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Ultraviolet Irradiation Disrupts Somatic Pili Structure and Function

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Three piliated bacterial species were exposed to ultraviolet light (7×10^3 $\mu W/cm^2$), and the effect of increasing duration of irradiation on the integrity of the somatic pili was quantitated by negative-stain electron microscopy. Heavily piliated *Proteus mirabilis* became devoid of pili after 20 min of irradiation, but *Escherichia coli* and *Neisseria gonorrhoeae* required 40 min for complete depiliation. Partially purified proteus pili underwent progressive loss of structural integrity with increasing doses of irradiation as determined by negative staining and nephelometry, suggesting that ultraviolet light exerted an effect directly on the pili themselves. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis demonstrated that new, small molecular weight fragments appeared after irradiation of purified *E. coli* pili, suggesting that cleavage of the peptide chain rather than disassociation of pilin monomers accounted for the loss of pili structure. Ultraviolet irradiation also inhibited the ability of piliated bacteria to bind to human buccal epithelial cells. These observations indicate that the ultrastructural integrity and function of pili can be disrupted by ultraviolet light.

Irradiation with ultraviolet (UV) light is commonly believed to be one of the least destructive methods of killing microorganisms. Death has been shown to result from the dimerization of adjacent pyrimidines of deoxyribonucleic acid (DNA), which inhibits replication and transcription (2). Because exposure to UV light is also known to affect the conformation of many proteins (10), the possibility remains that the irradiated bacteria may be altered in other, less apparent ways. In this communication we report that UV irradiation of several gram-negative species results in loss of structural integrity and function of their somatic pili.

MATERIALS AND METHODS

Growth of bacteria. All bacteria were clinical isolates. *Proteus mirabilis* and *Escherichia coli* were cultured in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and passed at 48-h intervals until maximal piliation was achieved. *Neisseria gonorrhoeae* was grown on GC medium (Difco Laboratories, Detroit, Mich.) without hemoglobin, to which was added IsoVitaleX (BBL Microbiology Systems). The organisms were maintained in a piliated state by selective passage of type 1 colonies.

Preparation of isolated pili. *P. mirabilis* and *E. coli* were purified as recently described (9a). Briefly, organisms were depiliated in a blender and the pili were precipitated with 45% ammonium sulfate. Most flagella and membrane fragments were removed by ultracentrifugation at 100,000 × *g* for 1 h. *Proteus* pili were used after this point. *E. coli* pili were further purified by sucrose density centrifugation and precipitation with 0.1 M magnesium chloride. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of purified pili was carried out in 12.5% polyacrylamide gels by the method of Laemmli (6). Samples of 50 μl (100 μg of protein) were combined with 50 μl of 0.01 M Tris(hydroxymethyl)aminomethane (pH 7.5) containing 10% SDS, 1% 2-mercaptoethanol, and 10% glycerol and were heated at 100°C for 10 min. After cooling, 10 μl of bromophenol blue and 1 drop of 2 M sucrose were added to the sample before application to the gels. Electrophoresis was performed at 2.5 mA per gel for 3 h. Afterwards, the gels were stained for 2 h with Coomassie brilliant blue and destained overnight in methanol and acetic acid.

UV irradiation. Bacteria were washed three times and resuspended in 0.9% sodium chloride. Concentrations were adjusted to ca. 5×10^8 organisms per ml by optical density. UV radiation was provided by two Sylvania G15T8 germicidal lamps (principal wave length, 253 nm) positioned 2.5 cm above the surface. At this distance the radiant energy was ca. 7×10^3 $\mu W/cm^2$, as determined by a model J225 UV meter (Ultraviolet Products, San Gabriel, Calif.). For irradiation, suspensions of bacteria or isolated pili were placed in 60-mm plastic petri dishes under the UV source and slowly agitated on a rotating platform shaker. A duplicate control sample was agitated concurrently, but was shielded from the UV light. Both the irradiated and control samples were kept on ice during this procedure to minimize heating. Aliquots were removed at intervals, and the effect on the pili was assessed by negative staining and turbidimetric methods as described below.

Negative staining. Copper grids coated with Formvar and carbon were floated for 1 min on a drop of the bacteria or pili sample to be examined, washed

with distilled water for 10 s, and stained for an additional 30 s on a drop of 0.5% uranyl acetate. The grids were allowed to dry and were photographed in an AEI 6B electromicroscope. The degree of piliation of a culture was defined as the percentage of organisms with 50 or more pili. The degree of photodestruction of isolated pili was evaluated blindly as follows. Electronmicrographs of the irradiated preparations were coded, after which a square (4 by 4 cm) was drawn on a random portion of the surface of each print. The length of every pilus present within each square was measured, and the results were summed. Three prints were evaluated for each interval of irradiation.

Nephelometry. A 5-ml suspension of the partially purified pili (50 μg/ml) was irradiated as described above, and portions were removed at 0, 10, 20, and 40 min for determination of their optical density by using a spectrophotofluorometer (American Instrument Corp., Silver Spring, Md.). For these determinations both the exciting and recording wave lengths were adjusted to 550 nm. Operated in this mode, the photometer recorded light reflected off suspended particles in the test solution, and thus any change in the size or number of pili was represented by a corresponding change in optical density.

Attachment of irradiated bacteria to buccal cells. To determine whether the function, as well as the structure, of pili was altered by UV irradiation, we evaluated the effect of UV light on the ability of *E. coli* and *P. mirabilis* to adhere to human buccal epithelial cells (8, 9). Adherence was measured by the method of Ellen and Gibbons (3). Buccal cells were collected and washed in phosphate-buffered saline, pH 7.4. Bacteria were irradiated for 0, 3, 10, or 20 min and combined with the cells in a ratio of 100:1. The mixture was rotated end-over-end for 30 min at 37°C, after which the buccal cells were separated from unattached bacteria by filtration, fixed to glass slides, and stained with crystal violet. The percentage of all cells with 25 or more attached bacteria was determined by light microscopy.

RESULTS

The three species were not uniformly sensitive to effects of UV light. Thus, although only an occasional *Proteus* pilus was seen after 20 min of irradiation (Fig. 1A and B), *E. coli* (Fig. 2a and b) and *N. gonorrheoae* (Fig. 3a and b) required 40 min for complete depiliation. The effect of UV light on piliated *Proteus* and *E. coli* was explored further. For both species there was a progressive decrease in the degree of piliation with increasing duration of irradiation (Table 1). There was about a 5-log reduction in viability after 1 min (Table 1).

A decrease in pili length and number was also observed when isolated *Proteus* pili were exposed to increasing duration of UV light (Fig. 4a to e). Morphometric studies of negatively stained preparations revealed that there was a 60% reduction in the aggregate pili length during the initial 5 min. Thereafter, pili disappeared at a slower but constant rate (Fig. 5). Pili appeared to be more sensitive to the effects of UV light than were flagella (Fig. 4a to e).

Because the results of negative staining might have been influenced by changes in the degree of adherence of the irradiated particles to the grid surface, we employed a turbidometric method to confirm the photolytic effects of UV light on *Proteus* pili. After a 10-min lag period, the optical density of the pili preparation decreased progressively so that by 40 min the amount of light reflected from the irradiated suspension was less than 10% of the nonirradiated control sample (Fig. 6).

To determine whether dissolution of pili was accompanied by cleavage of the peptide chain, 50 μl of purified *E. coli* pili which had been irradiated for 30 min and a sample of unirradiated pili were subjected to SDS-polyacrylamide electrophoresis. The electrophoretic pattern of the unirradiated preparation displayed a single band at ca. 17,000 daltons, which is characteristic of the *E. coli* pili monomer (Fig. 7) (8). In contrast, with the irradiated sample the portion of the gel between the position of the pilin band and the electrophoretic front was diffusely stained. This suggests that smaller molecular weight fragments of various sizes had been formed during irradiation.

The ability of both *E. coli* and *Proteus* to bind to the surface of buccal epithelial cells was reduced by irradiation for as little as 1 min in the case of *E. coli* and 5 min for *Proteus* (Table 1). In general the reduction in adherence paralleled the loss of pili. Most organisms were killed within 1 min.

DISCUSSION

The observations described above demonstrate that the structure and function of the somatic pili of several gram-negative species can be disrupted by UV irradiation. The effect appears to be exerted directly on the pili themselves because pili in a cell-free suspension were disrupted by doses of irradiation comparable to those required to depiliate intact cells. The photolytic effect was demonstrated by both morphometric and turbidometric techniques and is thus unlikely to be an artifact of the particular method employed. There was also a correlation between the degree of pili destruction and inhibition of pili-mediated binding to buccal epithelial cells.

Novotny and co-workers have reported that *E. coli* produced fewer and shorter F pili when irradiated with doses of UV light similar to those employed here (7). In contrast to the present study, however, these authors attributed the loss of sex pili to an effect of the UV light on intracellular DNA because the loss of pili was most

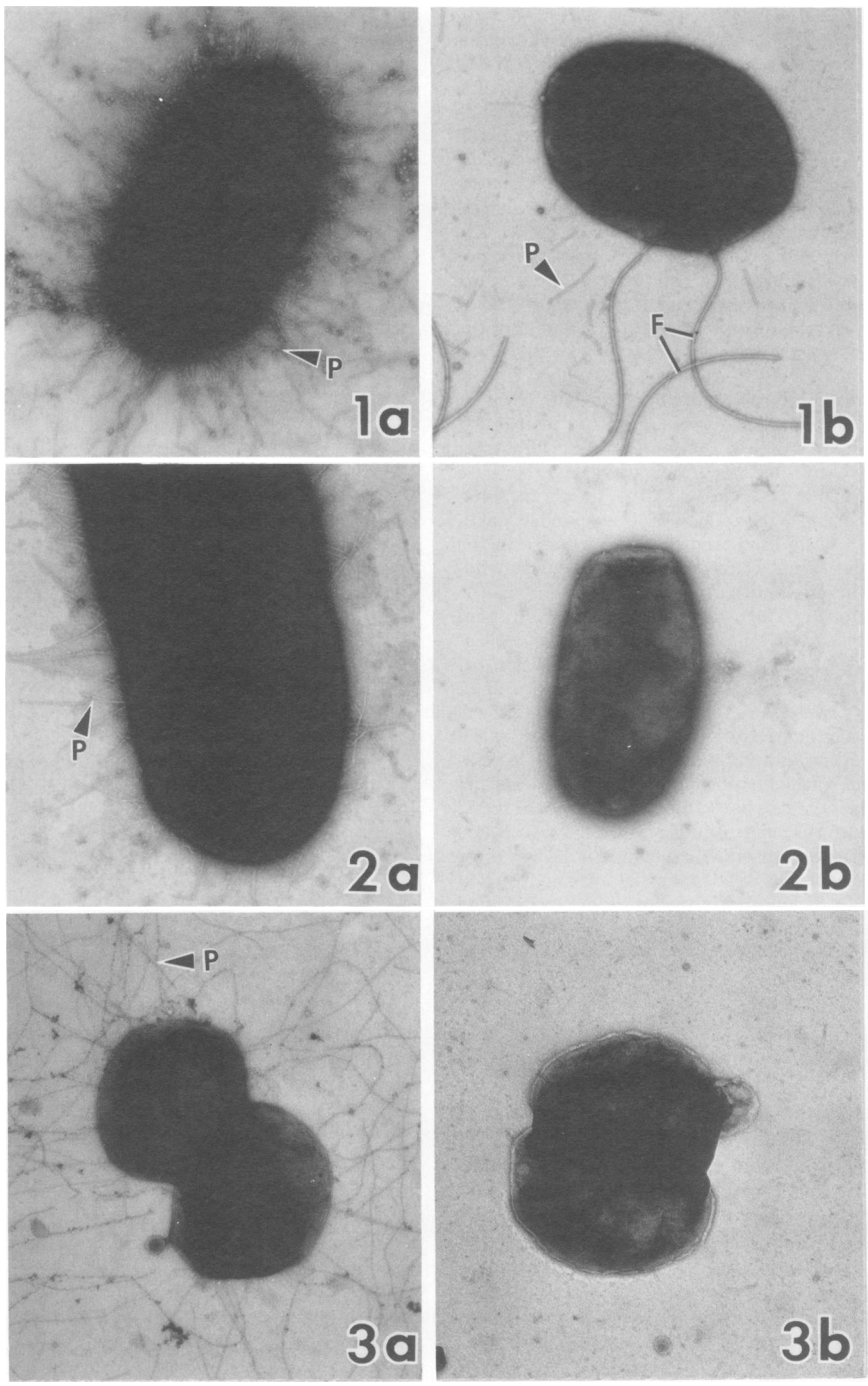

FIG. 1 to 3. *Electronmicrograph of bacteria negatively stained with 0.5% uranyl acetate.*

FIG. 1. *P. mirabilis before (a) and after (b) 20 min of UV irradiation.*

FIG. 2. *E. coli before (a) and after (b) 40 min of UV irradiation.*

FIG. 3. *N. gonorrhoeae before (a) and after (b) 40 min of irradiation. All magnifications are ×25,000 except for Fig. 1b, which is ×40,000. P, Pili; F, flagella.*

TABLE 1. *Effect of UV light on the viability, degree of piliation, and ability of E. coli and P. mirabilis to adhere to buccal cells*

Duration of irradiation (min)	*E. coli*			*P. mirabilis*		
	Colony count	Piliation (%)[a]	Adherence (%)[b]	Colony count	Piliation (%)[a]	Adherence (%)[b]
0	5×10^8	70	93	3×10^8	61	90
1	2×10^3	64	77	1×10^3	43	90
5	0	38	63	0	37	60
10	0	22	39	0	6	32
20	0	6	30	0	0	0
40	0	0	23	0	0	0

[a] Percentage of organisms with 50 or more pili.
[b] Percentage of buccal cells with 25 or more bacteria.

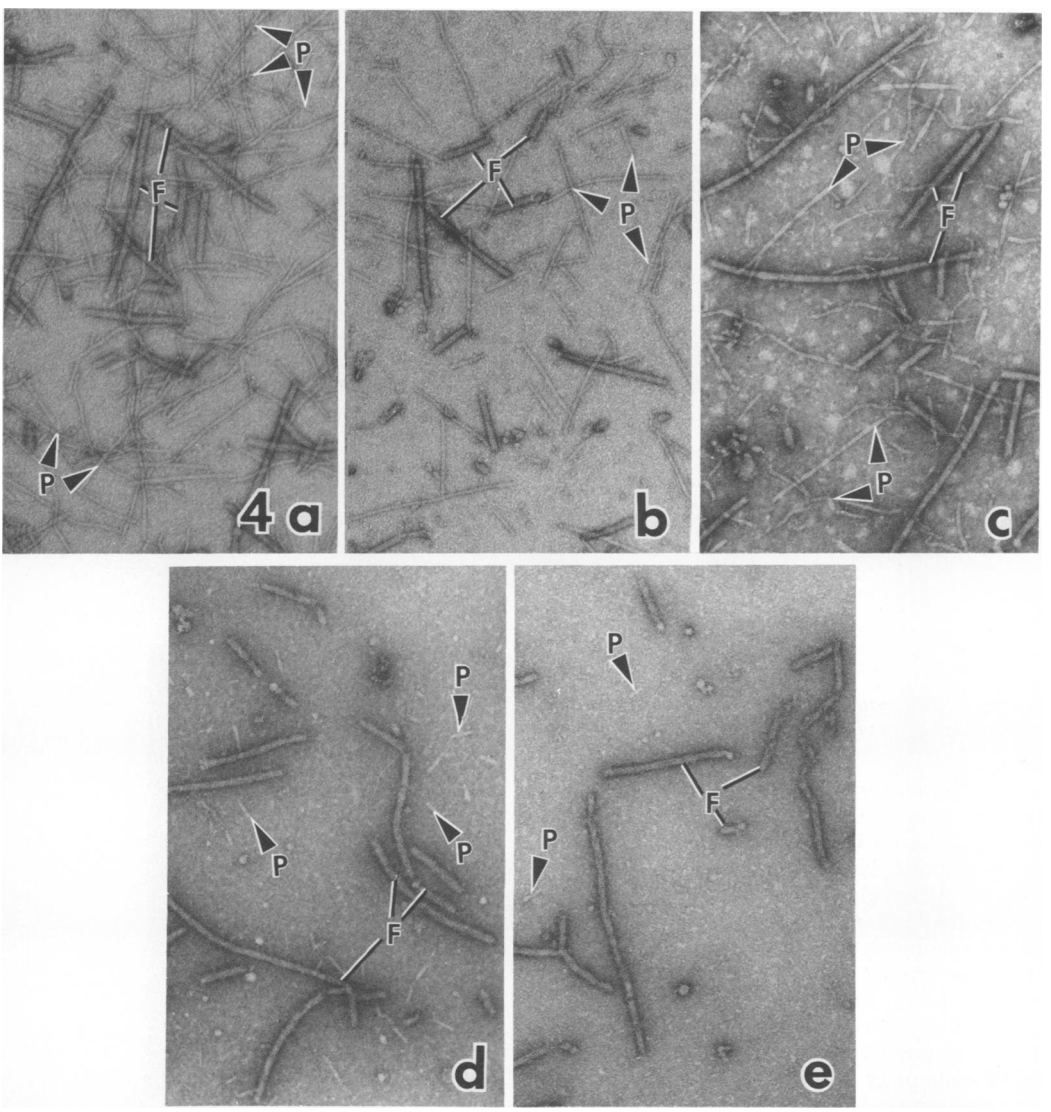

FIG. 4a to e. *Negatively stained isolated Proteus pili irradiated for 0 min (a), 5 min (b), 10 min (c), 20 min (d), and 40 min (e). ×55,000 P, Pili; F, flagella.*

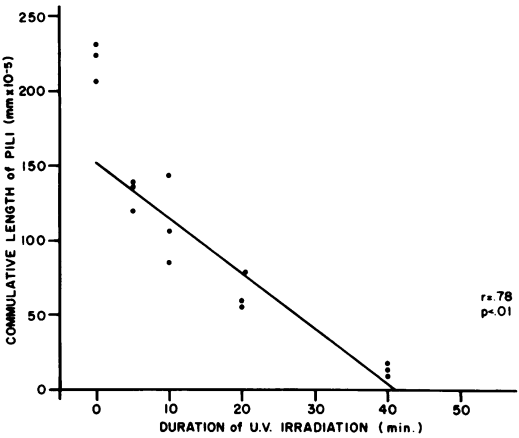

FIG. 5. *Effect of increasing duration of UV irradiation on the optical density of a suspension of partially purified Proteus pili.*

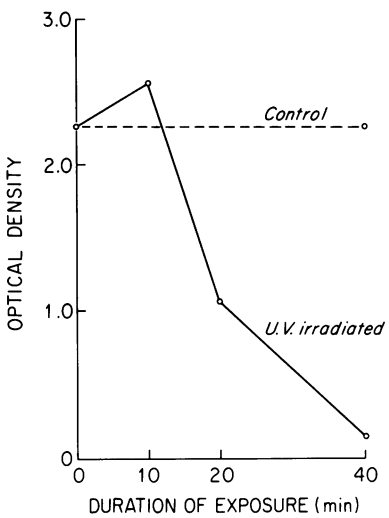

FIG. 6. *Disruption of isolated pili by UV light as determined by nephelometry.*

marked in cultures which had been grown in a medium containing 5-bromouracil (4). Incorporation of this antimetabolite into DNA is known to render cells more sensitive to photoinactivation (4). Although somatic pili were reported not to be affected by the UV irradiation, these authors' results do not necessarily conflict with those reported here. Differences in the experimental design of the two studies such as the composition of the medium through which the UV light passed might have affected the ultimate amount of radiation absorbed by the pili and thus make direct comparison difficult.

How might UV light destroy pili? All pili that have been studied thus far have been shown to be composed of polymerized protein subunits. UV light is known to inactivate proteins by photochemical attack on the aromatic amino acids phenylalanine, tryptophane, and tyrosine and by cleavage of the disulfide bridges of cystine (10). Of the three species studied here the amino acid composition of gonococcal and *E. coli* somatic pili are known. Brinton has shown that the subunits of type I *E. coli* pili contain tyrosine, phenylalanine, and cystine but not tryptophane (1), and Hermodsen et al. have recently reported that all four of these aromatic amino acids are present in the pili of *N. gonorrhoeae* (5). Photodestruction of one or more of these residues might have altered the tertiary structure of the subunits causing them to disaggregate. Alternatively, UV irradiation may cause more profound damage of pili. The observation that irradiation of purified *E. coli* pili generated new, smaller molecular weight protein entities when examined by SDS-polyacrylamide gel electrophoresis suggests that the peptide chains of the pilin molecule had been cleaved by the irradiation. Pili appear to be more sensitive to UV light than flagella. The comparison between these two molecules is pertinent, since both are polymerized protein subunits and are surface structures.

Finally, the results of this study suggest that UV irradiation should be used cautiously (if at all) to inactivate gram-negative bacteria when their pili are to be preserved. Although the amount of irradiation required for complete depiliation far exceeded the lethal dose, a measurable degree of structure and considerable pili function was lost after exposure to UV approaching the bacteriocidal dose.

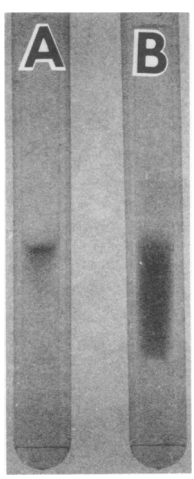

FIG. 7. *SDS-polyacrylamide gel electrophoresis of purified E. coli pili: (A) unirradiated pili; (B) pili irradiated for 30 min.*

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