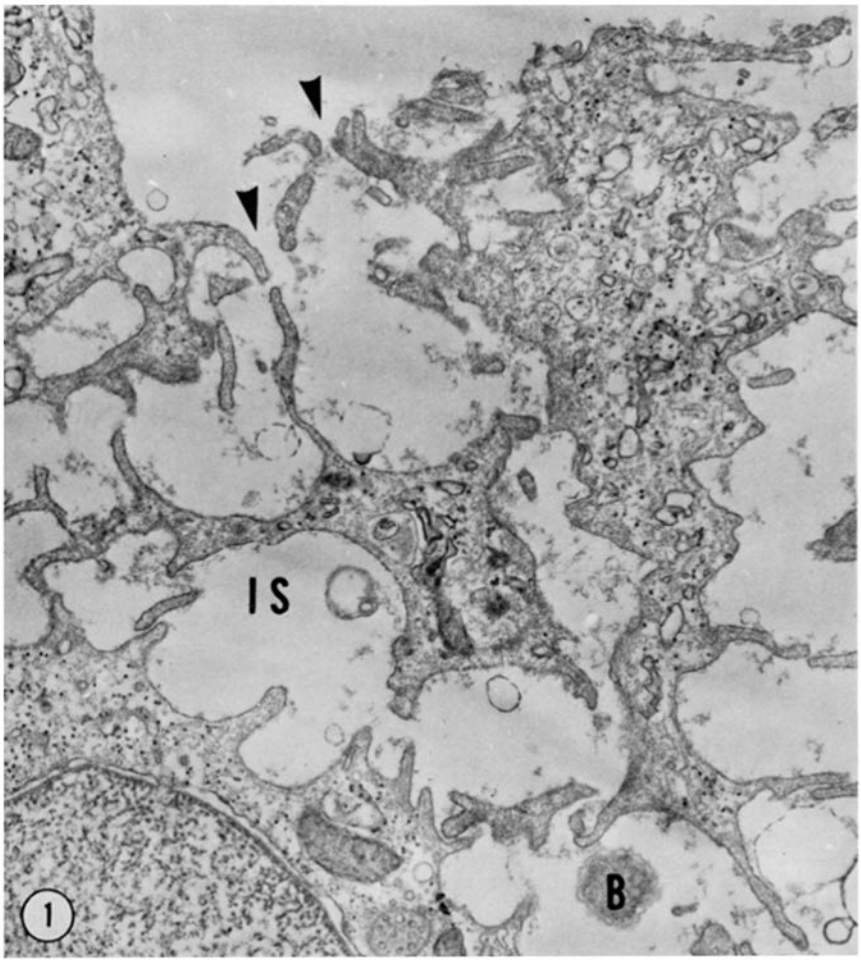

FIG. 1. Animal sacrificed at 1 h. In this portion of the fornical epithelium the cells have separated (arrow heads) allowing a bacterium (B) to penetrate the intercellular space (IS). Normally, tight junctions seal the apical end of the intercellular space from the pelvis. × 17,400. All rats were inoculated intravesicularly with 10^8 *P. mirabilis* from a 4-h growth phase and sacrificed at from 1 to 24 h thereafter.

papillary epithelial (Fig. 6) and transitional cells (Fig. 7). The morphological appearance of intracellular bacteria did not differ from that of microorganisms present in the pelvic space. In general, intracellular bacteria were not numerous and were encountered only after many serial sections were examined. Many bacteria not adherent to the mucosa were also observed within the pelvic space. These were often surrounded by fibrin and amorphous electron-opaque material (Fig. 8).

Inflammatory cells, predominantly polymorphonuclear leukocytes, were present along the margins of medullary capillaries, between pelvic epithelial cells (Fig. 9) and in the pelvic space. In the latter location, polymorphs frequently contained intracellular bacteria (Fig. 8).

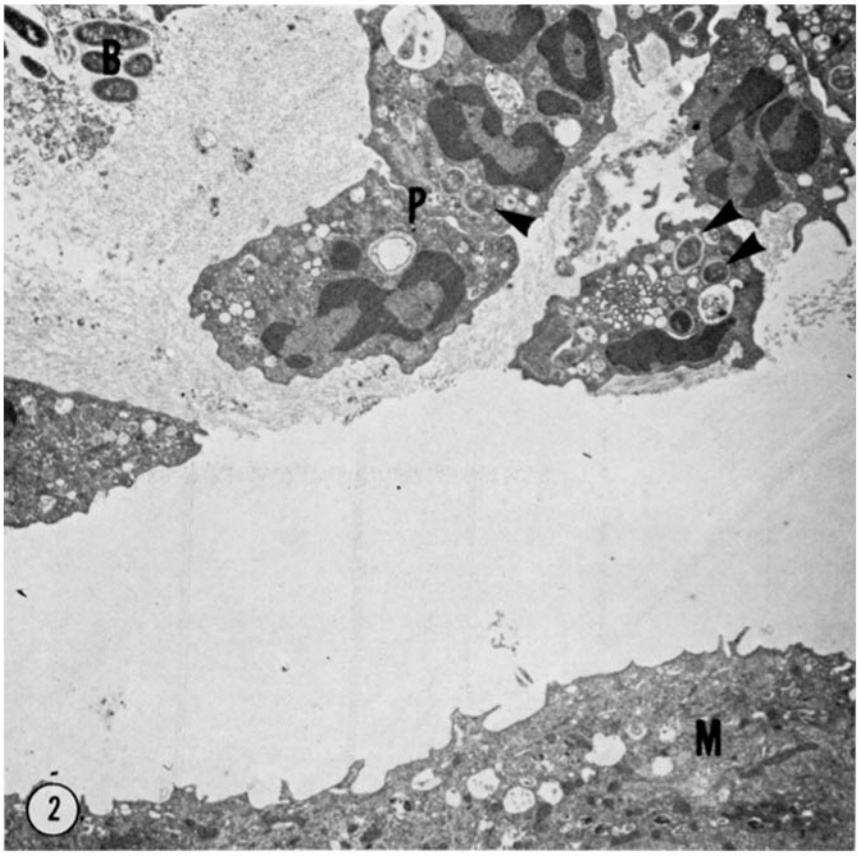

FIG. 2. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 8 h. Bacteria were seen more frequently by 8 h. As depicted here, there was evidence of an intense inflammatory reaction in some fornices. A clump of bacteria (B) amidst some cell debris lies nearby some polymorphonuclear leukocytes (P). Ingested bacteria can be seen within the polymorphs (arrows). Pelvic mucosa, M. × 4,950.

Adherence of bacteria to the epithelial cells was not invariably associated with cell injury (for example, see Fig. 3). Likewise, the ultrastructure of the epithelial cells containing intracellular bacteria did not differ from that of their neighbors. Rather it appeared that the most severe injury was seen in areas of intense inflammatory reaction where active phagocytosis of bacteria by neutrophils was occuring. In these regions discontinuities of the epithelial cell border were seen, which were due to the loss of necrotic cells. An occasional bacterium was observed to have penetrated these gaps (Fig. 10). In other areas the response of the epithelial cell was marked only by an increased number of dense bodies, ribosomes, and fat droplets. A careful search of the renal parenchyma revealed additional bacteria within the lumina of renal tubules and within the urinary space of cortical glomeruli (Fig. 11).

INFECTED ANIMALS (3 days to 1 wk). In addition to the changes present at 1 day, by 72 h there were focal microscopic abscesses in the medulla and by 1 wk abscesses could be seen in the cortex by the unaided eye. Histological

examination of these kidneys revealed the characteristic appearance of acute pyelonephritis: wedge-shaped areas of infection consisting of necrotic cortical tubules surrounded by neutrophils and medullary collecting ducts packed with bacteria, debris, and inflammatory cells. In many places the pelvic epithelium was now several layers thick.

Surface Features of P. Mirabilis Studied by Negative Staining. In order to more effectively delineate the topographical features of their surface, bacteria in various phases of growth were examined by negative staining. Two different types of pili were observed. In cultures 4-h old, bacteria bearing 40-Å-thick pili predominated (Fig. 12). On the other hand, after one to six serial subcultures of 48 h each, bacteria with pili measuring 70 Å emerged (Fig. 13). In addition to differences in their size, the two types of pili were distinguishable by the fact that the axial region of the 70-Å-thick pilus was permeable to uranyl acetate (inset Fig. 13). These two kinds of pili correspond to types III (40 Å) and IV (70 Å) of Brinton's classification (20). On the basis of their similar size, the pili of bacteria seen in the thin sections of the infected kidneys were presumed to be type IV.

To quantitate the relative proportion of type III and IV pili present on organisms from cultures grown under these two different conditions, photographs of at least one hundred randomly selected, negatively stained bacteria of each type were made at magnifications of 25,000 times. Pili counts were made directly from the negatives. As can be seen in Table I, a relatively homogenous population of one pili type predominated in each circumstance. Both types were rarely present on the same cell. In a few instances a single type IV pilus was seen close to the surface of a bacterium otherwise pilated with type III pili and it was impossible to tell whether it originated from that cell. A variable number of flagella, 125 Å in thickness, were seen on both pilated variants.

Comparison of the Pathogenicity of Type III or Type IV Pilated Organisms for the Kidney by the Ascending Route. The availability of methods to obtain these two phases of pilation in almost pure culture made it possible to compare the effect of pili type on virulence in this model. Cultures in which either type III or type IV pilated organisms predominated were diluted serially with saline to give inocula ranging from 10^7–10^{10} organisms/ml. Groups of 10 rats each were inoculated intravesicularly with 0.5 ml of one of the four dilutions of the type III or type IV organisms. After 7 days the surviving animals were sacrificed and the kidneys were visually examined for presence of cortical abscesses. If one or both kidneys were infected, the animal was counted as positive.

As shown in Fig. 14, type IV pilated organisms were more virulent than type III with respect to both frequency of infection and inoculating dose in ascending infection. Although within individual groups the numbers of animals were too small for statistical comparison, when the results of all dilutions were summed, the difference between the virulence of type III and type IV was significant ($P < 0.02$ by the chi-square test with the Yates corrections).

Discussion

The manner in which bacteria ascending from the lower urinary tract cross the renal pelvic epithelium is not completely understood. The ultrastructural

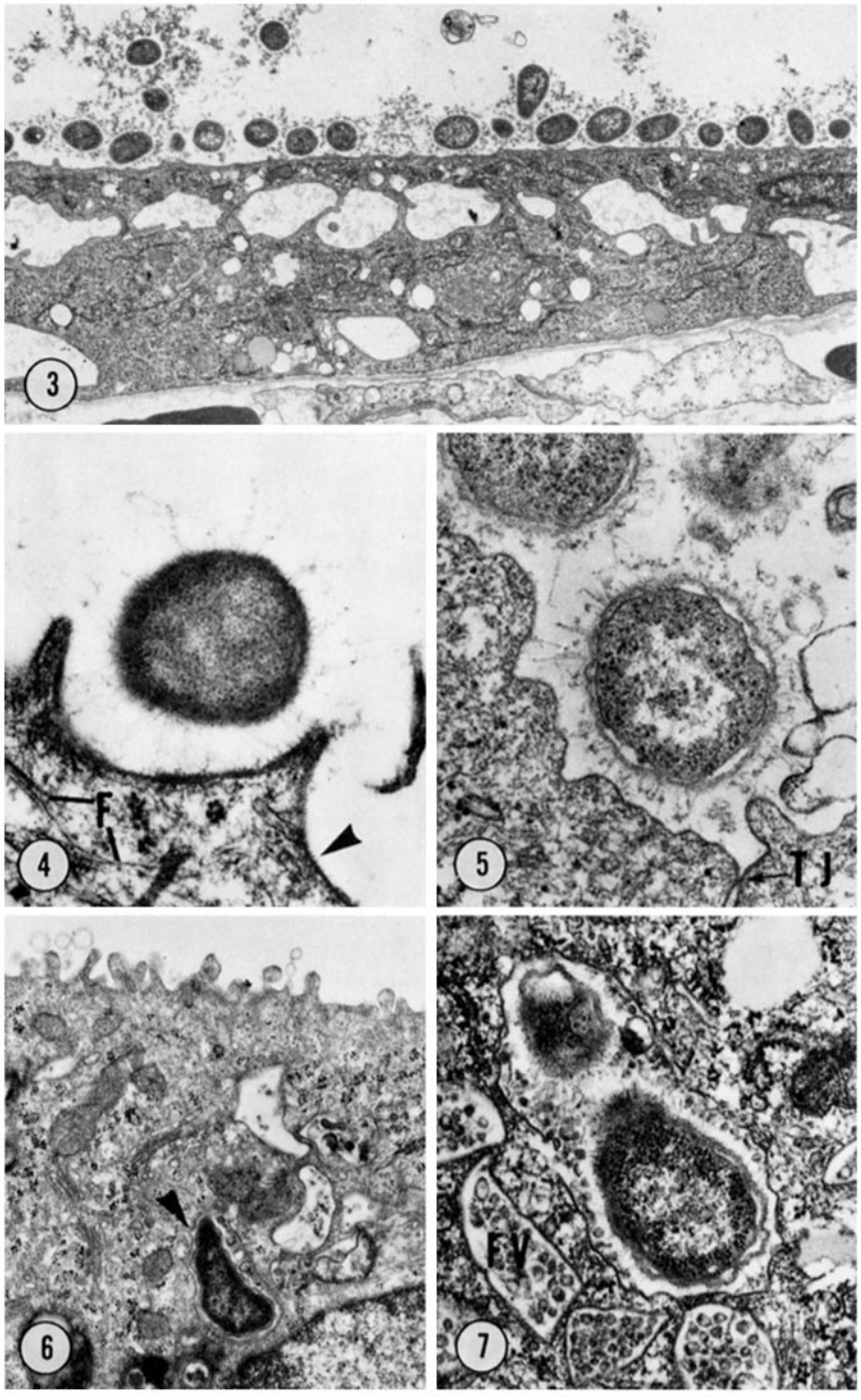

FIG. 3. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 24 h. The apical surface of these pelvic epithelial cells is lined with many closely adherent bacteria. × 6,250.
FIG. 4. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 24 h. The apical region of a transitional epithelial cell is depicted here. The plasma membrane (arrow head) is

observations detailed here suggest that possibly more than one mechanism of transepithelial spread may be involved. It seems likely that in some of the rats, bacteria first gained access to the renal parenchyma during vesicoureteral reflux. Fornical structures are known to be fragile and are easily damaged by the excessive hydrostatic pressure which can be generated if the bladder is overdistended during inoculation (21). Using a model similar to the one employed here, Fierer et al. found that pyelonephritis resulted only when there was some evidence of fornical injury, such as transient bacteremia occurring during inoculation (22). Use of inoculation vol in excess of 0.5 ml and withholding fluids before inoculation were shown to predispose the rats to fornical injury. In the present experiments, care was taken to avoid these factors which overdistended the bladder and all animals with bacteremia after bladder inoculation were eliminated from the study; nonetheless there was evidence that the epithelial cells in the fornix of some animals had pulled apart, allowing bacteria to penetrate the mucosa. While an occasional bacterium may have reached the renal parenchyma in this fashion, the subsequent pathological changes which occurred in these kidneys were most likely manifestations of the virulent properties of the invading organisms themselves, as no infection occurred when a strain of *E. coli* was substituted for the *Proteus*. (F. Silverblatt, unpublished observations.)

Other possible mechanisms for parenchymal invasion were indicated by electron micrographs obtained at a later stage of the pelvic infection. In some regions of the pelvis, bacteria were observed within gaps of the mucosa left by necrotic cells. Proteus also appeared capable of penetrating intact epithelial cells and it is possible that the organisms may have been passing through the cells in a manner similar to the mechanism whereby *Shigella* (8) or *Salmonella* (9) reach the intestinal submucosa. *P. mirabilis* may have a specific ability to parasitize renal epithelial cells as Braude and Siemienski have reported that monkey kidney epithelial cells grown in tissue culture are susceptable to intracellular colonization by *P. mirabilis* but not by *E. coli* (23). Finally, the discovery of bacteria in Bowman's space and in occasional tubular lumina suggest that bacteria may also reach the cortex as a result of calicotubular backflow. This

unusually thick (130 Å) and its outer leaflet is thicker than the cytoplasmic leaflet. This unique membrane, termed the asymmetric unit membrane, is characteristic of transitional epithelial cells. The angular profiles of the cell surface and the bundles of 60 Å filaments (F) are also characteristic of this cell type. A bacterium lies in a cuplike depression of the cell surface. Many pili 50–70 Å in diameter and up to 0.2 μm in length radiate from the bacteria and extend between the bacterium and the epithelial cell surface. × 50,800.

FIG. 5. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 24 h. Pilated bacteria are adherent to the apical surface of these two papillary epithelial cells. The apical plasma membrane is thinner (75 A) than the asymmetric unit membrane of transitional cells (see Fig. 4). Note that the plasma membranes of these cells are fused at the apical end of the intercellular space to form a tight junction (TJ). × 40,000.

FIG. 6. All rats inoculated and sacrificed as in Fig. 1. Animals sacrificed at 24 h. This epithelial cell from the fornix contains a bacterium enclosed within a membrane-bound body (arrow head). × 16,500.

FIG. 7. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 24 h. A vacuole which contains a bacterium and many small vesicles can be seen in this region of a contrapapillary transitional epithelial cell. Nearby are fusiform vesicles (FV). × 35,000.

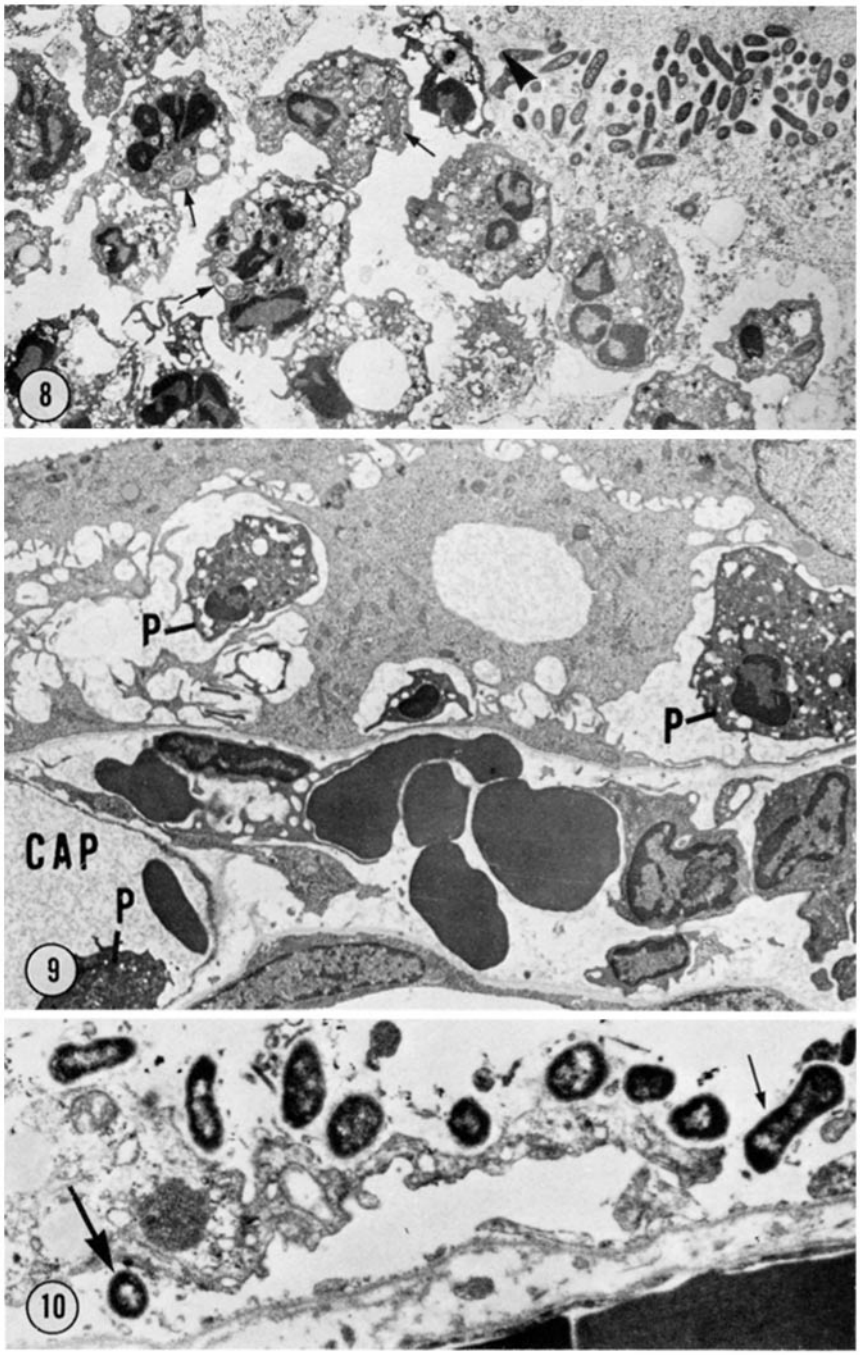

FIG. 8. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 24 h. A large collection of bacteria and polymorphonuclear leukocytes enmeshed in fibrin are seen in a portion of the pelvis. Several polymorphs contain intracellular organisms (arrows). × 6,900.
FIG. 9. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 24 h. In this region of the pelvic epithelium, polymorphonuclear leukocytes (P) infiltrating into the pelvis can be seen along the margin of a capillary (CAP) and between the pelvic epithelial cells. × 4,180.
FIG. 10. All rats inoculated and sacrificed as in Fig. 1. Rat sacrificed at 24 h. A region of necrotic pelvic epithelium which may have been a site of transepithelial spread. One bacteria (thin arrow) can be seen penetrating a gap in the continuity of epithelial cells. Another (thick arrow) lies between the epithelium and the basal lamina. × 10,900.

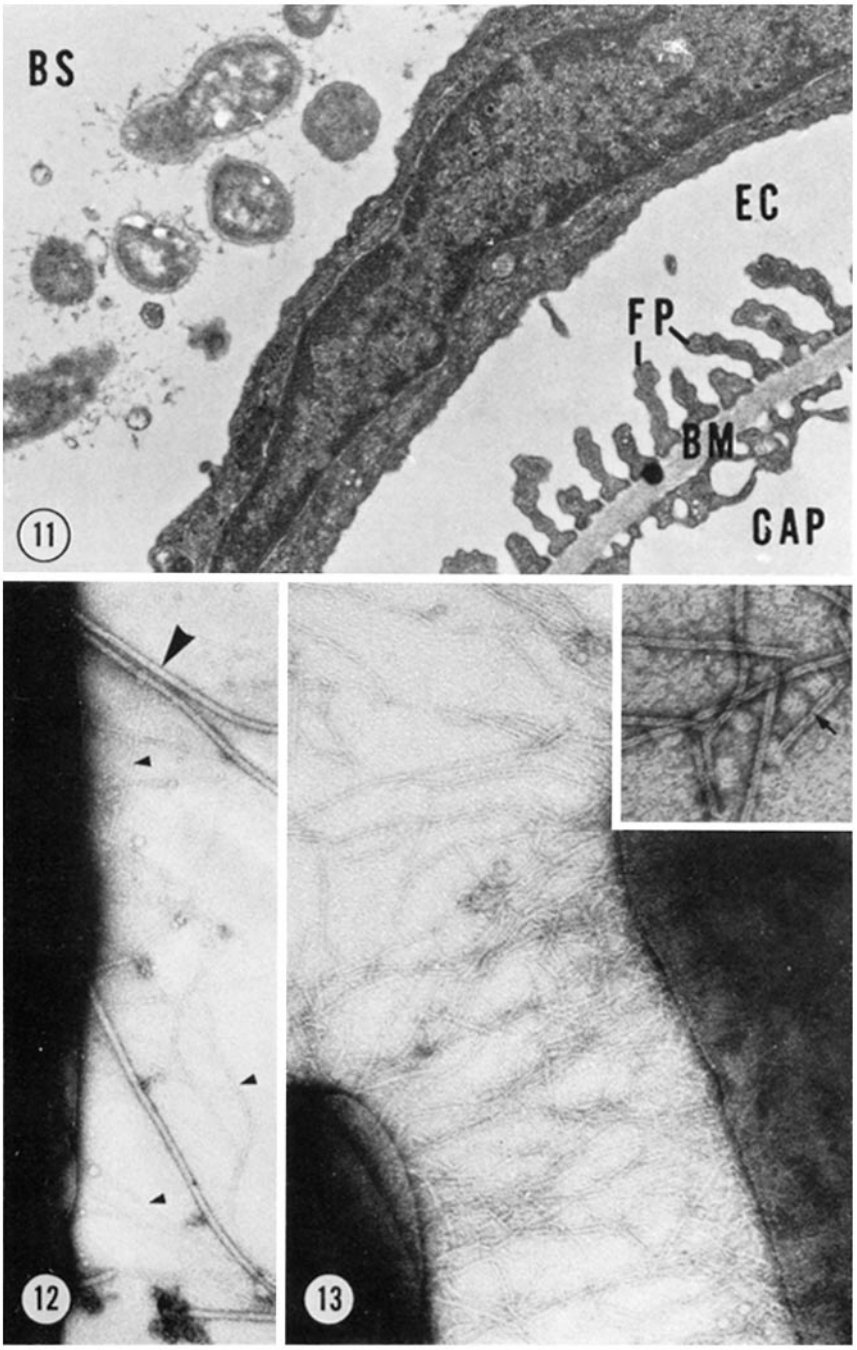

FIG. 11. All rats inoculated and sacrificed as in Fig. 1. Animal sacrificed at 24 h. A cortical glomerulus showing bacteria in Bowman's space (BS), epithelial cell (EC), epithelial cell foot processes (FP), basal membrane (BM), and capillary (CAP). × 18,600.

FIG. 12. Electron micrograph Negative stain preparation of *Proteus* from 4-h broth culture. Type III pili (small arrow head) measure about 40 Å in diameter. Flagella (large arrow head). × 63,000.

FIG. 13. Electron micrograph. Negative stain preparation of *Proteus* from 48-h culture in broth. Type IV pili are more numerous than the type III seen in Fig. 12 and measure about 70 Å in thickness. × 81,400. Inset shows the characteristic uranyl acetate-permeable axial hole of type IV pili (small arrow). × 150,000.

TABLE I
Effect of Growth Conditions on Frequency Distribution of Pili Types in P. Mirabilis

Type of pili present on bacteria	Age of culture	
	4 h*	96 h‡
	%	%
Type III	79	1
Type IV	2	98
No pili	19	1

* Bacteria incubated in trypticase soy broth for 4 h in a shaking waterbath.
‡ Bacteria grown for 48 h in stationary tubes of trypticase soy broth and subcultured for an additional 48 h.

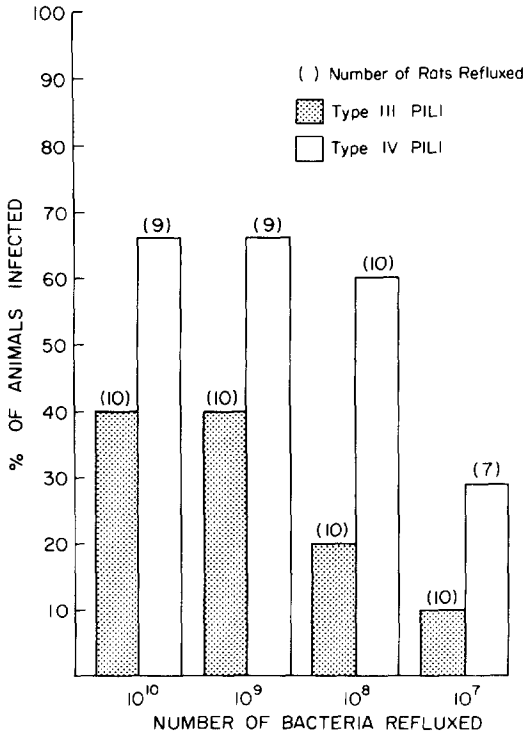

FIG. 14. Comparison of the pathogenicity of type III or type IV pilated *P. mirabilis* in retrograde pyelonephritis. Serial 10-fold dilutions were made of cultures containing almost pure growths of either type III or type IV pilated organisms. A portion of each dilution was inoculated intravesicularly into groups of rats and their kidneys were examined by eye for the presence of cortical abscess after 1 wk.

phenomenon also occurs in humans. Amar observed calicotubular backflow of contrast media during voiding cystography in eight patients with vesicoureteral reflux (24).

There is increasing evidence that on many mucosal surfaces, bacterial

adhesion is an essential step for colonization or invasion. For example, the distribution of dental plaque-forming *Streptococci* in the mouth is determined by their ability to attach to certain oral surfaces (25). Similarly, attachment to the intestinal epithelium is believed to be a prerequisite for mucosal invasion by *Shigella* (26) and certain strains of *E. coli* (11).

Recently Ellen and Gibbons reported that *E. coli*, a common urinary tract pathogen, adhered more readily to urinary bladder epithelial cells than to oral epithelial cells, whereas the affinity for these cell types of *Streptococcus pyogenes*, an organism not usually found in the urinary tract, was reversed (27). This tissue specificity suggests that adherence may be important for colonization and infection of the urinary tract mucosa. Observations reported in the present study support this thesis, demonstrating that during the course of experimental pyelonephritis pathogenic bacteria adhere to the urinary tract epithelium in vivo.

The ability to stick onto the pelvic wall may benefit bacteria at several stages during the pathogenesis of pyelonephritis. First, conditions present in the renal pelvis may make such attachment necessary for initiation of the infection. Peristaltic contractions of the pelvic walls rapidly eliminate most bacteria refluxed from the lower urinary tract, therefore those microorganisms that can readily stick to the pelvic wall might be expected to have a greater opportunity to persist in the pelvis.

Second, as noted above, bacterial attachment may facilitate invasion of the renal parenchyma. The closely packed layers of adherent bacteria seen in this study may have injured the underlying epithelial cells, either through the cytotoxic effects of a bacterial product such as urease (23, 28) or indirectly, by hydrolytic enzymes released from phagocytosing leukocytes (29). Adherence may also be a prerequisite for the intracellular penetration of intact mucosal cells.

Third, bacterial adhesiveness may play a role in perpetuating the infection. Unlike the renal cortex, the normal antibacterial host defenses of the pelvis are inhibited by the hypertonicity of the pelvic urine (30, 31). Intrapelvic organisms are maintained, therefore, in a relatively protected environment and may serve as a reservoir for reinfection long after the cortex has been sterilized. This thesis is supported by the observations of Cotran and his colleagues who found that months after the initiation of *Proteus* pyelonephritis, bacteria could be seen adhering to the pelvic mucosa, when they no longer could be demonstrated in the renal parenchyma (3).

Adhesion of *Proteus* to the renal pelvis appeared to be mediated by pili. These filamentous appendages are present on the surface of many microorganisms including all the gram-negative pathogens which cause human urinary tract infections (20). There is accumulating evidence that the possession of pili is important for the pathogenesis of genitourinary tract infection. Swanson and his colleagues have shown that virulent colony types 1 and 2 of gonococci are pilated, whereas avirulent types 3 and 4 lack these structures (15). Also, pyelonephritis in cattle is often caused by *Corynebacterium renale*, a pilated gram-positive organism (32). Finally Brinton has reported that bacteria present in the urine of patients with urinary tract infections are heavily pilated (33).

Several types of pili have been recognized which differ in size and function. Properties thus far associated with pili include exchange of genetic material (F

pilus) (33), susceptibility to certain phages (34), attachment of *Enterobacteriaceae* (13) and *Gonococci* (14) to mammaliam cells, resistance to phagocytosis (35, 36), and most recently, with a peculiar twitching movement of moraxella across an agar surface (37).

P. mirabilis has at least two kinds of pili which can be distinguished in the electron microscope by differences in their size and appearance (33). In the present study, organisms bearing one pili type or the other could be made to predominate by varying the conditions of growth in vitro. A similar shift in the phase of pilation responsive to changes in the cultural environment is a feature of many pilated organisms (38). In general, the pilated phase develops upon repeated subculture in stationary tubes of broth, and disappears during exponential growth or after cultivation on agar. Duguid and Gilles have shown that pilated *Shigella* can outgrow the nonpilated bacteria when concentrations of oxygen are low, e.g. in static tubes of broth, and that the emergence of pilated organisms in a culture is heralded by the appearance of a membranous pellicle composed of closely packed heavily pilated bacteria (12). They have suggested, therefore, that the pilated phase represents an adaptation to hypoxic conditions, enabling the bacteria to concentrate at the surface, in proximity to a readily available supply of oxygen. While the type IV pili of the *P. mirabilis* strain used in this study responded to similar enviromental cues, type III pili behaved antithetically, that is, type III pilated organisms predominated in exponential cultures in broth or when cultured on agar, whereas very few type III pilated bacteria were found in static tubes of broth which were repeatedly subcultured.

In the present study, the phase of pilation was also noted to vary in vivo; bacterial pilation changed from type III to IV after reflux into the renal pelvis. This transition may well represent an adaptive response by the *Proteus* to conditions in the kidney for which the type IV phase was especially suited. Indeed, when the ability of type III and IV pilated organisms to initiate retrograde infection was compared, bacteria with type IV pili were found to be more virulent.

How might type IV pili enhance virulence? While it is true that these pili appeared to mediate adherence to mucosal cells in vivo, they may possess other attributes useful for successful colonization such as confering resistance to phagocytosis (35, 36). On the other hand it is possible that types III and IV pili have similar properties and that the superior ability of type IV pilated organisms was due to the higher percentage of pilated organisms which were present in the 48-h subcultures (Table I). In either case (whether the results reflected qualitative or quantitative difference), the fact that the infectivity of *P. mirabilis* was altered by changing the type of pilation suggests that pili play some role in the pathogenesis of retrograde pyelonephritis. Ideally this thesis should be tested by comparing the virulence of this strain of *Proteus* with that of a nonpilated mutant. Regrettably, efforts at isolating such a mutant have been unsuccessful.

Summary

The initial interaction between bacteria and the renal pelvic epithelium may determine whether intrarenal infection occurs. A model of retrograde pyelone-

phritis was employed to study these events by electron microscopy. Female rats received an intravesicular inoculation of a 0.5-ml suspension of *Proteus mirabilis* containing 10^8 organisms. At intervals after inoculation, the kidneys were fixed by intravascular perfusion and the tissues were prepared for electron microscopy.

During the first 24 h, increasing numbers of bacteria were seen to be attached by pili to the renal pelvic epithelial cells. The organism appeared to cross the mucosal barrier by several mechanisms: (*a*) penetration into the cytoplasm of intact epithelial cells, (*b*) passage between epithelial cells that were separated by excessive hydrostatic pressure generated during bladder inoculation, (*c*) passage across necrotic regions of the pelvis, and (*d*) translocation to the cortex by calicotubular backflow.

Whereas at inoculation bacteria possessed pili 40 Å in diameter (type III pili) 24 h after reflux, the predominant type of pili measured 70 A in thickness (type IV pili). Repetitive subculture induced a similar transition in vitro. To assess the influence of pili type on virulence in this model, 80 rats were challenged with either type III or type IV pilated organisms and the frequency of rats with cortical abscesses were compared at 1 wk. A significantly greater number of rats inoculated with type IV pilated *Proteus* manifested macroscopic evidence of infection. These results suggest that pili play a role in the pathogenesis of ascending pyelonephritis.

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