The Cohesive Properties of Variants of *Neisseria gonorrhoeae* Strain P9: Specific Pilus-mediated and Non-specific Interactions

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(Received 9 January 1980)

The cohesive properties of virulent pilated *Neisseria gonorrhoeae* strain P9 (P++) have been compared with those of a non-pilated isogenic variant (P−) possessing the same outer membrane components. The binding of P++ gonococci to buccal epithelial cells was dependent on pH, with an optimum at pH 6.5 to 7.0. This adhesion was markedly inhibited by treatment of the buccal epithelial cells with a neuraminidase/exoglycosidase mixture. In contrast, the binding of P++ gonococci to erythrocytes was unaffected by pH. A possible explanation is that pili bind to a carbohydrate receptor present on buccal epithelial cells but lacking on erythrocytes. The adhesion of P− gonococci to erythrocytes and to buccal epithelial cells was unaffected by pH but enhanced by treatment of the cells with neuraminidase or periodate. Presumably, neuraminic acid residues on host cell surface carbohydrates inhibit adhesion. The finding that P− gonococci bind to amphipathic gels suggests hydrophobic interactions as a possible non-specific mechanism attaching P− gonococci to host cell surfaces.

**INTRODUCTION**

The mucosal surfaces of man are the natural habitat of *Neisseria gonorrhoeae*. A critical determinant of the ability to grow in such an hostile environment is the possession of a mechanism for anchorage to the mucosal cells whose surfaces are continuously washed by flows of mucus and other secretions. This adhesion to the host cell must, of necessity, be mediated by macromolecules expressed at the surface of the gonococcus. The function of the outer envelope in such Gram-negative bacteria is complex (DiRienzo *et al.*, 1978) with different components being involved in structural integrity, in the formation of non-specific diffusion pores as well as in uptake systems for iron, sugars, vitamins, etc. For gonococci, this is further complicated by the need for surface structures to form a diffusion barrier protecting the vulnerable cytoplasmic membrane from the lytic action of antibody and complement (Ward *et al.*, 1978). Clearly, outer membrane molecules with different functions will contribute to the physical characteristics of the gonococcal surface and by their effects on charge density, hydrophobicity and polymer interactions influence the cohesive properties of specific mediators of adhesion.

Pili, protein filaments protruding up to 1 μm from the gonococcal surface, facilitate the binding of gonococci to cells in tissue culture, to human sperm and to Fallopian tube organ cultures (Buchanan, 1977). However, pili are not the sole effectors of gonococcal adhesion since outer membrane proteins have been shown to mediate gonococcal attachment to buccal mucosal cells, erythrocytes and polymorphonuclear (PMN) leukocytes (Lambden *et al.*, 1979). In order to identify the function of pili in gonococcal attachment and investigate
the likely effect of outer membrane components on pilus-mediated adhesion we have compared the cohesive properties of the virulent, pilated gonococcus strain P9 with a non-piliated variant, both of which possess only protein I and lipopolysaccharide as major components of the outer membrane.

METHODS

Bacterial strains and growth conditions. Neisseria gonorrhoeae strain P9 variants were grown on clear typering medium as previously described (Lambden et al., 1979). Colonies of transparent pilated P++ (P9-2) (Lambden et al., 1980) and non-piliated P- (P9-1) variants were picked, purified by single colony isolation and stored in liquid nitrogen.

3H-Labelled gonococci were harvested from plates containing 25 µCi d-[1-3H]glucose ml-1 [500 mCi mmol-1 (18.5 GBq mmol-1); The Radiochemical Centre, Amersham] into complete Dulbecco phosphate-buffered saline pH 7.4 (PBS; Oxoid). The gonococci were suspended by vortex mixing and aggregates were removed by centrifugation at 150 g for 1 min. The resulting gonococcal suspensions were diluted to a cell density of 2.8 x 10^8 colony-forming units (c.f.u.) ml-1 in Basal Medium Eagle (Modified) with Hanks’ balanced salts (Flow Laboratories) buffered with 0.05 M-N-2-hydroxyethylpiperazine-N'2-ethanesulphonic acid (HEPES) to pH 7.4 (TCM).

Determination of gonococcal attachment. Gonococcal attachment to host cells was determined as described previously (Lambden et al., 1979). Buccal epithelial cells and erythrocytes were collected from volunteers, washed three times in PBS and finally suspended at a packed cell volume (p.c.v.) of 10% in TCM. 3H-Labelled gonococci and host cells were incubated together with gentle shaking at 37°C. Host cells with adherent gonococci were separated from free gonococci by centrifugation through a cushion of dextran. The pellet was recovered following aspiration of the dextran containing unattached gonococci and finally counted in a Packard TriCarb liquid scintillation counter. Before estimation of the numbers of bound gonococci, erythrocytes were lysed with 1 M-NaOH and decolorized by incubation with 30% (v/v) H2O2 at 50°C for 1 h. Attachment of gonococci to various alkyl-substituted agarose gels (Miles Laboratories Ltd, Stoke Poges, Slough) was performed using 20% (v/v) gel suspensions.

Under control conditions with tests repeated 16 times the mean value for binding P++ gonococci to octylagarose was 11.3% with a standard deviation of 0.59%; P- gonococci showed 10.5% binding with a standard deviation of 0.4%. Experiments using gonococci at different concentrations (1 x 10^7 to 1 x 10^9 c.f.u. ml^-1) and varying the binding time demonstrated that under standard experimental conditions binding sites for P++ and P- gonococci on alkyl-agarose gels were unsaturated.

Experiments were performed to study the effect on gonococcal attachment of (i) time; (ii) temperature; (iii) pH (determined in TCM adjusted to the desired pH by the addition of 1 M-Tris or 1 M-maleic acid); (iv) the cations Ca2+, Mg2+, Mn2+ and Fe3+ (using host cells washed in PBS containing 5 mM-EDTA); (v) the amino acids histidine, tyrosine and tryptophan at a final concentration of 1 mM; (vi) the sugars lactose, melibiose, mannose, a-methyl mannoside, galactose, a-methyl galactoside, β-methyl galactoside, fucose, N-acetylgalactosamine and N-acetylglucosamine; (vii) the gangliosides GM1, GD1a, GT1 and mixed; and (viii) several substituted agarose gels.

Periodate oxidation of gonococci and host cells. Gonococci were harvested from solid medium into 0.1 M-sodium acetate buffer pH 5.5 containing 9 mg NaCl ml-1 and 1 mg CaCl2 ml-1 to give a final cell density of about 1 x 10^9 to 5 x 10^9 c.f.u. ml^-1. The gonococcal suspension was diluted with an equal volume of acetate buffer containing 20 mM-sodium periodate and incubated with gentle shaking for 30 min at 4°C. The organisms were then washed twice, resuspended in 0.1 M-Tris/maleate pH 7.4 containing 9 mg NaCl ml-1 and 1 mg CaCl2 ml-1, and added to an equal volume of the same buffer containing 10 mM-sodium borohydride. After incubation at 4°C for 30 min, the gonococci were washed twice in PBS and diluted to the required cell density in TCM. Control organisms were treated in an identical manner except that sodium periodate was omitted.

Buccal epithelial cells and erythrocytes were subjected to periodate oxidation by the above procedure at a p.c.v. of 1% and 4%, respectively.

Enzyme treatment of host cells. (i) Erythrocytes were treated with trypsin as follows: erythrocytes were recovered from fresh heparinized blood by centrifugation, washed three times in PBS and resuspended in PBS to a p.c.v. of 10%. Trypsin (EC 3.4.21.4; Sigma Type III) was added to the erythrocyte suspension to give a final concentration of 1 mg ml^-1 and the cells were incubated for 1 h at room temperature. Finally, cells were washed twice and resuspended to the required cell density in TCM. (ii) Buccal epithelial cells and erythrocytes were treated with neuraminidase as follows: buccal epithelial cells and erythrocytes were suspended at a p.c.v. of 10% in 0.05 M-sodium acetate buffer pH 5.5 containing 9 mg NaCl ml-1 and 1 mg CaCl2 ml-1. Neuraminidase (EC 3.2.1.18; Behring) was added to give 0.1 U ml^-1 and the cells were incubated at 37°C for 1 h. The release of N-acetylmuraminic acid was monitored using the thiobarbituric acid method.
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(Waravdekar & Saslaw, 1959). The cells were finally washed and resuspended in TCM. (iii) Buccal epithelial cells were treated with mixed exoglycosidases (Miles Laboratories) at a final concentration of 1 or 10 mg ml⁻¹ in 0.05 M-sodium acetate buffer pH 5.5. Neuraminidase (0.1 U ml⁻¹) was also added to the exoglycosidase mixture as this activity was absent from the preparation. The exoglycosidase preparation contained the following enzyme activities: α- and β-N-acetyhexosaminidase, α- and β-mannosidase, α- and β-glucosidase, α- and β-galactosidase, α-L-fucosidase and β-xylanase.

Preparation of ¹²⁵I-labelled polysaccharide-methyl-bovine serum albumin conjugate. Lipopolysaccharide (LPS) from N. gonorrhoeae P9 was prepared from a cholate extract of outer membrane material (Heckels, 1977). Core oligosaccharide was prepared by partial acid hydrolysis of LPS according to the method of Perry et al. (1975) and keto groups were reduced with sodium borohydride. Reduced P9 core oligosaccharide was coupled through free carboxyl groups on 2-keto-3-deoxyoctoic acid (KDO) to free amino groups on the methyl ester of bovine serum albumin (methyl-BSA; Sigma) using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.HCl (EDC). Reduced core oligosaccharide (10 mg) was mixed with methyl-BSA (10 mg) in 2 ml water. EDC (50 mg) was added to the mixture and the pH was adjusted to 4.5. The pH was maintained at 4.5 for 1 h and then the reaction mixture was allowed to stand at room temperature overnight. The methyl-BSA–oligosaccharide conjugate was removed from the reaction products and uncoupled polysaccharide by chromatography on a Sephadex G-50M column (1.5 x 35 cm). The amount of carbohydrate associated with the protein peak was determined by the phenol/sulphuric acid method (Dubois et al., 1956). The analysis of the conjugate showed a ratio of 5 mol polysaccharide to 1 mol methyl-BSA. Samples of the P9 polysaccharide–methyl-BSA conjugate and methyl-BSA were then radiolabelled with ¹²⁵I using the chloramine-T method (Greenwood et al., 1963).

Gangliosides. The gangliosides GM1, GD1a, GT1 and mixed gangliosides were a generous gift of W. E. van Heyningen (Sir William Dunn School of Pathology, University of Oxford). Gangliosides were dissolved in chloroform/methanol (1:1, v/v) and dried to a film in a flask under a stream of N₂. The ganglioside film was shaken with a small volume of TCM and the resulting suspension was sonicated (MSE, 100 W) for 2 min under a stream of N₂. The ganglioside micelles thus produced were used in attachment-inhibition experiments.

RESULTS

Factors affecting gonococcal attachment

The time course for gonococcal attachment to buccal epithelial cells is shown in Fig. 1. The binding of both pilated (P+++) and non-piliated (P⁻⁻⁻) variants was essentially maximal after 4 h, although the overall binding of the P++ variant was at least twice that of the P⁻ variant. This adhesion was temperature-dependent. The binding of P++ organisms decreased from 17% at 37 °C to 8.7% at 4 °C, while P⁻ organisms showed a decrease from 8.7% binding at 37 °C to 6.3% binding at 4 °C.

The influence of pH on gonococcal attachment to buccal epithelial cells produced striking differences between P++ and P⁻ organisms (Fig. 2). The binding of P++ organisms increased almost fivefold over the pH range 4.5 to 7.0, with a decrease in attachment above pH 7.0, whereas the binding of P⁻ organisms was progressively reduced as the pH was raised from 4.5 to 7.5. Interestingly, at pH 4.5 the binding of P⁻ organisms was always greater than that of P++ organisms. In contrast, the attachment of gonococci to erythrocytes was totally unaffected by pH over the range tested (Fig. 2), demonstrating a clear difference in the mechanisms of attachment to the two host cells. As with buccal epithelial cells, P++ organisms showed a twofold greater binding to erythrocytes than did P⁻ organisms. The binding of gonococci to EDTA-washed host cells was not dependent on the presence of divalent cations in the attachment medium. The addition of either 5 mM Ca²⁺, Mg²⁺ or Mn²⁺ increased the binding of the P++ variant from 10 to 13%, but was without effect on the P⁻ variant. The presence of 5 mM Fe³⁺ caused marked aggregation of P++ gonococci preventing meaningful assessment of the effect of Fe³⁺ on the binding of gonococci to host cell membranes.

Nature of the attachment mechanism

A variety of compounds was tested for their ability to inhibit attachment of gonococci to host cells. The amino acids histidine, phenylalanine, tyrosine and tryptophan at a final concentration of 1 mM had no effect on gonococcal binding to host cells, and neither did
The sugars lactose, melibiose, mannose, α-methyl mannoside, galactose, α-methyl galactoside, β-methyl galactoside, fucose, N-acetylglucosamine and N-acetylgalactosamine.

The effect of gangliosides on the attachment of intact gonococci to buccal epithelial cells is shown in Table 1. Clearly, binding of the P⁻ variant was unaffected by the presence of gangliosides in the attachment medium. The P++ variant, however, aggregated much more in the presence of ganglioside GT1 which resulted in an apparent inhibition of binding to buccal epithelial cells when the control counts were subtracted from the test.

Non-specific interactions

The extent to which hydrophobic interactions influence binding of intact gonococci was measured using agarose gels, substituted with non-polar ligands (Table 2). The degree of adsorption to the gels was similar for both P++ and P⁻ variants, both demonstrating a marked increase in attachment according to the chain length of the alkyl substituent.

Non-specific interactions between charged groups were investigated by measuring binding to CM Bio-gel A and DEAE Bio-gel A (Bio-Rad) over the pH range 5.0 to 7.5. In each case, binding over the entire pH range was constant and there was no apparent difference between P++ and P⁻ organisms, although attachment of gonococci was greater to diethylaminoethyl groups on DEAE Bio-gel (16.6%) than to carboxyl groups on CM Bio-gel (0.8%).

Nature of the gonococcal adhesin

In an attempt to understand the molecular basis of the attachment mechanism, the gonococcal surface was modified by a variety of agents (Table 3). Ultraviolet irradiation of whole gonococci did not affect the binding of the P⁻ variant whereas the attachment of the P++ variant was decreased by some 50%, presumably due to denaturation of surface pili. Modification of surface carbohydrates was achieved using glycosidases and by periodate oxidation followed by borohydride reduction. Binding of the P⁻ variant was little affected although
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Table 1. Effect of gangliosides on the binding of §H-labelled pilated (P++) and non-pilated (P-) variants of N. gonorrhoeae strain P9 to buccal epithelial cells

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Percentage of P++ variants sedimenting</th>
<th>Percentage of P- variants sedimenting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With buccal cells</td>
<td>Alone</td>
</tr>
<tr>
<td>None</td>
<td>21.1 5.7</td>
<td>8.8 0.6</td>
</tr>
<tr>
<td>GM1</td>
<td>23.3 9.1</td>
<td>7.3 0.9</td>
</tr>
</tbody>
</table>
| GD1a       | 28.0 21.5 | 7.5 0.9 | * Gonococci were exposed to ganglioside micelles at a concentration of 120 μg ml⁻¹.

Table 2. Comparative adsorption to hydrophobic gels of §H-labelled pilated (P++) and non-pilated (P-) variants of N. gonorrhoeae strain P9

Values given are the percentage of organisms recovered with the gel beads and are the mean of several experiments.

<table>
<thead>
<tr>
<th>Non-polar ligand on agarose gel</th>
<th>Variant</th>
<th>None</th>
<th>Ethyl</th>
<th>Butyl</th>
<th>Hexyl</th>
<th>Octyl</th>
<th>Decyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-</td>
<td>1.2</td>
<td>4.4</td>
<td>4.1</td>
<td>7.9</td>
<td>10.5</td>
<td>14.1</td>
<td></td>
</tr>
<tr>
<td>P++</td>
<td>1.1</td>
<td>4.1</td>
<td>3.7</td>
<td>9.7</td>
<td>11.3</td>
<td>12.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Effect of modifying gonococcal surface components on the binding of §H-labelled pilated (P++) and non-pilated (P-) variants of N. gonorrhoeae strain P9 to buccal epithelial cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variant P++</th>
<th>Variant P-</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>32</td>
</tr>
<tr>
<td>U.v. irradiation†</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td>Periodate/NaBH₄</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>NaBH₄ control</td>
<td>100</td>
<td>39</td>
</tr>
</tbody>
</table>

* To facilitate comparison of results the percentage binding of untreated P++ variants to buccal epithelial cells (31%) is expressed as 100.
† U.v. irradiation: 254 nm at 5 cm (1240 μW cm⁻²) for 1 h.

borohydride reduction resulted in a small increase in attachment. In contrast, the binding of the P++ variant to buccal epithelial cells was greatly decreased (70%) to the level obtained for the P- variant. Attempts to block pilus-mediated adhesion using the galactose-specific lectin Ricin I were unsuccessful. In the presence of 0.08 μM-Ricin I the apparent binding of P++ to cells was increased from 13 to 24% presumably due to cross-linking by the multivalent lectin.

The possibility that lipopolysaccharide (LPS) in the gonococcal outer membrane was contributing to the attachment process was examined using a gonococcal core oligosaccharide–methyl-BSA conjugate. No significant binding of the conjugate to buccal cells was detected over a 100-fold range of concentration (1 to 100 μg ml⁻¹). Approximately 3% of the added conjugate was recovered with the buccal epithelial cells, whereas a greater proportion (10%) of the unsubstituted methyl-BSA was found attached under identical conditions. Presumably, hydrophobic interactions accounted for the binding of §I-labelled methyl-BSA to buccal epithelial cells and this was significantly impaired in the core oligosaccharide–methyl-BSA conjugate.
Table 4. Effect of modifying the surface carbohydrates of buccal epithelial cells on the binding of \(^3\)H-labelled pilated (P++) and non-piloted (P-) variants of N. gonorrhoeae strain P9

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variant P++</th>
<th>Variant P-</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>92</td>
<td>30</td>
</tr>
<tr>
<td>Neuraminidase plus exoglycosidase (1 mg ml(^{-1}))</td>
<td>73</td>
<td>34</td>
</tr>
<tr>
<td>Neuraminidase plus exoglycosidase (10 mg ml(^{-1}))</td>
<td>22</td>
<td>35</td>
</tr>
<tr>
<td>Periodate/NaBH(_4)</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td>NaBH(_4) control</td>
<td>100</td>
<td>31</td>
</tr>
</tbody>
</table>

* To facilitate comparison of results the percentage binding of P++ variants to untreated buccal epithelial cells (26.6 %) is expressed as 100.

Table 5. Effect of modifying the erythrocyte surface on the binding of \(^3\)H-labelled pilated (P++) and non-pilated (P-) variants of N. gonorrhoeae strain P9

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variant P++</th>
<th>Variant P-</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>64</td>
</tr>
<tr>
<td>Trypsin</td>
<td>114</td>
<td>114</td>
</tr>
<tr>
<td>Neuraminidase</td>
<td>107</td>
<td>105</td>
</tr>
<tr>
<td>Periodate/NaBH(_4)</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>NaBH(_4) control</td>
<td>104</td>
<td>69</td>
</tr>
</tbody>
</table>

* To facilitate comparison of results the percentage binding of P++ variants to untreated erythrocytes (37 %) is expressed as 100.

**Modification of the host cell surface**

The effect of modifying the surface carbohydrates of buccal epithelial cells is shown in Table 4. The binding of the P- variant was unaffected by any of the treatments of buccal epithelial cells. By contrast, attachment of the P++ variant to buccal epithelial cells pretreated with a mixture of exoglycosidases was dramatically decreased to a level below that found for the P- variant. However, modification of buccal epithelial cell surface carbohydrates by treatment with neuraminidase or periodate oxidation followed by borohydride reduction failed to decrease the attachment of the P++ variant.

Modification of the erythrocyte surface presented a somewhat different picture (Table 5). Removal of sialic acid residues with neuraminidase or removal of surface glycoprotein with trypsin enhanced the binding of the P- variant to the same level as the P++ variant. In contrast to the buccal epithelial cells, periodate oxidation of the erythrocyte surface resulted in a decreased attachment of the P++ variant to the level obtained for the P- strain.

**DISCUSSION**

Gonococci infecting mucosal surfaces exist in a complex environment rich in mucins and varying in ionic composition. The pH optimum (pH 6 to 7) for attachment of P++ gonococci to buccal epithelial cells is appropriate to the preferred sites of infection in the urethra and endocervix. In women during the menstrual cycle the pH of endocervical mucus varies from 5.9 to 7.3 (Kroeks & Kremer, 1977) while the urethra may be flushed with urine with a pH range of 4.8 to 8.0 and, in men, prostatic secretions at a pH of 6.45. By contrast, the squamous lining of the vagina which is immune to gonococcal infection is bathed with acid secretions ranging in pH from 3.5 to 5.3. Mardh & Weström (1976) reported that gonococcal attachment to vaginal epithelial cells was enhanced threefold at pH 4.5 compared to pH 7.5, while Pearce & Buchanan (1978) showed comparable increases in the binding of purified,
radio-iodinated pili to buccal epithelial cells. Recent work in this laboratory (Lambden et al., 1980) has shown that two distinct types of pili produced by variants of a single strain of gonococcus P9 differ markedly in their pH optima for attachment to buccal epithelial cells. Whether pili with differing binding optima are important in gonococcal adhesion to different mucosal surfaces is unknown but there is now evidence of phenotypic change in gonococci induced by the physiological conditions of the host, for example, change in outer membrane composition induced by female sex hormones and proteases (James & Swanson, 1978).

Unlike the adhesion to epithelial cells, the attachment of P++ gonococci to erythrocytes was unaffected by pH over the range 4.5 to 7.5. This suggests differences in the mechanism of attachment of these cell types. One possibility is that the erythrocyte membrane, which is significantly different from that of other cells in the absence of HLA antigens (Barnstable et al., 1978), lacks receptors for gonococcal pili. Certainly, gonococcal pili readily bind to non-specific surfaces such as polystyrene and, like adhesion to cell membranes, this attachment is temperature-dependent (Buchanan, 1978). Further, the suggestion that pili serve to penetrate the electrostatic repulsive barrier between the negatively charged surfaces of the gonococcus and host cell membranes (Heckels et al., 1976) is consistent with the finding that removal of charged groups from the erythrocyte surface by treatment with trypsin or neuraminidase increases the binding of P– gonococci to the same value as P++ gonococci. In this case, adhesion of P– gonococci to the modified erythrocyte surface must be due to other surface components such as outer membrane protein I or LPS. There is evidence to suggest that the adhesion of Salmonella typhimurium to macrophages results from the interaction of sugar residues on the bacterial LPS with 'receptors' on the host cell surface (Freimer et al., 1978). This adhesive mechanism does not apply to gonococcal epithelial cell interactions since the gonococcal core oligosaccharide–methyl-BSA complex does not attach either to erythrocytes (results not presented) or buccal epithelial cell membranes. The non-specific nature of the attachment of P– gonococci to erythrocyte surfaces is further supported by the finding that variants possessing additional outer membrane proteins show specific adhesion to epithelial cells but impaired binding to erythrocytes (Lambden et al., 1979).

The importance of hydrophobic interaction in gonococcal attachment could not be assessed using currently available methods. The usual technique of hydrophobic interaction chromatography permits bacteria to bind to the gel in the presence of 1 M-ammonium sulphate and then measures the elution profile on stepwise reduction in the concentration of ammonium sulphate while increasing the concentration of Triton X-100 from 0 to 0·1 % (v/v) (Stjernström et al., 1977). Unfortunately, the outer membrane of gonococci is extremely fragile and using 3H-labelled gonococci we have shown that the eluate consists of solubilized gonococcal components and not intact organisms. The alternative approach of partition in an aqueous two-phase system consisting of 4·4 % (w/w) polyethylene glycol 600 and 6·2 % (w/w) Dextran T500 (Stendahl et al., 1973) could not be applied to gonococci since polyethylene glycol causes aggregation of gonococcal pili (unpublished observations). The simple technique described here permitted comparison of the relative hydrophobicity of gonococcal variants at ionic concentrations comparable to those on mucosal surfaces. The binding of gonococci to the amphipathic gels showed a direct relationship between gonococcal binding and the length of the alkyl substituent from agarose Co to C10. The concentration of alkyl groups is comparable on all gels [0·2 mol substituent (mol galactose)-1; manufacturers' data] suggesting that lipophilic interaction between the gonococcal surface and the hydrocarbon chains accounts for the marked increase in binding to agarose C6, C8 and C10.

These results clearly establish that the outer membranes of gonococci possess hydrophobic pockets or regions capable of interactions with any like groups on host cell membranes. Further, pili confer minimal advantages in binding to agarose C6, C8 or C10. This finding is in marked contrast to the report that the pilus-like K88 antigen was solely responsible for the binding of smooth Escherichia coli to amphipathic gels (Smyth et al., 1978). The adhesion
forces.

of the gonococcal outer membrane to the mucosal cell surface occurs in an aqueous environ-

ment; the exclusion of hydrophobic groups from the water lattice will facilitate close

approximation of hydrophobic surfaces permitting bonding by London-van der Waals’

forces.

Treatment of buccal epithelial cells with an exoglycosidase mixture decreased pilus-

mediated adhesion to 22 % of the control value. Although we cannot exclude pilus-mediated

binding to a single sugar with a unique conformation, this is unlikely because adhesion was

unaffected by the presence of the constituent sugars of cell surface glycolipids and glyco-

proteins. One explanation may be that the pilus receptor is a polysaccharide with a specific

unaffected by the presence of the constituent sugars of cell surface glycolipids and glyco-

structures.

approximation of hydrophobic surfaces permitting bonding by London-van der Waals’

forces.

This work was supported by a Medical Research Council Programme Grant.

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