Brit. J. vener. Dis. (1974) **50**, 354

Surface structures of the gonococcus

A. GRIMBLE AND L. R. G. ARMITAGE
Guy's Hospital, London SE1 9RT

The fine structures of *Neisseria gonorrhoeae*, especially the surface structures, are of interest at this time, when the organism and the infection it causes are being more widely investigated. Ward, Watt, and Glynn (1970) noted the loss of a virulence factor on subculture of *N. gonorrhoeae*. Kellogg, Cohen, Norins, Schroeter, and Reising (1968) had previously described the colonial morphology of the fresh pathogenic strains in contrast to the laboratory subcultures. They noted that, although the nonpathogenic laboratory strain (Type 4 colony) sometimes existed in fresh isolations along with the virulent (Type 1 colony) strain, for the most part the former appeared to arise in subculture out of the latter. They surmised that the nonpathogenic strain usually arose from a previously virulent strain. By suitable methods the pathogenic strain can now be preserved in subculture.

The surface appearance of the freshly isolated micro-organisms seen under the electron microscope differs from that of the subcultured, laboratory strains. Most obviously fimbriae, or pili, are to be seen on virulent strains as opposed to subcultured strains.

The surface structures or appendages which may be seen on bacteria consist of: *flagellae*, whose function is primarily that of motility; and *fimbriae* or *pili*, which are finer appendages, functioning (depending upon the type of pilus) either as something imparting 'adhesiveness' to the organism, or as a means by which metabolites and genetic material are transferred from one bacterium to another. The former pili, or common pili, are shorter; the latter sort are longer.

The accompanying electron photomicrographs are presented to show the kinds of surface structures which appear in the freshly isolated gonococcus.

Material and methods

A negative staining technique was applied. Studies were made of strains of *N. gonorrhoeae* taken direct from patients, and of cultured strains grown for 12 to 48 hrs on ordinary chocolate agar plates and on Thayer-Martin medium. Two stock laboratory strains from Burroughs Wellcome, No. 890 and No. 1000, were also examined. At the time of harvesting, the laboratory strains had usually completed their log-phase growth, whereas in most instances the fresh strains had only arrived at the middle to end of their log-phase growth.

The specimens from the patients and harvests from the plates were obtained with a loop and suspended (using a Pasteur pipette) in 5 drops of distilled water. One drop of 3 per cent. potassium phosphotungstate was added. The suspension was transferred without delay to the electron microscope grids which had been lightly carbon-coated on a film of polyvinyl formol Formvar (stock solution of 1 per cent. w/v Formvar in chloroform, diluted 1 in 20 in chloroform for use). Prolonged suspension of the cocci in distilled water resulted in rupture of the cell membrane by an osmotic pressure effect, and this had to be avoided. The difficulty with fresh strains direct from patients was the larger quantity of debris harvested, and the pus, which has to be scanned. The illustrations provided in the Figures were not taken from any bacterium seen within a leucocyte.

The microscope used was RCA E.M.U3 at 50 KV; 100 KV gave no noticeable improvement in resolution.

Findings and discussion

Fig. 1 demonstrates the 'bald' appearance of the laboratory strains. Fig. 2 *et seq.* demonstrate the features of the long pili to be seen on pathogenic strains of gonococci. Also presented are 'larger appendages' to be seen on cells in material direct from patients and from fresh cultures (24 hrs). These larger surface structures differ from the pili previously described in the literature.

Pili are frequently to be seen in electron micrographs of bacteria. Several methods have been used to distinguish the functions of these structures, and the subject was reviewed some years ago by Meynell, Meynell, and Datta (1968).

Swanson, Kraus, and Gotschlich (1971) published their electronmicrographs of pili on Type 1 colonial strains of gonococci, noting their absence in the nonpathogenic (Type 4 colonial) strains. These authors in their discussion speculated upon the antigenic

Received for publication December 11, 1973

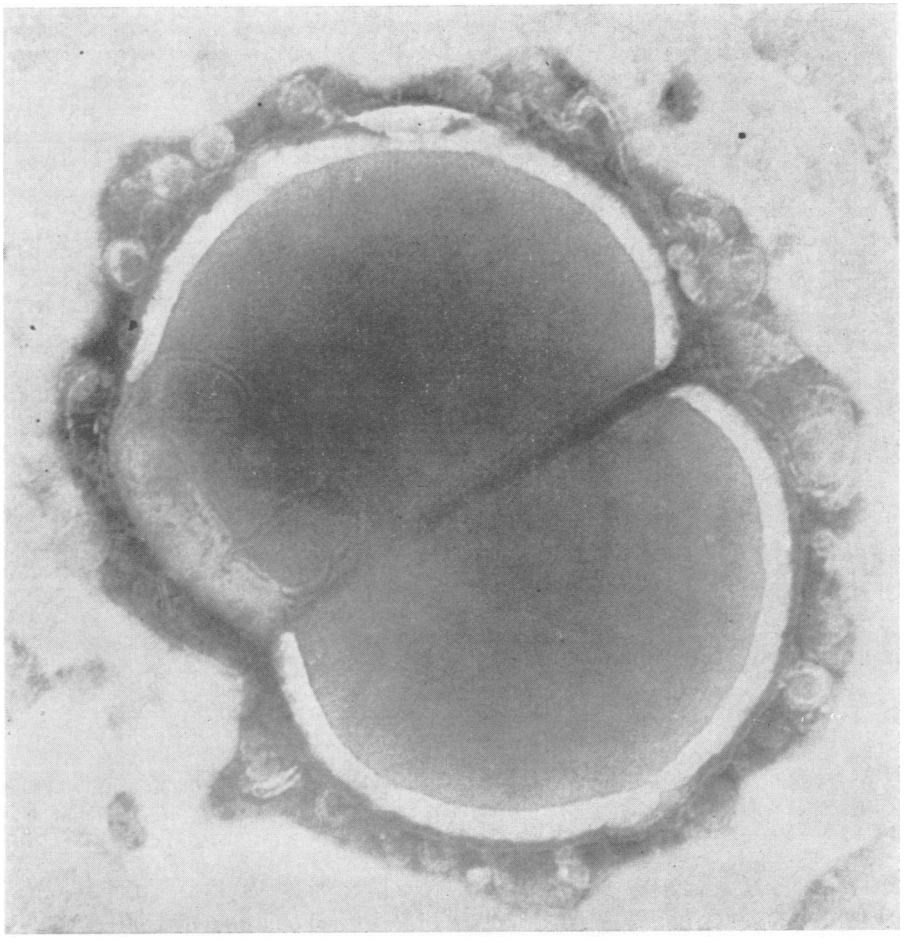


FIG. 1 *Laboratory strain of gonococcus, showing 'bald' appearance of cell-wall outline.* × 50,000

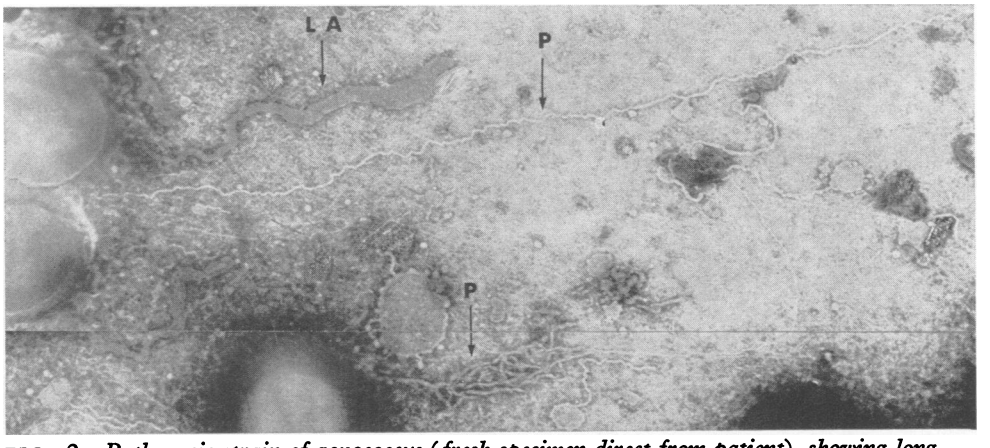


FIG. 2 *Pathogenic strain of gonococcus (fresh specimen direct from patient), showing long pili (P) and a broken 'larger appendage' (LA).* × 7,060

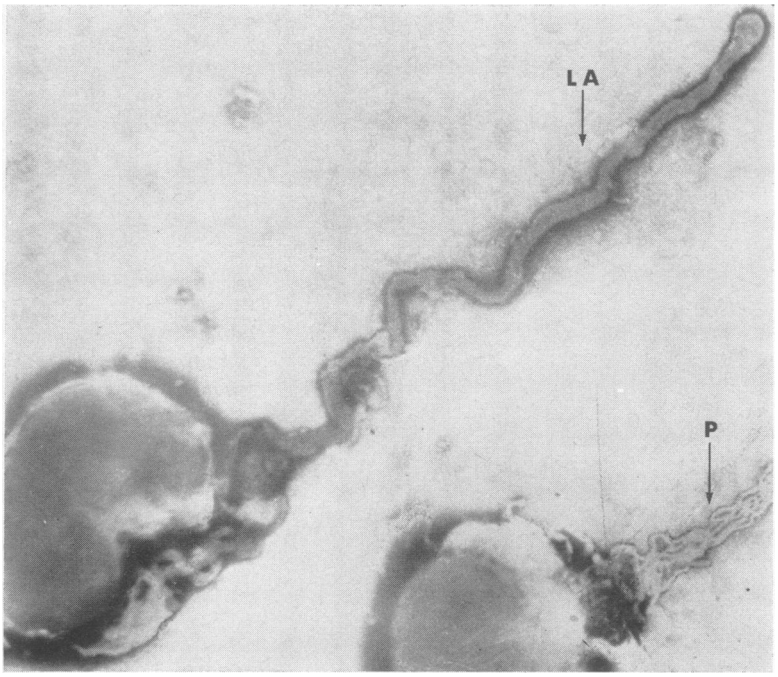


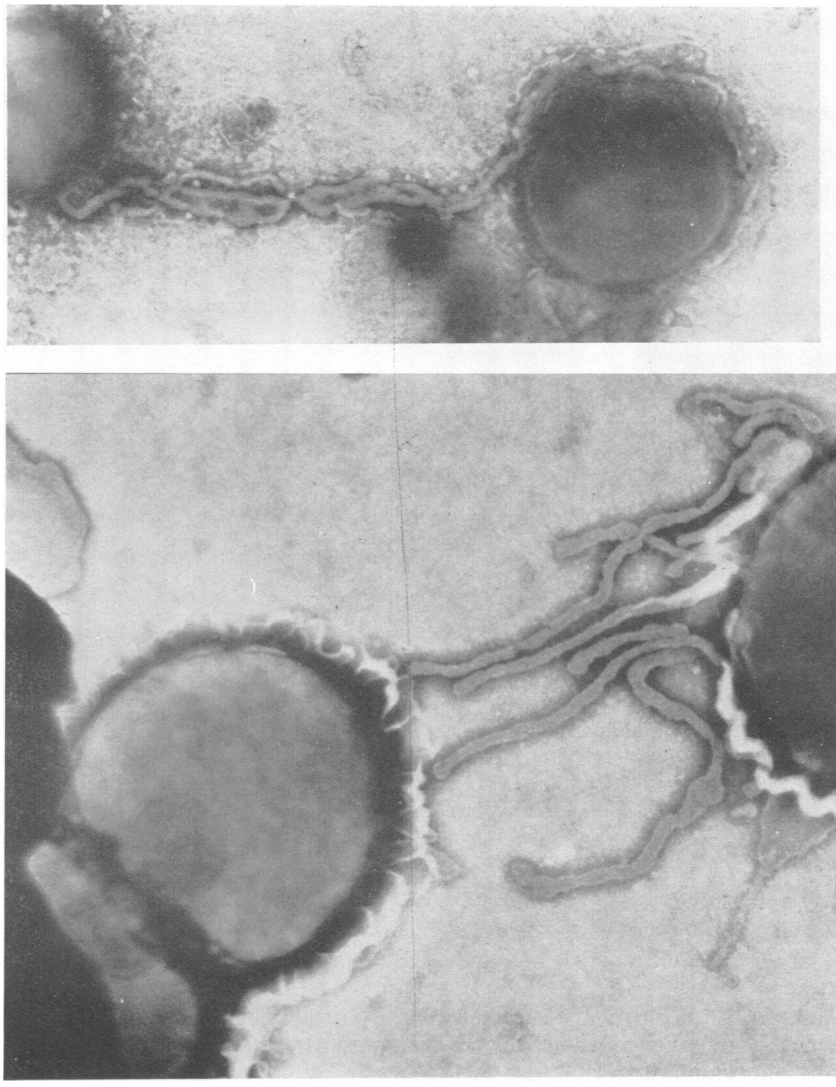
FIG. 3 *Pathogenic strain of gonococcus, showing 'plaited' bundle of pili (P) together with a 'larger appendage' (LA).* × 14.600

role which these pili might play. The pili were long rather than short and were similar to those shown in Figs 2 and 3. These slim structures (Figs 2 and 3 and, less noticably, in other accompanying Figures) appear in all probability to be related to the long slim 'sex pili', F and R specialized pili (the latter responsible for the transference of antibiotic resistance in *E. coli*) investigated by Harden and Meynell (1972). Moreover, they appear to be in the same morphological category of surface structure as the ones demonstrated on the gonococcus by Swanson and others (1971). There do not appear to be represented, in the photographs of Swanson and others, the shorter 'common pili', whose function is one of stabilization or adhesiveness, which would enable the gonococcus to stick to the epithelial cell and resist being washed away. It was not certain whether or not these short pili appeared in our material either, although the surface of pathogenic gonococci appeared 'coated' and piliform in contrast with the 'bald' appearance of laboratory strains (compare Fig. 1 with Fig. 9).

In addition, electronmicrographs of larger appendages are presented (Figs 3 to 8). They were often to be seen broken off and lying free in the grid, singly or in groups. The diameter of these latter surface appendages is in the region of 20-30 μm, whereas the long pili (Figs 2 and 3; and see Swanson and others, 1971) are about 3 to 7 μm in diameter. The larger appendages are present singly or sparsely on the cell, or occasionally exist in profusion, over what appears to be the whole cell surface. At times they may be seen beginning to appear in a cluster from the central fissure of the organism.

One feature which was fairly common to these appendages was the terminal bulb, also to be seen in other pili. Another feature was a 'beading' along the length of the appendage itself: these 'beads' occurred singly or in clusters on the appendage (Figs 6 and 7). Occasionally a break was visible in the wall. These larger appendages seemed to make contact with another bacterial cell (Figs 4 and 5), which might indicate that some metabolic function was being fulfilled.

Whether these larger tube-like structures are, as suggested, surface appendages, or whether they are possibly related in kind to the lipopolysaccharide 'surface blebs' described by Devoe and Gilchrist (1973) in *Neisseria meningitidis*, requires further investigation. Devoe and Gilchrist observed that the origin of their 'blebs' was at the outer membrane layer of the cell-wall, the suggested site of endotoxin



FIGS 4 AND 5 *Pathogenic strain of gonococcus. Further 'larger appendages' demonstrating contact between these structures and other cell surfaces. Fig.* 4: × 10,600. *Fig.* 5: × 32,000

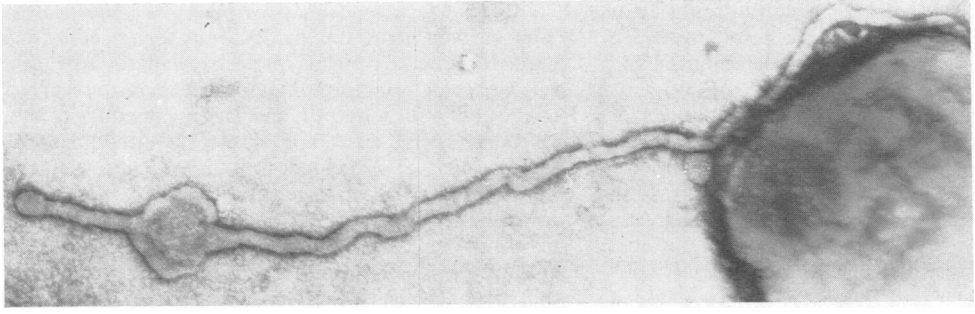


FIG. 6 *Pathogenic strain of gonococcus, showing 'beading' along appendages.* × 17,500

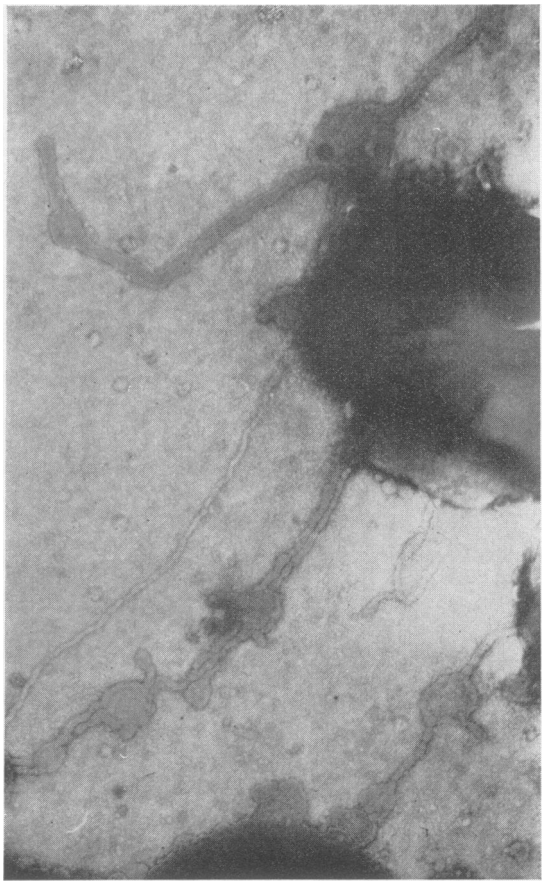


FIG. 7 *Pathogenic strain of gonococcus, showing more frequent 'beading' than in Fig.* 6. × 14,600

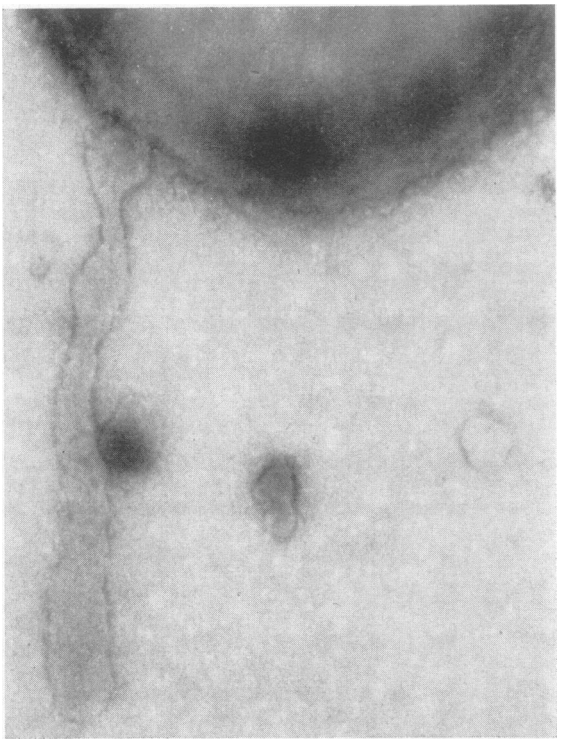


FIG. 8 *Pathogenic strain of gonococcus, showing a 'larger appendage' with its point of junction with the cell-wall and the appearance of material along the outer side of the cell-wall.* × 33,300

production. Surface material with similar features to these so-named 'blebs' is visible in the outer regions of the cell-wall presented by us at high magnification (Fig. 8).

The structure and function of bacterial fimbriae or pili, as well as of the cell-wall, are receiving much attention at the present time. These surface areas and appendages are of importance in respect of antigenic roles as well as metabolic and genetic functions or attributes. In the case of the gonococcus, these aspects are only beginning to receive attention. On the question of their antigenic role and their relationship to pathogenicity, it should be mentioned that pili have been noted in *Neisseria catarrhalis*, *perflava*, and *subflava*, organisms hitherto considered non-pathogenic (Wistreich and Baker, 1971). It is hoped that their study in the gonococcus may produce some of the answers to the problems of antigenicity and cell metabolism posed by this organism.

Summary

The surface structure of *Neisseria gonorrhoeae* has been examined by means of electron microscopy. The cell-wall, material proximal to the external surface of the cell-wall, long pili, and larger appendages have all been noted, and are shown in accompanying illustrations. It is hoped that investigation of this region of the bacterial cell may help to reveal facts concerned with bacterial endotoxin production and may thus be of assistance in clarifying the at present obscure position of the host immune defence against the gonococcus, whether naturally or artificially induced.

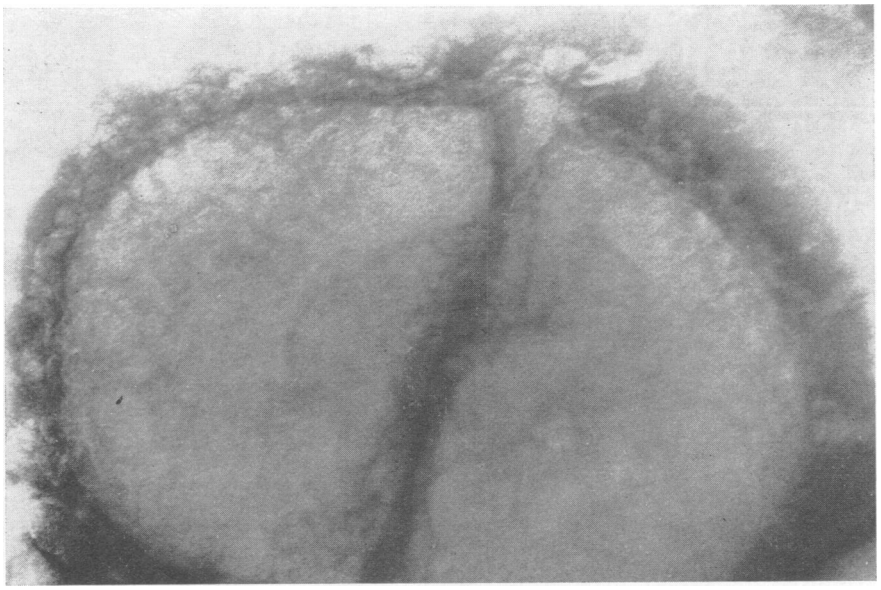


FIG. 9 *High magnification of cell-wall of a pathogenic strain of gonococcus, showing layers of material outside cell-wall: for comparison with Fig.* 1. × 33,300

References

DEVOE, I. W., and GILCHRIST, J. E. (1973) *J. exp. Med.*, **138,** 1156

HARDEN, V., and MEYNELL, E. (1972) *J. Bact.*, **109,** 1067

KELLOGG, D. S., COHEN, I. R., NORINS, L. C., SCHROETER, A. L., and REISING, G. (1968) *Ibid.*, **96,** 596

MEYNELL, E., MEYNELL, G. G., and DATTA, N. (1968) *Bact. Rev.*, **32,** 55

SWANSON, J., KRAUS, S. J., and GOTSCHLICH, E. C. (1971) *J. exp. Med.*, **134,** 886

WARD, M. E., WATT, P. J., and GLYNN, A. A. (1970) *Nature* (*Lond.*), **227,** 382

WISTREICH, G. A., and BAKER, R. F. (1971) *J. gen. Microbiol.*, **65,** 167

Les structures de surface du gonocoque

SOMMAIRE

La structure de surface de *Neisseria gonorrhoeae* a été examinée à l'aide du microscope électronique. La paroi cellulaire, le matériel voisin de la surface externe de cette paroi, de longs poils et des appendices plus importants ont tous été notés comme le montrent les illustrations de cet article. On espère qu'une recherche dans cette partie de la cellule bactérienne pourrait aider à révéler des faits en rapport avec la production d'endotoxines bactériennes et pourrait ainsi servir à clarifier la question jusqu'ici obscure de la défense immunitaire de l'hôte contre le gonocoque, qu'elle soit naturelle ou artificiellement provoquée.