SPECIFIC LECTIN-LIKE INTERACTIONS ASSOCIATED WITH BACTERIAL ATTACHMENT TO HOST CELLS

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FRONTISPIECE

What is firmly established cannot be uprooted.
What is firmly grasped cannot slip away.
It will be honoured from generation to generation.

Lao Tsu (Sixth century B.C.)

To my wife, Mary, for her support and
encouragement throughout these studies

CONTENTS

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ABSTRACT

1. Lectin-like material has been extracted from Escherichia coli NCTC 10418, Escherichia coli NCTC 4428, Salmonella typhimurium LT2 and Corynebacterium parvum NCTC 10390. The crude, lectin-containing extracts from these organisms were purified by affinity chromatography on Sepharose 4B, to which yeast mannan had been covalently linked. Fractions were screened for mannose-specific lectin activity by a yeast agglutination assay. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to study the protein profiles of the extracts, and to relate lectin-like activity of the purified extracts to particular proteins. The possible role of these mannosephilic lectins is discussed in relation to adherence of the organisms to host tissues, particularly to phagocytic cells.

2. An adherence assay has been developed for the study of attachment of group B streptococci (GBS) to buccal epithelial cells (BEC). Some general features of this interaction are described.

3. The characteristics of a sonicate of type III GBS, which inhibits the binding of GBS to BEC, have been investigated. The inhibitory component of the sonicate is shown to be heat-labile, but unaffected by periodate oxidation.

4. Results are presented which indicate that a protein on the surface of GBS mediates adherence of the organism to BEC. Membrane lipoteichoic acid, a major adhesin for group A streptococci, is shown not to play a major role in the adherence mechanism of GBS.

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5. Evidence gained from experiments in which BEC were pretreated with sodium metaperiodate has suggested that the epithelial cell receptor for GBS is a carbohydrate residue. This finding was extended by the results of sugar-inhibition studies, which have shown that N-acetyl-D-glucosamine inhibits, in a dose-response fashion, the binding of GBS to BEC. It is postulated that a bacterial lectin, with a specificity for N-acetyl-D-glucosamine, may be responsible for attachment of GBS to BEC.

6. The effects of growth of GBS in sub-minimal inhibitory concentrations of penicillin, on their subsequent attachment to BEC, are described. At very low concentrations of penicillin there was some evidence for a reduction in the degree of binding observed.

DECLARATION

The composition of this thesis, and the investigative procedures described, were personally designed and performed by the author.

ABBREVIATIONS

αMM	α-methyl-D-mannoside
BEC	Buccal epithelial cell(s)
CAMP	Cyclic adenosine monophosphate
CFA	Colonisation factor antigen
Con A	Concanavalin A
DEAE	Diethylaminoethyl
EDTA	Ethylenediaminetetraacetic acid
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
GBS	Group B streptococcus/streptococci
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MR	Mannose-resistant
MS	Mannose-sensitive
MW	Molecular weight
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline (0.01M, pH 7.4, unless stated otherwise)
PGP	Polyglycerophosphate
RES	Reticuloendothelial system
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
WGA	Wheat germ agglutinin

INTRODUCTION

The 'Introduction' to this thesis will be presented as eight separate sections, because the diversity of material which has to be covered precludes its presentation as a single unit. Various aspects of bacterial adhesion, together with related information, are described in the first five sections. This is followed by two sections which deal individually with the immunological activities of Corynebacterium parvum, and with group B streptococcal infections, both of which are central to the study. The aims of the investigation are listed in a concluding section.

A note on terminology

There is disagreement among research workers regarding the terminology for nonflagellar bacterial appendages. In recent years, the two terms that have gained general acceptance are 'fimbriae', introduced by Duguid et al. (1955), and 'pili', the designation which Brinton (1965) has suggested should replace the term 'fimbriae'. In order to avoid confusion, the term 'fimbriae' has been used predominantly throughout this thesis to describe all nonflagellar bacterial appendages other than those involved in the transfer of nucleic acid, as suggested by Ottow (1975). There are two exceptions to this rule. The term 'pili' has been used to describe the filamentous appendages associated with adhesion of gonococci to epithelial surfaces. This decision was taken because all workers in the field of gonococcal attachment have referred to these structures as 'pili', not 'fimbriae'.

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Secondly, the structures shown by electron microscopy to radiate from the surface of group A streptococci are referred to as 'fibrillae'. This term has been proposed by Beachey, Eisenstein and Ofek (1981) because unlike true fimbriae, fibrillae are a network composed of lipoteichoic acid and cell surface proteins, and do not, therefore, have a distinct structure.

1 Background

Mechanisms of microbial attachment have stimulated intense interest over the past decade, since it has become obvious that the ability of an organism to attach to a surface can be an important factor in its survival. In the field of medical microbiology this finding is of particular relevance, as the initial attachment of a micro-organism to host tissue surfaces is an important first step in the pathogenesis of many types of infectious diseases (Gibbons and van Houte, 1971).

Duguid et al. performed the earliest work concerning the attachment of micro-organisms to eukaryotic cells. In a series of papers (Duguid and Gillies, 1957; Duguid and Gillies, 1958; Duguid, 1959; Duguid, Anderson and Campbell, 1966) it was shown that many enterobacteria caused agglutination of human and animal red blood cells. The strains of bacteria which caused agglutination were shown by electron microscopy to possess nonflagellar filamentous appendages, termed fimbriae. These fimbriate strains were further shown to exhibit reversibility between fimbriate and nonfimbriate phases, depending on the cultural conditions. In many cases the agglutination reaction could be inhibited by small concentrations of D-mannose, α-methyl-D-mannoside or yeast mannan, and those fimbriae exhibiting mannose-sensitive agglutinating activity were termed type I fimbriae. Fimbriate bacilli were shown to adhere rapidly to epithelial cells of guinea pig and human colon, whereas nonfimbriate bacilli did not adhere to the cells (Duguid and Gillies, 1957). The implications of this work in terms of the relationship between bacterial attachment to host cells, and the pathogenesis of infectious disease were largely unrecognised until the early 1970's.

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In 1971, it was shown that in the mouth there is a correlation between the relative adherence of various Streptococcus species to particular tissue sites, and their proportional distribution found naturally on oral epithelial surfaces (Gibbons and van Houte, 1971). It was therefore proposed that the ability of an organism to attach to a particular surface may be a critical ecological determinant, on which any subsequent colonisation depends. The adhesive mechanisms of many species of bacteria have since been studied, with a view to obtaining a greater understanding of the early stages of infection. It should be noted, however, that just as attachment to epithelial cells may help an organism to colonise a body site, attachment of the organism to phagocytes has implications in host defence mechanisms. This apparently paradoxical situation is well illustrated by strains of Escherichia coli (E. coli) which attach to epithelial cells in a mannose-sensitive manner (Ofek and Beachey, 1978). Pre-incubation of E. coli with D-mannose or its derivatives inhibits epithelial cell adherence, and can also inhibit attachment of the organism to human polymorphonuclear leucocytes (Bar-Shavit et al., 1977) or mouse peritoneal macrophages (Bar-Shavit et al., 1980). It therefore appears that the mannose-binding activity of some Gram-negative organisms may play a dual role in the host-parasite relationship.

Although the material in this thesis is concerned with the attachment of micro-organisms to mammalian tissues, it is worthy of mention that microbial attachment to other surfaces can be of great biological, and economic, importance. Adhesion of Rhizobium to legume root hairs, thus forming root nodules which fix atmospheric nitrogen for synthesis of ammonium,/...

ammonium, is an example of a very specific interaction between components on the bacterium and on plant tissue (Dazzo, 1980). In some industrial processes, such as the trickle filter used for water purification, the ability of micro-organisms to attach to an inert support is being exploited (Ash, 1979). Conversely, bacterial adhesion to, and fouling of, surfaces in other flowing water systems may create problems. Examples include heat transfer equipment, in which the insulating effect of the bacterial film alters the heat transfer resistance of the metallic structures, and ship or pipeline surfaces, where the bacterial film increases surface roughness, thus creating greater fluid frictional resistance (Characklis, 1981).

It is thus clear that a thorough understanding of the mechanisms of microbial attachment would be of great benefit to medicine, industry, and agriculture. Once these processes have been properly characterised, then attempts can be made to modify attachment patterns, in order to overcome the problems which are at present associated with microbial adherence.

2 Physico-chemical aspects of microbial adhesion

Much of the work performed by biologists in the study of microbial attachment has been concerned with specific interactions between particular organisms and host cells. It is also important, however, to have some understanding of the general nature of the forces acting between a micro-organism and the surface to which it is attaching, because if the two surfaces cannot be sufficiently closely approximated, then the specific interactions cannot become operative. The physical chemistry underlying these phenomena is very complex, and for a full understanding requires knowledge of electrodynamics. This section will outline some of the basic principles to be considered in the analysis of microbial adherence mechanisms.

Contact between two immiscible bulk phases creates an interface. The interface which has been studied most closely in terms of microbial adhesion is that between a solid collector and the aqueous system in which it is immersed. In most cases, the liquid is an aqueous solution, and when a solid is immersed in such an aqueous environment it usually acquires a surface charge, either by ionisation of surface groups or by adsorption of ions. It has been shown, for example, that surfaces presenting carboxyl, amino or phosphate groups to the environment exhibit changes in surface charge with changing pH (Healy et al., 1978; Homola and James, 1977; James, 1979). Once a surface has acquired a charge, it attracts counter-ions from the surrounding aqueous medium, this process being opposed by the thermal motion of the counter-ions, which tends to distribute them evenly through the aqueous phase. The net effect of these two opposing processes is the formation of a zone

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next to the charged surface, referred to as the 'Gouy-Chapman diffuse electric double layer', where the concentration of counter-ions is greater than in the rest of the aqueous phase (Fig. 1). It is usual for both the bacterial and collector surfaces to be negatively charged in aqueous environments. Thus, a bacterial cell will experience a repulsive force when its diffuse double layer approaches that of a collector. Charge effects have been demonstrated in studies of attachment of marine bacteria to different collectors, where it was demonstrated that more bacteria attached to positively charged (platinum) or neutral (germanium) surfaces than to negatively charged surfaces (glass and mica) (Fletcher and Loeb, 1979). Other types of electrostatic interaction involved in microbial adhesion have been discussed by Pethica (1961).

Analysis of these long range forces between cells and between cells and surfaces is based largely on the so-called DLVO theory of colloidal stability, originally developed independently by Derjaguin and Landau (1941) and Verwey and Overbeek (1948). For the model of microbial adhesion, contact interactions between bacteria and a collector may be considered in terms of the balance of electrostatic repulsion, and attractive interactions of the van der Waals' type. This general theory has been analysed by a number of workers (Pethica, 1961; Weiss, 1968; Brook et al., 1967; Weiss and Harlos, 1977). The analysis can effectively be described in terms of two flat plates, two large spheres or a sphere and a plate, because of the relatively large 'radius' of cells. The general shape of a potential energy-distance curve for the interaction between a particle and a substrate is shown schematically in fig. 2.

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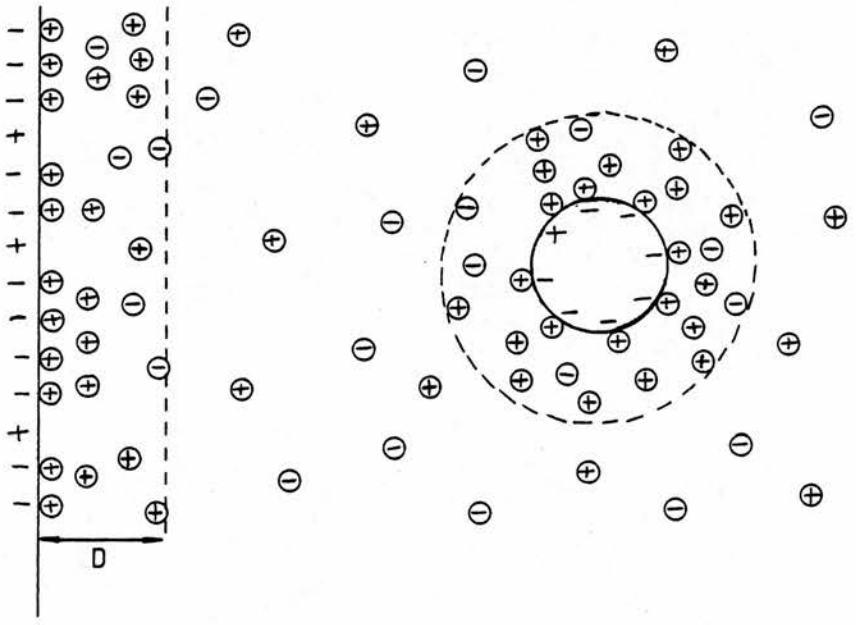

Fig. 1 The electrical double layer associated with a planar surface and a spherical particle. 'D' is the thickness of the diffuse double layer.
(Adapted from Fletcher et al., 1980)

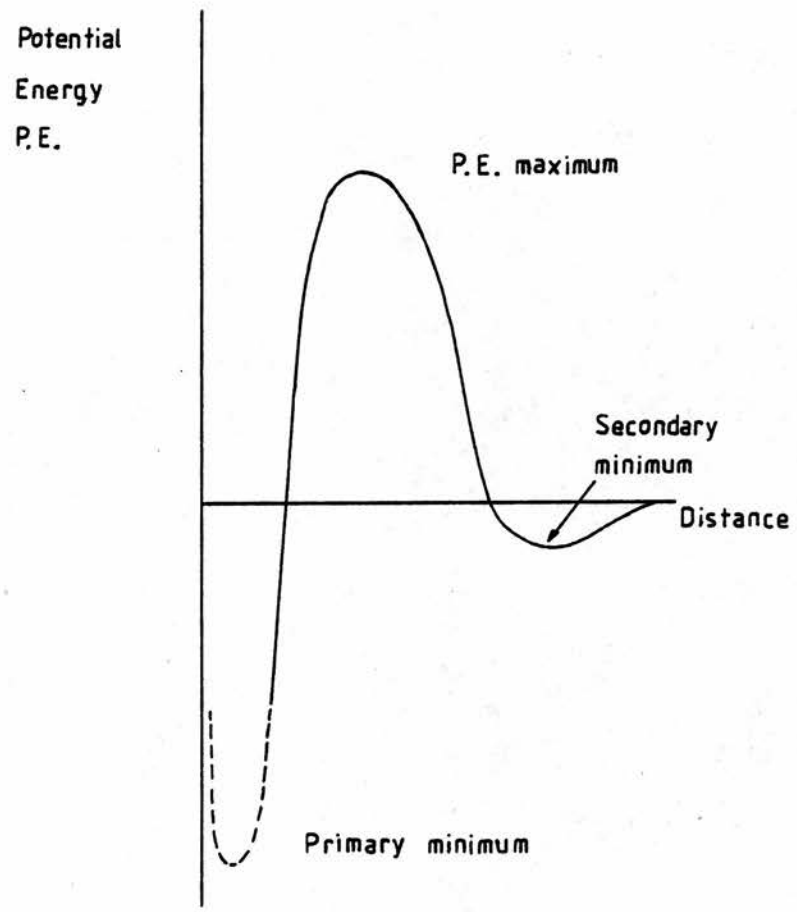

Fig. 2 A potential energy-distance curve for the interaction between a particle and substrate (schematic). (Adapted from Tadros, 1980)

It will be noted that at short distances between surfaces there is a very high potential energy barrier. For direct interaction, this barrier must be crossed, requiring an energy supply either from bombardment by other molecules or from movement of the particle itself. It has been calculated (Marshall, Stout and Mitchell, 1971) that the kinetic energy developed by a pseudomonad sufficient to propel it at 33μm/s is far too small to overcome this energy barrier. Similarly, molecular bombardment, which produces Brownian motion of bacteria, is grossly insufficient for the purpose (Brook et al., 1967). Thus, the bodies of undeformable cells appear to be incapable of passing the energy barrier and making use of the deep energy well which lies at even shorter distances.

Further study of fig. 2, however, shows that an attractive secondary energy minimum may exist at a greater distance between bodies. The secondary minimum interaction is relatively weak, and unlikely to account for the strong interaction termed 'adhesion'. Nevertheless, it may perform an important function in halting a cell for long enough to allow other events to take place, in particular the formation of hydrogen and ionic bonds by cell extensions or deformations. The importance of cell extensions may be explained as follows. The energy of interaction between spheres or between a sphere and a plate is related to the radius. As the radius of the sphere is reduced, the potential energy barrier becomes smaller, as shown in fig. 3, and for a body with a radius of 0.05 to 0.1μm the barrier is small enough to be overcome by either forces of locomotion of micro-organisms, or molecular bombardment. Thus, if the cell has probes of narrow diameter projecting from its surface,/...

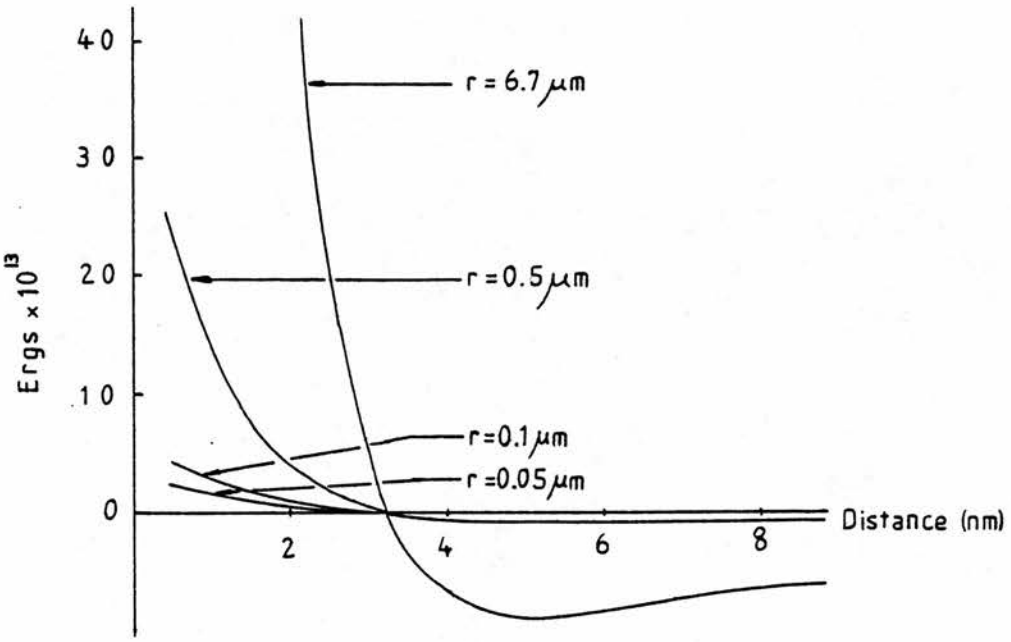

Fig. 3 The energies of interaction at different distances between the flattened region of a cell and probes from another cell of different radius (r) of curvature.

(Adapted from Weiss and Harlos, 1977)

surface, these can easily come close enough to a surface for hydrogen or ionic bonds to form. Surface structures of bacteria such as fimbriae or flagella form ideal probes for this purpose, and when a number of these bonds act in unison, they can produce a very strong attachment of the organism to a surface.

Another way in which the potential energy barrier between cells and surfaces can be overcome is for the micro-organism to form extracellular material such as polysaccharides. These polymeric molecules are able to approach closely to a surface. In the case of a mammalian tissue surface, this is also likely to have polysaccharide, in the form of a glycoprotein coat, outside the cytoplasmic membrane. This coat will contain polymers able to form bonds with either the microbial surface or the extracellular polymers produced by the organism. In considering such a mechanism, the 'wettability' of the surface by the microbial polymers must also be considered (Baier, Shafrin and Zisman, 1968). If two smooth surfaces are brought together with a layer of liquid between them which wets them (that is, one with a low contact angle to the surface), there will be strong resistance to any attempt to pull them apart, although the surfaces can easily slide across one another. If the fluid layer is viscous, or is a gel, there will also be resistance to shear forces.

As can be seen from the above discussion, interactions between micro-organisms and interfaces, followed by subsequent attachment processes, are extremely complex. The initial interaction between a micro-organism and a surface is, however, of vital importance for strong and enduring adherence of the organism to occur. The importance of fimbriae, in particular, will be described in later sections of this thesis.

3 Lectins: their properties, and their association with bacterial cells

One of the most common biological recognition and binding mechanisms is the lectin type of interaction. There has been some controversy over a suitable definition of the term 'lectin' (Goldstein et al., 1980; Kocourek and Hořejší, 1981; Dixon, 1981), but the definition proposed by Goldstein et al. (1980), which has been accepted by the Nomenclature Committee of the International Union of Biochemistry, will be quoted in this thesis. Thus, a lectin is defined as a 'sugar-binding protein or glycoprotein of non-immune origin which agglutinates cells and/or precipitates glycoconjugates'.

A number of excellent review articles have been published on lectins and their biological properties (Sharon and Lis, 1972; Hart, 1980; Goldstein and Hayes, 1978). Lectins were first discovered in plant seeds, some notable examples being concanavalin A from jack bean (Sumner and Howell, 1936), soy bean agglutinin (Liener and Pallansch, 1952) and wheat germ agglutinin (Burger and Goldberg, 1967). They have since been detected in many organisms from bacteria to mammals. Lectins may occur as soluble proteins or as cell-membrane associated proteins, and they also differ in their degree of sugar specificity. Some lectins will bind to more than one sugar, an example being concanavalin A, which recognises both α-D-glucopyranosides and α-D-mannopyranosides (Goldstein, Hollerman and Smith, 1965). Others, such as wheat germ agglutinin, which recognises only N-acetyl-D-glucosamine (Burger and Goldberg, 1967), are highly specific.

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Only a limited number of lectins have been prepared in a highly purified form, which makes it difficult to isolate properties that may be generally characteristic of these substances as a group. However, some similarities are evident. Most lectins are glycoproteins, though concanavalin A, which is devoid of sugar residues, is a notable exception (Agrawal and Goldstein, 1968). The carbohydrates found in lectins are typical of those found in animal glycoproteins, and include mannose, glucosamine and galactose.

With respect to amino acids, lectins are generally relatively rich in aspartic acid, serine and threonine, which may compose up to 30% of their amino acid content. They tend to be relatively low in, or even completely lacking, sulphur-containing amino acids, this overall pattern being characteristic of many plant proteins (Sharon and Lis, 1972). The metal ions Mn^{2+} and Ca^{2+} are contained in lectins, and it has been shown that metal ions are a prerequisite for the carbohydrate binding and agglutinating activity of lectins (Galbraith and Goldstein, 1970).

The range of molecular weights reported for various lectin molecules varies widely, from 26,000 for wheat germ agglutinin (Burger and Goldberg, 1967) to 400,000 for the horseshoe crab lectin (Marchalonis and Edelman, 1968). Of perhaps more practical interest is the fact that markedly different values have sometimes been reported for the same lectin. For example, concanavalin A has been ascribed molecular weights from 55,000 (Kalb and Lustig, 1968) to 96,000 (Sumner, Gralén and Eriksson-Quensel, 1938). One of the reasons for this apparent discrepancy is that most, if not all, lectins are composed of subunits, and undergo association-dissociation/...

dissociation reactions. Furthermore, the actual subunits may be composed of polypeptide fragments that are complementary, and which assemble to form the intact subunit, as shown for concanavalin A (Wang, Cunningham and Edelman, 1971).

Another interesting finding is that in a number of seed extracts, for example from Pisum sativum (Entlicher, Koštíř and Kocourek, 1970), as well as in extracts from the vineyard snail (Hammarström and Kabat, 1969), the presence of several very similar lectins has been observed. These multiple molecular forms of lectins, termed isolectins, differ from one another in their electrophoretic mobilities. They may be products of closely related genes, or they may be formed prior to, or during, isolation as a result of side chain modifications. In the case of isolectins which are glycoproteins, the differences may reside in the carbohydrate side chains.

A lectin molecule must possess at least two binding sites if it is to cause agglutination of cells, or form precipitates with complementary macromolecules. Concanavalin A, for example, has one binding site for methyl-α-D-mannopyranoside or methyl-α-D-glucopyranoside per subunit of molecular weight 32,000 (Yariv, Kalb and Levitzki, 1968). However, this subunit does not exist as such, since concanavalin A usually occurs as a dimer of molecular weight 55,000, with two binding sites (Kalb and Lustig, 1968). Similarly, there are six sites for N-acetyl-D-galactosamine in the lectin from the vineyard snail (Hammarström and Kabat, 1979). In all cases, the combining sites are homogeneous and identical with respect to their distribution in different molecules of the same lectin.

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If one considers the expression of carbohydrate moieties on cell membranes, it has been shown that the membrane-associated glycoproteins and glycolipids are oriented so that most of the carbohydrate is on the external surface of the plasma membrane (Singer, S.J., 1971; Steck and Dawson, 1974). In the majority of cases the function of the carbohydrates expressed at the cell surface is unclear, but one of the most common postulates is that they may perform a role as biological receptors. Specificity of the carbohydrate, both in terms of cellular distribution and at the level of the carbohydrate itself, where variations in linkage and configuration can occur, could provide a high level of variability. Such variability may help to explain various biological phenomena such as tissue tropisms, and inherent resistance or sensitivity to particular agents or organisms. It is the potential flexibility of this system, and the ubiquity of cell membrane glycoproteins, which make it possible that carbohydrate recognition could play a role in the adherence of bacteria to mammalian cells.

Several species of bacteria have been shown to possess lectin-like molecules. A summary of a number of these is shown in table 1. In many cases the presence of the lectin is inferred on the basis of haemagglutination patterns, or the inhibition of haemagglutination or bacterial attachment by particular sugars. The first bacterial lectin to be purified and characterised was the mannose-specific lectin from E. coli 7343 (Eshdat et al., 1978). The purified lectin agglutinated yeast cells in a mannose-sensitive fashion, and was shown to exist as an aggregate composed of subunits with individual molecular weights of 36,500. The evidence available to support the role of lectins in bacterial attachment will be outlined in section 4 of the 'Introduction', when mechanisms of adherence of particular species are described.

Table 1 Lectin-like molecules associated with bacteria

Organism	Sugar specificity	Reference
Escherichia coli (mannose-sensitive)	α-D-mannose	Eshdat et al. (1978)
Escherichia coli (mannose-resistant)	Various	see 4.1
Vibrio cholerae	L-fucose	Jones and Freter (1976)
	D-mannose	Bhattacharjee and Srivastava (1978)
Pseudomonas aeruginosa	D-galactose	Gilboa-Garber (1972)
	D-mannose	Gilboa-Garber, Mizrahi and Garber (1977)
Aeromonas hydrophila	L-fucose D-galactose D-mannose	Atkinson and Trust (1980)
Fusobacterium nucleatum	D-galactose or N-acetyl-D-galactosamine	Mongiello and Falkler (1979)
Eikenella corrodens	D-galactose or N-acetyl-D-galactosamine	Yamazaki, Ebisu and Okada (1981)
Actinomyces naeslundii	D-galactose or lactose	Saunders and Miller (1980)
Corynebacterium parvum	D-mannose	Bagg, Poxton and Weir (1981)

4 Specific mechanisms of bacterial attachment

In this section of the 'Introduction', a description will be given of some of the better characterised mechanisms of bacterial adhesion. In order to avoid confusion, the material will be presented in terms of individual species, but the multiplicity of mechanisms by which micro-organisms attach to surfaces will become self-evident to the reader.

4.1 Escherichia coli attachment

It was first reported in 1908 that E. coli would agglutinate erythrocytes of humans and other animals (Guyot, 1908). Collier and de Miranda (1955) later showed that D-mannose strongly inhibited the haemagglutination reactions, whereas other sugars had no such effect. The work of Duguid (Duguid et al., 1955; Duguid and Gillies, 1957; Duguid and Gillies, 1958) established the importance of fimbriation in the agglutination of red blood cells by many species of enterobacteria, including E. coli. It was also shown by these workers that in many cases the haemagglutinating activity, and adherence of the organisms to intestinal epithelial cells could be inhibited by low concentrations of mannose or its derivatives (Duguid and Gillies, 1957; Duguid, 1959; Duguid, Anderson and Campbell, 1966). These strains were termed mannose-sensitive (MS). The strains causing agglutination that was not prevented by mannose were described as mannose-resistant (MR).

Many clinical isolates of E. coli possess a mannose-specific cell-surface lectin which may bind to mannose residues on epithelial cells (Ofek,/...

(Ofek, Mirelman and Sharon, 1977; Ofek and Beachey, 1978). The mannose-binding activity of E. coli strains can be easily detected, either by the ability of the organism to cause agglutination of mannan-containing yeast cells (Ofek, Mirelman and Sharon, 1977) or by mannose inhibition of E. coli-induced agglutination of guinea pig erythrocytes (Old, 1972).

E. coli has been shown to attach to a variety of eukaryotic cells, including erythrocytes (Old, 1972), intestinal epithelial cells (Thaler, Hirschberger and Mirelman, 1977), leucocytes (Bar-Shavit et al., 1977) and buccal epithelial cells (Ofek and Beachey, 1978). The binding to each of these cell types can be inhibited by D-mannose, thus indicating that mannose residues are widely distributed among mammalian cells, and that they can serve as receptors for micro-organisms possessing mannose-specific cell surface lectins.

Each of the strains of enterobacteria demonstrating MS adherence was shown to bear surface fimbriae (Duguid and Gillies, 1957; Duguid, Anderson and Campbell, 1966; Duguid, 1959). Type I fimbriae were purified by Salit and Gotschlich (1977a) from a strain of E. coli K12 which had no sex pili or flagella. These highly purified fimbriae caused MS agglutination of guinea pig erythrocytes, indicating that fimbriae alone could induce haemagglutination by binding to mannose-like residues on the erythrocyte surface. Furthermore, the purified fimbriae were shown to adhere rapidly to vero cell monolayers (Salit and Gotschlich, 1977b) by a mechanism that could be inhibited by D-mannose and its analogues, or by pre-incubation of tissue cells with mannose-specific plant lectins. These results indicated that bacterial binding/...

binding could occur via fimbriae which behaved as lectins and presumably bound to mannose-containing glycoproteins on mammalian cell surfaces.

A different molecule, but with similar mannose-binding activity has also been isolated from E. coli 7343, a strain that causes rapid MS agglutination of yeast cells (Eshdat et al., 1978). It is a high molecular weight protein aggregate, composed of subunits with individual molecular weights of 36,500. Antibodies raised against the purified protein specifically agglutinated cells of the strain of E. coli from which it had been extracted, thus demonstrating its location on the bacterial surface. The relationship of this protein to fimbriae has not been established.

As mentioned previously, a small proportion of enterobacteria produces haemagglutination which cannot be blocked by D-mannose. It has been demonstrated that 10% of enterotoxigenic strains of E. coli (ETEC) isolated from humans with diarrhoea cause mannose-resistant (MR) haemagglutination (Levine and Rennels, 1978), and it is likely that these strains possess adhesins which are specific for receptors other than mannose residues. MR adhesins also mediate adhesion of many uropathogenic strains of E. coli to uroepithelial cells, as described later.

The first of the MR adhesins to be characterised in any detail was the capsular antigen, designated K88, which was isolated from piglets suffering from diarrhoea (Ørskov and Ørskov, 1966). This protein antigen was shown to be a fimbrial surface structure (Stirm et al., 1967), but morphologically distinguishable from type I fimbriae.

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K88 fimbriae are plasmid-mediated (Ørskov and Ørskov, 1966) and preferentially expressed after growth on solid medium at 37°C, but not after growth at 18°C or in broth (Ørskov and Ørskov, 1966). Presence of the K88 antigen confers on ETEC the ability to agglutinate guinea pig erythrocytes in the presence of D-mannose (Stirm et al., 1967). The K88 antigen was later shown to be an essential virulence factor for K88-positive strains of ETEC which cause diarrhoea in newborn piglets (Smith and Linggood, 1971). Removal of the K88 plasmid from a K88-positive strain was accompanied by loss of its diarrhoea-producing capacity, but this property was restored by introducing a K88 plasmid from another strain of E. coli. The K88-positive strains exhibited adhesive properties which allowed them to attach to the mucosa of the anterior small intestine and proliferate, rather than being carried along with the movement of the chyme, as the K88-negative ones appeared to be. Thus, despite being comparable in terms of enterotoxin production, K88-negative mutants of K88-positive strains are avirulent in pigs, because they cannot attach and propagate in the upper small intestine. It has been observed, however, that K88-positive strains do not adhere to intestinal tissue of all piglets (Rutter et al., 1975). In consequence, two host phenotypes have been described, namely 'adhesive' pigs and 'non-adhesive' pigs. The gene for the 'adhesive' phenotype behaves as an autosomal dominant, inherited in a simple Mendelian manner (Rutter et al., 1975).

An analagous, fimbrial, surface protein antigen, which was associated originally with ETEC in calves and lambs, has been identified by Ørskov et al. (1975) and termed K99. This antigen has also been found/...

found in atypical piglet ETEC. The properties of K99 antigen have been shown to be analagous with those of K88 antigen in terms of morphology (Burrows et al., 1976), plasmid coding (Ørskov et al., 1975) and production of MR agglutination of sheep erythrocytes (Burrows et al., 1976). It therefore seems likely that K99 performs the same role as K88 antigen in mediating attachment of ETEC to the intestinal mucosa.

The receptors on host tissue for K88 and K99 antigens have not been well defined, but in the case of K88 antigen there is an indication that N-acetylhexosamines may play some role in the attachment of the antigen to hog brush border membranes (Anderson, Whitehead and Kim, 1980). The formation of complexes between K88 and porcine intestinal brush border membranes was inhibited by glycoproteins with terminal N-acetyl-glucosamine or N-acetyl-galactosamine residues, and to a lesser extent by free N-acetyl-hexosamines.

Although many ETEC strains isolated from piglets possess K88 or K99 antigens, many strains isolated from piglets with diarrhoea lack these antigens. Furthermore, they are clearly pathogenic and adhere to porcine intestinal epithelium. One such strain, E. coli 987, has been studied intensively (Isaacson et al., 1978; Nagy et al., 1977), and has been shown to adhere to intestinal epithelium via a class of fimbriae distinct from K88 or K99. These fimbriae have been purified (Isaacson and Richter, 1981) and shown to be morphologically identical with E. coli type I fimbriae. Both are rigid appendages with a diameter of 7nm and have what appears to be an axial hole (Brinton, 1965). However, 987 fimbriae do not possess haemagglutinating activity against guinea/..

guinea pig or other erythrocytes (Isaacson and Richter, 1981), and are distinct both biochemically and antigenically (Isaacson et al., 1978; Nagy et al., 1977).

Mannose-resistant adhesion fimbriae have also been studied with respect to human diarrhoeal disease. Experiments with adult volunteers (DuPont et al., 1971) have shown that enterotoxigenicity alone is insufficient to cause diarrhoea in humans. Since then, two antigens similar to K88 and K99, in addition to type I fimbriae, have been identified on strains of ETEC pathogenic in humans. While working with a strain of ETEC (H 10407) that had been isolated originally from a case of watery diarrhoea in Bangladesh, Evans et al. (1975) noted that their laboratory strain suddenly failed to exhibit a population increase in their infant rabbit assay. It also failed to induce diarrhoea, despite a continued production of enterotoxin. This mutant strain (H 10407P) was shown to lack a plasmid present in the parent strain. By absorbing antiserum to the fully-virulent parent strain with H 10407P organisms, Evans et al. (1975) produced an antiserum to the surface antigen present on H 10407 but missing on H 10407P. This specific serum protected infant rabbits from challenge with living E. coli H 10407, although the serum did not possess bactericidal activity. Subsequent studies have shown this antigen to be a fimbrial organelle, distinct from the type I somatic fimbriae also present on H 10407 (Evans et al., 1978). These fimbriae are now termed 'colonisation factor antigen I' (CFA/I). Their properties are analagous to those of K88 antigen of porcine ETEC strains, in particular by the production of MR agglutination of human types A and B and bovine/...

bovine erythrocytes (Evans et al., 1977). Its morphology is similar to that of K88 antigen (Evans et al., 1978), and it is plasmid-mediated (Evans et al., 1975).

Evans et al. (1978) and Ørskov and Ørskov (1977) noted that CFA/I occurred commonly in only certain serogroups, including O15, O20, O25, O63 and O78. It was not evident on ETEC of serotypes O6 and O8, which are also frequently associated with acute diarrhoea in humans. A second fimbrial antigen, termed CFA/II, which is also plasmid-mediated and resembles CFA/I morphologically, has now been identified (Evans and Evans, 1978). However, whereas CFA/I mediates MR agglutination of human group A erythrocytes, CFA/II does not. Colonisation factor antigen II mediates MR agglutination of bovine erythrocytes, this reaction being rapid only at a reduced temperature (4°C). Serogroups O6 and O8 frequently express CFA/II (Evans and Evans, 1978).

Controversy exists over the prevalence of CFA/I and CFA/II in human ETEC strains, and over whether these particular antigens are required by all, or most, ETEC strains if they are to be pathogenic for human. Evans et al. (1978) reported that 86% of their ETEC strains from travellers' diarrhoea possessed CFA/I, and that 98% of ETEC belonging to serogroups O6, O8, O15, O25, O63 and O78 produced CFA/I or II (Evans and Evans, 1978). In contrast, Gross et al. (1978), who examined 89 ETEC strains from patients with diarrhoea, found that only 10% of the strains exhibited MR haemagglutination, and only 7% reacted with CFA/I antibody.

Levine et al. (1980) examined the various ETEC and enteropathogenic E. coli (EPEC) strains that have been used at the University of Maryland in volunteer challenge studies, for the presence of CFA/I, CFA/II and type I somatic fimbriae. The degree of virulence of these strains was thus indisputable. Of the ten strains examined, only one had CFA/I, and another CFA/II. Six other strains, which did not cause MR agglutination of human, bovine or guinea pig erythrocytes and lacked CFA/I or CFA/II, were nevertheless virulent for humans, resulting in intestinal colonisation and diarrhoea (Levine et al., 1980). Clearly, there must exist in human E. coli enteric pathogens other classes of adhesion fimbriae not associated with MR haemagglutination, or other surface structures which serve as colonisation factors. These could include extracellular polysaccharides, slime layers or lectins. After static growth in broth, most of the ETEC and EPEC strains demonstrated type I fimbriae (Levine et al., 1980), but since these appendages were also found in 11 of 15 E. coli strains from the normal flora, their role as a virulence factor was not established. It is, however, very possible that they serve as colonisation factors in some ETEC and EPEC strains that lack the MR type of fimbriae.

E. coli is also an important organism in the pathogenesis of urinary tract infections. In man, the ability to become attached to normal epithelial cells from the urinary tract is much greater in E. coli bacteria isolated from the urine of patients with acute symptomatic pyelonephritis or cystitis than in those isolated from urine of patients with asymptomatic bacteriuria (Svanborg-Edén et al., 1976). Thus, adherence may be related to the virulence of the organism. The presence of fimbriae on E. coli isolated/...

isolated from the urine of patients with urinary tract infection has been shown to be related to the ability of the bacteria to attach to human uroepithelial cells (Svanborg-Edén and Hansson, 1978). These fimbriate strains of E. coli agglutinated guinea pig erythrocytes in a mannose-specific fashion, and D-mannose blocked attachment of the same strains to buccal cells. However, D-mannose, D-galactose, α-methyl-D-mannoside and L-fucose did not affect attachment of these strains to uroepithelial cells (Svanborg-Edén and Hansson, 1978). These studies suggested that the E. coli isolates possessed two distinct mechanisms of adherence, and that the non-mannose-mediated mechanism was restricted to uroepithelial cells.

Mannose-resistant fimbriae have been prepared from three E. coli strains isolated from patients with acute pyelonephritis (Korhonen et al., 1980b). The haemagglutination patterns of the isolated fimbriae were identical with those of whole fimbriate bacteria (Korhonen, 1979), and fimbriae labelled with ^{125}I bound specifically to epithelial cells of the human urinary tract. This binding was inhibited by unlabelled fimbriae and was unaffected by D-mannose (Korhonen et al., 1980a). Thus, for these strains, fimbriae were likely to participate in the binding reactions. More recently, Hull et al. (1981) have cloned the genes encoding the so-called 'pyelonephritis-associated pili' and transferred them to an E. coli K12 derivative, thus endowing the new host with all the adherence properties of the urinary tract infection isolate. In addition, they performed a similar experiment with type I fimbriae, which indicated that unlike the adherence genes from bovine, porcine and human diarrhoeal isolates, both type I fimbriae and 'pyelonephritis-associated pili'/...

pili' are chromosomally encoded (Hull et al., 1981).

The human host cell receptors on uroepithelial cells for uropathogenic E. coli are believed to be a special class of glycosphingolipids, the globoseries glycolipids (Svanborg-Edén et al., 1981). Globotetraosylceramide and, to a lesser extent, globotriaosylceramide inhibited bacterial attachment, and the bacterial agglutination of erythrocytes from various species paralleled their content of globoseries glycolipids (Svanborg-Edén et al., 1981). Furthermore, erythrocytes not normally agglutinated by the E. coli strains became agglutinable after being coated with globotetraosylceramide (Leffler and Svanborg-Edén, 1980). The extract structural feature and site that the bacteria recognise in the saccharide chain is believed to be the disaccharide α-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl (Källenius et al., 1981).

The role of the MS fimbriae (type I) in urinary tract infection is unclear. A recent suggestion (Ørskov, Ørskov and Birch-Andersen, 1980) is that type I fimbriae may bind to mannose-containing receptors in urinary slime and mucus, which would function as a non-immune resistance mechanism. In the large intestine, however, where large amounts of mucus are produced, type I fimbriae may, by the same token, play a role as colonisation factors. It is interesting that in contrast to the human studies of uropathogenic E. coli, experimental infections with E. coli in mice (Aronson et al., 1979) suggest that adherence of the organisms to bladder mucosal surfaces of these animals is mannose-sensitive.

Thus far the discussion has been concerned with attachment of E. coli to particular mucosal surfaces and the subsequent establishment of infection. However, mannose-sensitive fimbriae also mediate adhesion of/...

of E. coli to phagocytic cells, presumably via mannose-containing structures of the plasma membrane (Bar-Shavit et al., 1977; Bar-Shavit et al., 1980; Mangan and Snyder, 1979; Rottini et al., 1979; Blumenstock and Jann, 1982). In contrast, E. coli with mannose-resistant fimbriae do not adhere to rat peritoneal macrophages or human polymorphonuclear granulocytes (Blumenstock and Jann, 1982), and are not, therefore, readily phagocytosed in the absence of antibody, a situation commonly encountered at the onset of infection. Thus, it seems likely that E. coli strains with MS fimbriae will be more readily eliminated in vivo than are those with MR pili. In view of their ability to mediate adhesion of E. coli to epithelial cells but not to phagocytic cells, MR pili can certainly be considered as virulence factors.

4.2 Group A streptococcal attachment

Group A streptococci populate the epithelial surfaces of the nasopharynx and the skin of humans. They have been shown to bind in vitro to washed pharyngeal or buccal cells (Beachey, 1975), and electron microscopy has revealed that the streptococci bind to the mucosal cell membrane via fibrils which project from the surface of the organism (Beachey and Ofek, 1976; Ellen and Gibbons, 1972). A surface antigen called M protein, which has long been associated with the virulence of group A streptococci, is associated with these fibrils (Swanson, Hsu and Gotschlich, 1969). It has been suggested that M protein is the molecule which mediates binding of the organism to oral epithelial cells (Ellen and Gibbons, 1972). This hypothesis was based on experiments which showed that M protein-possessing strains adhered well/...

well to human buccal cells *in vitro*, whereas an avirulent strain lacking M protein adhered only feebly (Ellen and Gibbons, 1972). The limitation of this technique was that no workers had demonstrated whether or not the fibrils were composed of M protein alone. Consequently, the differences in behaviour of the streptococcal variants which lacked fibrils (the so-called M(-) strains) could not be attributed solely to lack of M protein.

Evidence gained in later work (Ofek *et al.*, 1975; Beachey, 1975) suggested that lipoteichoic acid (LTA), which binds spontaneously to mammalian cell membranes via ester-linked fatty acids, may play a central role in the binding of group A streptococci to mucosal surfaces. It was shown that mild peptic digestion at pH 5.8 of group A streptococci removed virtually all the M protein from the cell surface, but had little visible effect on the surface fibrils (Beachey and Ofek, 1976). Furthermore, the treated organisms, which lacked M protein, adhered equally as well as untreated bacteria to human oral mucosal cells (Beachey and Ofek, 1976).

The evidence for implicating LTA in group A streptococcal adherence is as follows. Lipoteichoic acid prepared from group A streptococci has been shown to block group A streptococcal binding to oral mucosal cells in a dose-response fashion (Beachey, 1975). None of the other cell wall substances (including streptococcal M protein, C carbohydrate and peptidoglycan sonicate, and lipopolysaccharide from *Serratia marcescens* and *E. coli*) tested were capable of inhibiting attachment. The effect on binding of human sera that contained anti-LTA antibody added further credence to the theory. Serum from a patient with acute rheumatic fever contained/...

contained an anti-LTA titre of 1:160, and inhibited streptococcal binding to a high titre. This inhibitory effect was markedly reduced by absorbing the serum with erythrocytes coated with purified LTA (Beachey, 1975).

In further experiments, the LTA was subjected to mild hydrolysis with ammonia (Beachey, 1975), and the hydrolysate separated into a chloroform-methanol soluble, fatty acid fraction and a water-soluble glycerophosphate fraction. The inhibitory effect of the hydrolysed LTA on streptococcal binding to mucosal cells was confined to the fatty acid fraction, suggesting that fatty acid moieties were involved in the binding reaction. The water-soluble material, which was antigenic, contained no binding activity, but such activity could be restored by esterification of this fraction with palmitoyl chloride (Beachey, 1975). This particular finding is somewhat difficult to explain in terms of the model proposed by Wicken and Knox (1975), which indicates that the lipid end of the LTA molecule is anchored by intercalation into the bacterial cytoplasmic membrane, whereas the polyglycerophosphate (PGP) backbone protrudes outward through the cell wall. The answer to this dilemma may lie in the fact that LTA and its deacylated derivative are constantly secreted into the surrounding medium by viable group A streptococci (Alkan and Beachey, 1978). It has been suggested that because of its polyanionic character, the PGP end of the molecule may form complexes with positively charged substances in the bacterial cell wall. Evidence for this theory has been forthcoming in recent experiments (Ofek et al., 1980) which have demonstrated that LTA can be quantitatively precipitated in the presence of M protein at low pH ranges, with optimal precipitation/...

precipitation occurring at pH 3.7. Complexes were formed equally well by deacylated LTA as by LTA itself, thus indicating that binding to the M protein is through the PGP backbone of the LTA. The binding between LTA and surface protein anchors the LTA molecules to the bacterial surface, leaving the lipid end free to interact with LTA receptors on host cell membranes. Beachey and Ofek have proposed a 'tree theory' (Beachey, 1981) in which the LTA and deacylated LTA form a complex network with the M protein and other LTA-binding proteins on the surface of the Streptococcus. These complexes allow the lipid ends of some LTA molecules to be intercalated into the cytoplasmic membranes of the bacterium, while others are free to interact with LTA receptors on host cell membranes. It is possible that the surface fibrillar structures seen by electron microscopy, and which appear to be involved in binding of the organisms to epithelial cells are, in fact, these LTA-M protein complexes. Fatty acid ends of LTA molecules expressed at the extremities of the fibrillae may then interact with receptors in the cell membrane.

Analysis of the binding of radiolabelled LTA to isolated erythrocyte membranes (Chiang, Alkan and Beachey, 1979) showed that binding was dependent on membrane concentration and time, and that the binding sites on the erythrocyte membrane were saturable. One population of approximately 5.5×10^6 binding sites per erythrocyte membrane was detected. Similar specificity of binding has been demonstrated for platelets (Beachey et al., 1977), lymphocytes (Beachey et al., 1979a), intact erythrocytes (Beachey et al., 1979b) and oral epithelial cells (Simpson et al., 1980). It therefore seems likely that host cell membranes contain specific binding sites (receptors) on their surfaces for group A streptococcal LTA. In addition, 'right-side-out' erythrocyte membrane ghosts

bound/...

bound ten times more LTA than 'inside-out' membrane ghosts (Chiang, Alkan and Beachey, 1979), suggesting that specific binding sites for LTA were present on the outer, but not the inner, surface of human cell membranes.

At present, the membrane receptor for LTA has not been isolated, but albumin, which contains fatty acid binding sites, has proved to be a good receptor analogue (Simpson, Ofek and Beachey, 1980a; Simpson, Ofek and Beachey, 1980b). Simpson has shown that albumin blocks the binding of streptococci in a dose-response fashion, and that the inhibition is specific for this organism. Absorption of the inhibitory effects of albumin solutions by group A streptococci, but not by epithelial cells, indicates that the inhibition is due to binding of the albumin to the streptococcal surface. Further proof that LTA binds to albumin is provided by the results of experiments which show that LTA can protect albumin from heat denaturation and can also form complexes which are demonstrable by immunoelectrophoresis (Simpson, Ofek and Beachey, 1980a).

It was also important to investigate whether albumin would bind to LTA which had formed complexes with M protein, and thus lend support to the 'tree theory' mentioned above. Insoluble complexes were prepared at pH 3.7 between M protein and LTA or deacylated LTA (Beachey, 1981). Once formed, the complexes remained stable, even at neutral pH. The complexes formed between M protein and LTA bound ten times more albumin than those formed between M protein and deacylated LTA. This result supports the theory that the lipid end of the LTA-M protein complex remains free to interact with receptor molecules (Beachey, 1981). Thus, although/...

although the actual cell membrane receptor for LTA is unknown, the putative receptor may well be a membrane protein or glycoprotein which bears fatty acid binding sites similar to those on albumin.

Despite the evidence in favour of LTA as a mediator of the specific adhesion of group A streptococci to mucosal surfaces, other workers have demonstrated that it may not be solely responsible. A recent paper (Botta, 1981) indicated that although LTA markedly inhibited adhesion of group A streptococci to pharyngeal cells, pre-incubation of these epithelial cells with C polysaccharide also reduced subsequent adhesion of group A strains by 98.7%. This is in contrast to the results of Beachey (1975) who showed that C carbohydrate had no inhibitory effect on adhesion of group A streptococci to buccal cells. Botta (1981) also found that A-variant strains of group A streptococci, whose C polysaccharide lacks the N-acetyl-D-glucosamine residue, showed a markedly depressed ability to adhere to oral epithelial cells. On the basis of these results, it has been suggested that the C polysaccharide may be the envelope component specifically responsible for adhesion of group A streptococci.

Grabovskaya et al. (1980) studied group A streptococcal attachment to HEP-2 tissue culture cells, and concluded that at least two mechanisms were involved, depending on the bacterial strain. In the first type, binding was dependent on LTA, whereas in the second type of binding, M protein played the major role.

Very recently, Tylewska and Wadström (1981 and personal communication) have reported that adherence of group A streptococci to pharyngeal epithelial/...

epithelial cells is dependent on the presence of M protein, and that pre-incubation of M protein positive strains with disialogangliosides, N-acetyl galactosamine and D-galactose inhibits their adherence to pharyngeal cells. Both M-positive and M-negative strains adhered equally well to buccal epithelial cells, the binding mechanism being unaffected by the presence of sugars in this system. It was suggested that group A streptococci may attach to human pharyngeal cells by specific lectin-like binding to galactose residues.

Thus, it seems likely that group A streptococcal adherence will prove to be a complex process, involving several bacterial and epithelial cell components, and a complete picture is not yet available.

4.3 Attachment and accumulation of *Streptococcus mutans* on teeth

Dental plaque formation requires two types of adherence interactions (Gibbons and van Houte, 1973). Firstly, bacteria must attach to the pellicle surface, then secondly, they must adhere to each other, thus permitting accumulation of the organisms and imparting cohesive properties to the plaque. The ability of *Streptococcus mutans* (*S. mutans*) to accumulate on the teeth of experimental animals, or on glass or wire surfaces immersed in broth cultures, is related to the synthesis of extracellular polysaccharides from sucrose. The polysaccharides formed are complex mixtures of glucan and fructan (Guggenheim, 1970). A particular form of glucan that is predominantly α 1:3 linked is highly insoluble, and has been named 'mutan'. The enzymes responsible for synthesis of these extracellular polysaccharides are glucosyl- and fructosyl-/...

fructosyl-transferases, which are elaborated constitutively.

Several observations indicate that glucans are more important than fructans in enabling S. mutans to accumulate on teeth. Glucan-defective mutants do not form large plaques or initiate decay in experimental animals (Tanzer et al., 1974), while preparations of dextranase and mutanase reduce plaque accumulation when administered to rodents infected with S. mutans (Fitzgerald et al., 1968; Guggenheim et al., 1972). In addition, the glucans, especially 'mutan', are less soluble than the fructans and are most resistant to degradation by other oral bacteria (Guggenheim, 1970).

Cells of S. mutans bind high molecular weight glucans, which results in aggregation of the bacteria (Gibbons and Fitzgerald, 1969), but other types of polysaccharide are ineffective. This indicates the probable mechanism by which glucan synthesis promotes accumulation of S. mutans cells on teeth and other surfaces. Several glucan-binding ligands have been identified in cultures of this organism (Germaine and Schachtele, 1976; McCabe et al., 1977; Russell, 1979), one type containing glucosyltransferase activity, while another appears to be a glucan-binding protein. Trypsin-treated or heat-treated streptococci will not bind glucan (Kelstrup and Funder-Nielsen, 1974), suggesting that the ligands involved are proteins.

Glucosyltransferases are thought to have only one glucan-binding site, but aggregates of enzymes complexed with glucan apparently bind directly to glucan-binding proteins on the organism's surface (Spinell

and/...

and Gibbons, 1974; Germaine and Schachtele, 1976), or to other glucan molecules already attached to these components. The interactions of these ligands with glucan molecules are believed to promote cohesion of S. mutans cells proliferating on the teeth, and thus encourage their accumulation. However, it has been reported (Tinanoff, Tanzer and Freedman, 1978) that organisms of serotypes c and e can accumulate to a significant extent in vitro in the absence of sucrose. It is not clear, therefore, whether the entire S. mutans group accumulates on teeth by similar mechanisms.

It was originally believed that glucan synthesis was also important for the initial attachment of S. mutans cells to teeth (Gibbons and van Houte, 1973). However, several strains of S. mutans have since been shown to attach to the teeth of rats sufficiently firmly to colonise the occlusal fissures in the absence of detectable glucan synthesis from sucrose (van Houte, Burgess and Onose, 1976). Furthermore, a mutant of S. mutans, forming little or no cell-associated glucosyltransferase, attached in numbers comparable with the parent strain to the teeth of rats and to saliva-treated hydroxyapatite (Clark, Bammann and Gibbons, 1978b). In vivo experiments have also shown that exposure to sucrose, glucose or saline of S. mutans cells adsorbed to the smooth surfaces of human teeth, has little influence on the rate of desorption (Clark and Gibbons, 1977). Overall, these studies together with other evidence indicate that S. mutans can interact directly with components of salivary pellicles in the absence of glucan synthesis from sucrose.

Recent experiments have demonstrated that the adsorption of eight strains of S. mutans to saliva-treated hydroxyapatite surfaces is inhibited/...

inhibited by galactose and melibiose, but not by other neutral sugars tested (Gibbons and Qureshi, 1979). This suggests that the streptococci are binding via a lectin-like component to exposed α-galactoside residues of salivary glycoproteins in the pellicle, and is consistent with the finding that purified salivary glycoproteins treated with α-galactosidase no longer aggregate S. mutans cells (Levine et al., 1978). Adsorption of S. mutans to saliva-treated hydroxyapatite is also inhibited by various amines (Gibbons and Qureshi, 1979) implying that the organism may be binding to basic residues in the pellicle. This could reflect the interaction of S. mutans with blood group reactive mucins, since this is also inhibited by amines (Gibbons and Qureshi, 1978).

All these findings have been corroborated by the results of Staat, Langley and Doyle (1980), who employed Persea americana agglutinin as a selective adherence inhibitor. On the basis of the data gained in their experiments, these workers developed a two-reaction model of S. mutans adherence. The first reaction is attachment to the tooth pellicle, which is mediated by cell-surface proteins, rather than by glucans or teichoic acids. This is followed by a cellular accumulation reaction mediated by sucrose-derived D-glucans and cell surface lectins.

4.4 Adherence of Actinomyces species

Strains of Actinomyces viscosus (A. viscosus) and Actinomyces naeslundii (A. naeslundii) isolated from humans possess fimbriae (Girard and Jacius, 1974; Ellen, Walker and Chan, 1978; Cisar and Vatter, 1979). Some/...

Some strains agglutinate erythrocytes (Ellen et al., 1980) and attach in high numbers to buccal epithelial cells (Ellen, Walker and Chan, 1978; Saunders and Miller, 1980). Removal of the fimbriae by blending in an homogeniser impairs attachment of A. naeslundii to epithelial cells, thus implicating these structures in adherence related functions (Ellen, Walker and Chan, 1978). The haemagglutinating activity of strains of both species is significantly enhanced by neuraminidase (Costello et al., 1979; Ellen et al., 1980). Strains that haemagglutinate directly synthesise neuraminidase, while strains which are normally inactive acquire activity in the presence of the enzyme (Costello et al., 1979). Haemagglutinating activity is inhibited by lactose, galactose and β-galactosides (Ellen et al., 1980). Thus, Actinomyces haemagglutination appears to proceed via a two-step mechanism. Initially, terminal sialic acid residues are removed by neuraminidase, which is followed by lectin-like binding to the exposed β-galactoside-associated sites on the erythrocyte (Ellen et al., 1980).

Saunders and Miller (1980) have shown recently that pretreatment of A. naeslundii with galactose or lactose significantly inhibits attachment of the organism to buccal epithelial cells. Similarly, coaggregation reactions between certain strains of A. viscosus and Streptococcus sanguis can be inhibited by lactose, β-methyl-D-galactoside or D-galactose (McIntire et al., 1978; Kolenbrander and Williams, 1981). Thus, it would appear that mechanisms involving lectin-carbohydrate interactions may figure prominently in the various modes of attachment of strains of Actinomyces. Monoclonal antibodies specifically reactive with the fimbriae of A. viscosus T14V have been used to show that the lactose-sensitive lectin activity resides in these structures (Cisar et al., 1980).

A.viscosus/...

A. viscosus and A. naeslundii are prominent in dental plaque, and are thought to play a role in the induction of root surface caries and general periodontal pathology (Socransky, 1977). Both organisms adsorb well to saliva-treated hydroxyapatite (Clark, Bamman and Gibbons, 1978; Qureshi and Gibbons, 1979) and there is evidence that fimbriae may also be involved in this process (Brecher, van Houte and Hammond, 1978; Clark et al., 1981). Germ-free rats, fed a high sucrose diet, were inoculated with A. viscosus T14-Vi (virulent) or T14-Av (avirulent). The avirulent strain did not form gingival plaque, and adsorbed in much lower numbers than the virulent parent strain to hydroxyapatite treated with rodent saliva. Electron microscopy revealed that both the parental and mutant strains possessed numerous fimbriae, but the avirulent strain showed the presence of large amounts of cell-surface-associated polysaccharide which masked the fimbriae, thus preventing their interaction with the salivary pellicle (Brecher, van Houte and Hammond, 1978).

Many components of A. viscosus could foster accumulation of these organisms. Their surface fimbriae play a role in cellular aggregation (Cisar, Vatter and McIntire, 1978). A. viscosus also synthesises an extracellular heteropolysaccharide slime composed of N-acetyl-glucosamine and smaller amounts of glucose and galactose (Rosan and Hammond, 1974), which may help to explain plaque formation by the organism. In addition, a fatty acid-substituted amphipathic heteropolysaccharide, containing mannose, glucose and galactose is elaborated by A. viscosus (Wicken et al., 1978). Finally, it has been suggested that the spontaneous agglutination of Actinomyces cells at low pH values may also promote their accumulation (Miller, Palenik and Stamper, 1978).

4.5/...

4.5 Adhesive properties of Vibrio cholerae

Colonisation of the intestinal epithelium by Vibrio cholerae (V. cholerae) has been studied in a number of different assay systems. The results obtained from the different types of assay show a high degree of variation, and may imply that V. cholerae as a species produces several adhesins (Jones, Richardson and Vanden Bosch, 1980).

A good correlation has been found between the ability of V. cholerae strains to adhere to ileal tissue of adult rabbits and their ability to cause disease (Srivastava, Sinha and Srivastava, 1980). There is evidence to suggest that adherence of V. cholerae to the intestinal mucous membrane is important because the toxin generated is inadequate to induce fluid secretion unless it is released by bacteria that are actually attached to the mucous membrane (Chitnis, Sharma and Kamat, 1982a). These workers performed studies with an adult-rabbit ileal-loop model, and showed that antibodies to the lipopolysaccharide somatic antigen component of V. cholerae gave passive protection against challenge with live V. cholerae. This protection was correlated with a 10-15-fold reduction in the number of V. cholerae adherent to the mucous membrane of the antibody-protected loops. The total amount of toxin released was the same, however, irrespective of whether the organisms were adherent to the mucous membrane or were in the lumen. Thus, the toxin caused symptoms of disease only when elaborated by organisms that were attached to the intestinal mucous membrane.

Vibrio cholerae cells agglutinate both guinea pig and human red blood cells (Tweedy, Park and Hodgkiss, 1968; Jones, Abrams and Freter, 1976)./...

1976). The haemagglutination reaction is only partially inhibited by D-mannose (Tweedy et al., 1968). Filamentous appendages resembling fimbriae have been demonstrated by electron microscopy (Tweedy et al., 1968), and the evidence indicates that these fimbriae are probably responsible for haemagglutination.

The adhesion of V. cholerae to rabbit brush borders (Jones et al., 1976; Jones and Freter, 1976) is one of the assay systems that has yielded much information regarding vibrio attachment mechanisms. This interaction appears to be a complex one, and may be prevented in several ways, including low temperature (0.5°C), the absence of divalent cations or the presence of the monosaccharide L-fucose. In addition, nonmotile vibrio mutants lack the ability to adhere to rabbit brush borders and to agglutinate human erythrocytes. Activation of the adhesin by divalent ions is a function of ion size as well as charge, which suggests that ions such as Ca^{2+} act by forming bridges between the opposing surfaces, rather than by reducing surface potential (Jones et al., 1976). It is believed that L-fucose, which inhibits adhesion in this system, constitutes the major part of the adhesin receptor. An interesting finding is that vibrios adhere to agar beads to which L-fucose has been covalently linked, but do not attach to untreated agar beads (Jones and Freter, 1976). This is a specific reaction, which is inhibited by L-fucose, but not by D-fucose. Comparison of the molar inhibitory concentrations of L-fucose and fucosides suggests, however, that the receptor is likely to be larger than a single L-fucose residue (Jones and Freter, 1976).

The same workers have also studied the association between V. cholerae and/...

and intact slices of rabbit ileum (Freter and Jones, 1976), but found significant differences in results gained from the two separate assays (Freter and Jones, 1976; Jones and Freter, 1976). The most important distinguishing characteristics of the interaction between vibrios and intact mucosa appear to be insensitivity to inhibition by L-fucose, and the fact that agar-grown vibrios or vibrios incubated in buffer, which have lost their ability to react with isolated brush border membranes, are still able to adhere to intact tissue slices (Freter and Jones, 1976). The authors explain these findings by proposing the existence of at least two specific mucosal receptors for vibrio adhesion. One of these is L-fucose sensitive, and is located on the brush border surface, whilst the second receptor is L-fucose resistant, and occupies a different site.

Although D-mannose did not inhibit haemagglutination by vibrios, Jones and Freter (1976) did show that mannose slightly inhibited adhesion of *V. cholerae* to brush borders, though its inhibitory activity was 100 times less than that of L-fucose. A more recent report (Bhattacharjee and Srivastava, 1978) has provided evidence that mannose-sensitive haemagglutinins play a role in the adherence of *V. cholerae*, biotype eltor to freshly isolated rabbit intestine discs. This contrasts with the findings of Freter and Jones (1976), who found that neither L-fucose nor D-mannose inhibited adhesion of vibrios to intact intestinal mucosa.

It has been suggested that flagellar antigens may be involved in adhesion of vibrios to mucous membranes (Jones and Freter, 1976). This was because although adhesion generally correlated with the presence of a/...

a functional flagellum, adhesion did not necessarily depend upon the motility of the vibrios. However, a recent paper (Chitnis, Sharma and Kamat, 1982b) has indicated that the somatic antigens of V. cholerae play a major role in adhesion of the organism. Antisomatic antiserum against V. cholerae Inaba, at a dilution of 1 in 200, prevented the in vitro adhesion of three different strains of V. cholerae Inaba to rabbit intestinal mucous membrane, but had no effect on the adhesion of two test strains of NAG vibrios (the NAG vibrios share flagellar antigens with the classical vibrios, but have different somatic antigens (Buchanan and Gibbons, 1974)). In addition, anti-live V. cholerae Inaba antiserum absorbed with boiled cells of Inaba and devoid of antisomatic antibody, could not inhibit adhesion of the same three strains of V. cholerae Inaba (Chitnis et al., 1982b). The absorbed antiserum had no anti-lipopolysaccharide or bacterial agglutinin activity, but its anti-flagellar antibody titre was 32,000. In this particular experimental system, therefore, flagellar antigens did not play a major role in the adhesion of V. cholerae to the adult rabbit ileal mucous membrane. Instead, the somatic antigens appeared to be more important.

A somewhat different view is now held by Freter and co-workers (Freter, 1981), who believe that the association of V. cholerae with intestinal mucus, rather than its adherence to epithelial cells, is the important factor in determining pathogenicity. Non-chemotactic mutants of V. cholerae that do not readily associate with intestinal mucus gel in vivo or in vitro, also demonstrate an impaired ability to colonise the gut of germ-free mice or to multiply in intestinal loops of adult rabbits (Freter, O'Brien and Halstead, 1978; Freter, O'Brien and Macsai, 1979)./...

1979). In contrast, the parent strain or chemotactic revertants readily colonise these *in vivo* models, outgrowing and displacing the non-chemotactic mutants in mixed infections. Light microscopic observation of frozen sections of infected intestinal mucosa showed the vibrios to be located in the mucus gel, rather than adherent to the mucosa (Freter, 1981). It was of interest that the parent strain, which had been selected for optimal chemotactic responsiveness by repeated isolation from the spreading edge of colonies in semi-solid agar, lost its original ability to adhere to rabbit, mouse or human intestinal brush border membranes (Freter, 1981).

The studies reported in this section show clearly that different experimental models can highlight particular aspects of adhesion phenomena. Thus, results from such systems should always be interpreted with caution, because it is obvious that individual organisms may associate with mucosal surfaces by more than one mechanism.

4.6 Adhesion of *Neisseria gonorrhoeae*

When grown on solid medium, *Neisseria gonorrhoeae* (*N. gonorrhoeae*) gives rise to a variety of different colony forms. Four frequently observed colony types (termed T1, T2, T3 and T4) were defined by Kellogg *et al.* (1963). Organisms from colony types T1 and T2 are of increased virulence (Kellogg *et al.*, 1963; Kellogg *et al.*, 1968), and adhere more readily to eukaryotic cells (Punsalang and Sawyer, 1973; Swanson, 1973) than do organisms from colony types T3 or T4. The principal difference between these colony types is that pili are present on/...

on the surfaces of T1 and T2 organisms, but not on T3 or T4 gonococci (Swanson, Kraus and Gotschlich, 1971).

Gonococcal pili (Brinton et al., 1978) are filamentous protein appendages which extend from the bacterial cell wall and mediate attachment to host cells. Purified preparations of pili inhibit this attachment in a dose response fashion (Tramont et al., 1980). Attachment of both heterologous and homologous strains is blocked by purified pili, but they are most efficient at inhibiting adhesion of the homologous strain (Tramont, 1981). This inhibition also appears to be species-specific, since fimbriae isolated from E. coli do not inhibit the attachment of gonococci, and gonococcal pili do not inhibit attachment of E. coli (Tramont et al., 1980; Tramont, 1981).

Local genital antibodies developing after natural infection with N. gonorrhoeae are capable of blocking the attachment of gonococci to human epithelial cells (Tramont, 1977). Both IgG and IgA classes of immunoglobulin are involved, and the principal antigen to which this antibody is directed is pili (Tramont et al., 1980). Furthermore, immunisation of human volunteers with a vaccine prepared from purified gonococcal pili raises antibody capable of blocking adherence of both homologous and heterologous gonococci to buccal epithelial cells (Tramont et al., 1981), which suggests that the immune response is directed at a common pilus determinant.

Other antigens also inhibit gonococcal attachment. One such antigen is lipopolysaccharide (LPS), but this is not an entirely species-specific/...

specific reaction (Tramont et al., 1980), in that LPS purified from Salmonella typhimurium, Pseudomonas aeruginosa and E. coli also blocks attachment of N. gonorrhoeae to buccal epithelial cells. Thus, the receptor sites for gonococci on the buccal cells are possibly covered in these experiments in a random, non-specific manner by the LPS. However, LPS purified from the parent gonococcal species was two to four times more efficient than LPS purified from other bacteria in blocking attachment (Tramont et al., 1980).

Additional evidence for the involvement of other antigens in gonococcal attachment is provided by the finding that absorption of vaginal secretions or sera with pili purified from either homologous or heterologous strains does not completely remove the ability of the secreted material to inhibit adhesion of gonococcal strains (Tramont, 1981). Lambden et al. (1979) have reported a correlation between variation in epithelial cell attachment and alterations in surface proteins of variants of N. gonorrhoeae P9. Variants that produced extra surface proteins all showed increased adhesion to human buccal epithelial cells, whereas the prototype strain, lacking additional proteins, demonstrated the greatest binding to red blood cells. Adhesion to buccal cells and erythrocytes, therefore, appears to be mediated by different mechanisms. Furthermore, Swanson et al. (1975) have demonstrated that adherence of gonococci to polymorphonuclear cells is mediated by a so-called 'leucocyte association factor'. Work in our own laboratories has shown that whereas human monocytes show an increased ability to bind pilate, rather than non-pilate gonococci, polymorphonuclear leucocytes do not discriminate between pilate and non-pilate variants (Kinane et al., in press). This finding/...

finding is therefore in accordance with those of Swanson et al. (1975). Thus, gonococcal attachment is probably not solely mediated by pili.

Cells of N. gonorrhoeae will adhere to a variety of human cells, including vaginal mucosal cells (Mårdh and Weström, 1976), cervical mucosal cells (Tramont, 1977), fallopian tube mucosal cells (Johnson et al., 1977), sperm (James-Holmquest et al., 1974) and buccal cells (Tramont, 1977). However, the receptor sites for the gonococcus have not been identified. None of eleven simple sugars tested by Punsalang and Sawyer (1973) inhibited agglutination of rabbit erythrocytes by type I gonococci, while treatment of human fallopian tube organ cultures with lectins which bind to fucose, N-acetyl-galactosamine and N-acetyl-glucosamine did not block gonococcal attachment (Watt et al., 1978). Buchanan et al. (1978) showed that neuraminidase and low concentrations of gangliosides inhibited attachment of gonococci to buccal cells, but that sialic acid itself had no inhibitory effect. This suggested that the receptor may resemble a ganglioside. These results have been largely corroborated by Lambden, Robertson and Watt (1980), who demonstrated that on some strains, two distinct types of pili, designated α and β, may exist. Removal of sialic acid residues from buccal cell surface carbohydrates by neuraminidase treatment markedly inhibited the binding of α-pili, but had little effect on β-pili. Treatment of the neuraminidase-modified buccal cells with a mixture of exoglycosidases further reduced the binding of α-pili to a level comparable to that of β-pili (Lambden et al., 1980). The α-pili were not inhibited by galactose, fucose, mannose, glucose, N-acetyl-glucosamine and N-acetyl-galactosamine, or by the mucopoly-saccharides hyaluronic acid and chondroitin sulphate A, B and C (Buchanan et al., 1978).

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It should be noted that iron salts greatly enhance attachment of gonococci (James et al., 1976), and gonococcal pili (Buchanan et al., 1978) to eukaryotic cells. This may indicate the possibility of ionic bridge formation between pili and the cell surface.

In summary, adhesion of N. gonorrhoeae to mucosal surfaces is a complex process involving both pili and outer membrane adhesins. The pili are likely to play an important role in penetrating the electrostatic barrier between gonococci and mucosal cell surfaces, to initiate adhesion. The host cell receptor for gonococcal pili has not been properly characterised, but may resemble gangliosides.

4.7 Adhesion of other bacteria

The preceding sections have described the present state of knowledge regarding attachment to host tissues of six different organisms. Adherence characteristics of a number of other bacteria have also been studied, though in less detail, and a selection of these will be summarised in this section.

Aeromonas hydrophila (A. hydrophila) is an opportunistic pathogen in debilitated or immunologically compromised humans, though it may cause systemic infections in apparently healthy people. Clinically it is usually involved in acute diarrhoeal disease. Atkinson and Trust (1980) studied the haemagglutination properties of eleven strains of enterotoxigenic A. hydrophila. Sugar-inhibition studies and yeast-coagglutination tests revealed six different haemagglutination mechanisms for this species. These/...

These were characterised by inhibition by L-fucose, inhibition by D-galactose, inhibition by D-mannose, two distinguishable mechanisms which were inhibited by either L-fucose or D-mannose, and one type which required a combination of D-galactose and D-mannose. The haemagglutinating strains also attached well to buccal epithelial cells, although quantitative and qualitative differences in the adherence to these two types of cell were evident. Thus, at least five lectins with different inhibition properties have been recognised in A. hydrophila, one strain producing two distinct lectins at once. This organism is particularly interesting because of its variety of attachment mechanisms, which cover a spectrum of sugar sensitivities exhibited by other genera.

Eikenella corrodens (E. corrodens) is a Gram-negative, facultative anaerobic rod, associated with deep subgingival dental plaque in patients with advanced periodontitis. Adherence of this organism to buccal epithelial cells was investigated by Yamazaki, Ebisu and Okada (1981). Trypsinisation or heat treatment of the bacteria abolished their capacity to adhere to the epithelial cells. Adherence was also inhibited by ethylenediaminetetraacetic acid, but could be restored by the addition of Ca^{2+} ions. Sugars containing D-galactose and N-acetyl-D-galactosamine caused a marked reduction in attachment of E. corrodens to the epithelial cells. These data suggest that E. corrodens may attach to buccal cells via the interaction of lectin-like proteins on the bacterial surface with galactose-like receptors on the epithelial cells. This hypothesis is supported by the finding that human erythrocytes, irrespective of the ABO blood group, are agglutinated by E. corrodens cells, the haemagglutination being inhibited by D-galactose or N-acetyl-D-galactosamine (Ebisu, Yamazaki and Okada, 1979).

Findings/...

Findings which bear a marked similarity to those described for E. corrodens have been reported for another oral organism, Fusobacterium nucleatum (F. nucleatum). F. nucleatum is a Gram-negative, anaerobic bacillus implicated in the immunopathology of periodontal disease. Strains of this organism possess the ability to haemagglutinate a variety of erythrocytes of human and animal origin (Falkler and Hawley, 1977). In most cases, haemagglutinating activity could also be observed in sonicated preparations of the organism, and appeared to be dependent upon a heat-labile protein component of the cell wall which required Ca^{2+} ions for activity. Fimbriae could not be demonstrated by electron microscopy. In a later publication (Mongiello and Falkler, 1979), it was shown that haemagglutination by both whole cells of F. nucleatum and sonicated preparations of the organism was inhibited by the addition of sugars containing D-galactose and by N-acetyl-D-galactosamine. Adsorption of sonicated preparations to buccal epithelial cells was also inhibited by D-galactose. A recent report (Falkler and Burger, 1981) has demonstrated that haemagglutination-active fragments in sonicated preparations of F. nucleatum attach to other organisms displaying D-galactose-containing residues on their surfaces. Such organisms include Bacteroides gingivalis, Bacteroides fragilis subspecies distasonis, Bacteroides corrodens, Streptococcus morbillonum and certain strains of Streptococcus sanguis and Streptococcus mutans. This binding is dependent on Ca^{2+} ions, and is inhibited by D-galactose. Such microbial interactions may play an important role in the initial stages of colonisation and plaque development.

Organisms/...

Organisms of Bacteroides spp. possess markedly differing abilities to attach to mammalian cells. Haemagglutinating activity of certain strains of Bacteroides melaninogenicus (B. melaninogenicus) was first described by Okuda and Takazoe (1974). They showed that 29 out of 59 strains of B. melaninogenicus isolated from human gingival crevices caused agglutination of sheep, horse, guinea-pig, rabbit and human erythrocytes. Heat treatment destroyed this activity, and fimbrial structures were observed in all strains that caused haemagglutination (Okuda and Takazoe, 1974). Such structures were not observed in strains without haemagglutinating activity. This work has been extended by Slots and Gibbons (1978) who studied the ability of cells of B.melaninogenicus subsp. asaccharolyticus 381 to adhere to surfaces relevant to colonisation in the mouth. Preliminary experiments showed that of 48 asaccharolytic strains of B. melaninogenicus, 47 agglutinated human erythrocytes, whereas none of 20 fermentative strains were active. Electron microscopy revealed that both asaccharolytic and fermentative strains possessed fimbriae, indicating that the presence of fimbriae did not correlate with haemagglutinating activities of B. melaninogenicus strains. Both asaccharolytic and fermentative B. melaninogenicus strains adhered in high numbers to buccal epithelial cells and to the surfaces of several Gram-positive bacteria, including Actinomyces viscosus, Actinomyces naeslundii, Actinomyces israelii, Streptococcus sanguis and Streptococcus mitis. B. melaninogenicus subspecies asaccharolyticus 381 also attached, but in low numbers, to untreated and to saliva-treated hydroxyapatite. Both fermentative and nonfermentative strains of B. melaninogenicus attached in high numbers to crevicular epithelial cells/...

cells derived from human periodontal pockets. Saliva and serum inhibited attachment of B. melaninogenicus cells to all of these surfaces, except for adherence to Gram-positive bacteria commonly present in plaque (as listed above). Collectively, these data suggest that the presence of dental plaque containing Gram-positive bacteria may be essential for the attachment and colonisation of B. melaninogenicus. Observations of the attachment of a streptomycin-resistant mutant of B. melaninogenicus 381 to oral surfaces in vivo agreed well with these in vitro findings. Bacteroides cells introduced into the mouths of volunteers attached in high numbers to pre-existing dental plaque, but not to other oral surfaces (Slots and Gibbons, 1978).

Okuda, Slots and Genco (1981) showed that all their study strains of Bacteroides gingivalis, Bacteroides asaccharolyticus and B. melaninogenicus subspecies possessed numerous fimbriae and capsule-like outer structures. B. gingivalis strongly agglutinated 16 erythrocyte species studied, while B. asaccharolyticus showed variable and weak agglutination of only a few erythrocyte species. B. melaninogenicus subsp. intermedius strains strongly agglutinated rabbit erythrocytes and exhibited variable, often weak agglutination of 8 other erythrocyte species. Preparations of capsular polysaccharide or lipopolysaccharide from B. gingivalis failed to agglutinate sheep, horse or rabbit erythrocytes, while preparations of fimbriae from the same organism possessed marked haemagglutinating activity with these cells. These results suggest that fimbriae, to a large extent, determine the adherence capability of B. gingivalis.

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The addition of 0.05 M D-glucosamine, D-galactosamine or N-acetylneuraminic acid inhibited the haemagglutinating activity of B. melaninogenicus subsp. intermedius, whereas the haemagglutinating activity of B. gingivalis was unaffected by any of 16 test sugars (Okuda et al., 1981).

It is clear, therefore, that cells of the various Bacteroides species and sub-species show markedly differing abilities to attach to mammalian cells. This variation is likely to be due to both differences in surface structure, and to the varied range of adhesins expressed at the cell surfaces of these organisms.

4.8 Concluding remarks

The attachment mechanisms described for the particular organisms quoted give an indication of the vast range and complexity of microbe-host cell interactions. However, it is possible to select individual characteristics which are common to a number of them. Of particular importance is the presence of fimbriae, or similar filamentous appendages. These structures are associated with many bacterial species, and are often intimately involved with adherence phenomena. Another common finding is that bacterial adhesins often recognise and bind to sugar residues expressed at the surface of eukaryotic cells or other bacteria. The specificity of binding mechanisms is often very marked, which may help to explain host and tissue tropisms, as well as the pathogenicity of certain micro-organisms in particular body sites. It is sometimes difficult, however, to be certain of the biological significance of conclusions drawn from in vitro assays. They point the way to possible attachment/...

attachment mechanisms used by micro-organisms, but until methods can be developed to assess their role in the whole animal, caution should be exercised in interpretation of the data. It is possible, nevertheless, that detailed study of such specific interactions will provide a more thorough understanding of the pathogenesis of infectious diseases, in addition to new therapeutic and preventive techniques.

5 Subminimal inhibitory concentrations of antibiotics and bacterial adhesion

There are two classical mechanisms by which antibiotics clear a host of pathogenic bacteria. Some antibiotics kill the invading micro-organism and are termed bactericidal, while others, the bacteriostatic type, interfere with bacterial multiplication. Concentrations of antibiotics that are greater than or equal to the minimum inhibitory concentration (MIC) or the minimum bactericidal concentration (MBC) thus produce dramatic changes in bacteria. An interesting finding, however, is that concentrations of antibiotics lower than the MIC or MBC usually show qualitatively different effects, rather than milder forms of those produced by higher doses of antibiotic (Lorian, 1975; Washington, 1979; Duguid, 1946). These effects may be categorised into those that cause morphologic or ultrastructural changes, and those that result in a decrease in the number of micro-organisms exposed to the agent relative to those not exposed.

Recent studies have indicated that one of the more subtle ways in which antibiotics may alter host-parasite relationships is by interfering with the formation and expression of bacterial adhesins (Alkan and Beachey, 1978; Eisenstein, Ofek and Beachey, 1979; Eisenstein, Beachey and Ofek, 1980). The reports which have been published are concerned largely with effects on the attachment of group A streptococci and *E. coli* to mucosal cells.

Group A streptococci have been shown to bind to mucosal cells via cell membrane-associated lipoteichoic acid (LTA) (Beachey, 1975). If these/...

these organisms are treated with sublethal concentrations of penicillin, either during the resting or log phases of growth, there is a greatly increased loss of LTA from the bacteria, and a subsequent reduction in attachment of the treated organisms to oral mucosal cells (Alkan and Beachey, 1978). It is conceivable that such a mechanism may indirectly displace group A streptococci from epithelial surfaces to which they have attached, as well as influencing the initial colonisation of a body site by the organism. However, other factors may also play a role in reducing the attachment. Tylewska et al. (1980) have shown that if group A streptococci are grown in the presence of sub-lethal doses of a number of antibiotics, including penicillin, tetracycline and rifampin, both the surface hydrophobicity of the bacteria and their subsequent attachment to pharyngeal cells are decreased. In general, pathogenic bacteria are often more hydrophobic than non-pathogenic ones (Tylewska et al., 1980), therefore antibiotic-mediated alteration of bacterial hydrophobicity may be another mechanism by which bacterial attachment is reduced.

Results gained from the work with E. coli are slightly more complex. Growth of a streptomycin-sensitive strain of E. coli in broth containing a sublethal concentration of streptomycin caused a decrease in the mannose-binding activity of the organism, and reduced its ability to adhere to epithelial cells (Eisenstein et al., 1979). This effect could not be obtained by adding streptomycin to stationary phase cells, thus appearing to be related to initial formation of adhesin. The mannose-binding activity and epithelial cell adherence of a streptomycin-resistant mutant/...

mutant of this organism were not affected by growth in streptomycin-containing broth. This suggests that the effects of streptomycin on the surface properties of E. coli are mediated through the classical streptomycin target site on bacterial ribosomes (Eisenstein et al., 1979). In contrast, experiments with a different streptomycin-resistant strain showed that structurally altered, nonfunctional type I fimbriae were produced when this particular organism was grown in the presence of streptomycin (Eisenstein, Ofek and Beachey, 1981). These bacteria were as heavily fimbriated as untreated bacteria, but the fimbriae on the drug-treated organisms were longer than those on untreated organisms, lacked mannose-specific agglutinating activity and adhered poorly to epithelial cells and leukocytes. Thus, even in the case of a streptomycin-resistant organism, it is still possible for the antibiotic to cause production of an aberrant fimbrial protein, probably resulting from drug-induced mistranslation of messenger RNA (Eisenstein, Ofek and Beachey, 1981).

It has been shown that exposure of E. coli to sublethal doses of penicillin during growth, causes the bacteria to lose their ability to agglutinate yeast cells or to adhere to epithelial cells (Beachey, Eisenstein and Ofek, 1981). This effect was found to correlate with transformation of the normal rod-shaped organisms into filaments devoid of fimbriae. Exposure of resting-phase E. coli to penicillin did not affect their ability to adhere, indicating that the antibiotic affects the initial formation of surface adhesin.

Sandberg,/...

Sandberg, Stenqvist and Svanborg-Edén (1979) studied the effects of subminimal inhibitory concentrations of ampicillin, chloramphenicol and nitrofurantoin on the adhesion of 15 clinical isolates of E. coli to human uroepithelial cells. After exposure of bacteria for 4h to one quarter of the MIC of each drug, bacterial elongation was obvious and consistent with ampicillin, less marked with nitrofurantoin and was not evident with chloramphenicol. At the same level of drug, ampicillin-treated organisms attached less than untreated bacteria, while neither chloramphenicol nor nitrofurantoin affected attachment.

Results gained by Vosbeck et al. (1979) are somewhat at variance with the above data. In studies of the adhesion of one strain of E. coli to a human epithelioid tissue culture cell line, it was shown that growth of the organism in subminimal inhibitory concentrations of tetracycline, clindamycin and trimethoprim-sulfametrole caused a significant reduction in adhesion, while nalidixic acid, at one quarter of the MIC, increased adhesion. Penicillin G, ampicillin, mecillinam, cephacetrile, cephalexin, cefotaxime, chloramphenicol and streptomycin did not affect adhesion significantly at the same concentration. It therefore seems likely that differences exist between sub-MIC effects of antibiotics in different assay systems, as well as between different bacterial strains in the same system.

The attachment of a vancomycin-tolerant strain of Streptococcus sanguis to aortic vegetations in rats was studied by Bernard, Francioli and Glauser (1981). Prophylactic, intravenous vancomycin given 30 minutes before bacterial challenge decreased the incidence of endocarditis from 88% to 8%, despite the fact that peak vancomycin levels were/...

were below the MBC. To investigate this effect further, Streptococcus sanguis were incubated with an inhibitory concentration of vancomycin for 10h, then washed. Whereas 85% of rats inoculated with control bacteria developed endocarditis, only 42% of those inoculated with vancomycin-exposed bacteria did so. When the rats were killed 30min after bacterial challenge, Streptococcus sanguis were detected by culture of the vegetations in 44% of rats injected with control bacteria, but in only 13% of those challenged with vancomycin-exposed bacteria. It was shown that the treated bacteria did not demonstrate enhanced clearance by phagocytic cells, therefore the results suggested that vancomycin exerted its protection by reducing adherence of tolerant Streptococcus sanguis to vegetations.

These results were supported and expanded by Scheld et al. (1981), who utilised an in vitro assay system to study the effect of sub-MIC levels of various antibiotics on streptococcal adhesion to a fibrin-platelet matrix. This matrix simulated nonbacterial thrombotic endocarditis. They showed that adhesion of Streptococcus sanguis and Streptococcus faecalis to the matrix was significantly reduced by exposure of the bacteria to one quarter MIC levels of vancomycin, penicillin, tetracycline, chloramphenicol and streptomycin, but not by rifampin or trimethoprim-sulfametrole. These effects were dose-dependent and increased with duration of exposure to antibiotic. There was a correlation of these in vitro findings with a diminished capacity of sub-MIC vancomycin-exposed Streptococcus sanguis to produce endocarditis in vivo. After intravenous inoculation of 10^6 colony forming units of pre-incubated organisms into rabbits with traumatised aortic valves, six/...

six of 22 developed endocarditis, against 17 of 22 controls (Scheld et al., 1981). Thus, the exposure of streptococci to low concentrations of certain bacteriostatic and bactericidal antibiotics may reduce their ability to attach to vegetations and cause endocarditis.

Finally, Peros and Gibbons (1982) have published a report concerning the influence of sublethal antibiotic concentrations on bacterial adherence to saliva-treated hydroxyapatite. Cells of Actinomyces viscosus LY7 and S2, Bacteroides gingivalis 381, Capnocytophaga ochraceus 6 and Actinobacillus actinomycetemcomitans N27 attached in lower numbers to saliva-treated hydroxyapatite after growth in the presence of half the MIC of tetracycline. However, cells of Actinomyces naeslundii L13 and S4 attached in higher numbers when grown in the presence of tetracycline, clindamycin, erythromycin, chloramphenicol or neomycin. Two strains of Streptococcus mutans also exhibited increased adherence to saliva-treated hydroxyapatite after culture in the presence of 50% or 25% of the MIC of penicillin.

Thus, it would appear that subinhibitory levels of several antibiotics are capable of suppressing the adhesive properties of bacteria, without greatly altering bacterial growth. Reduction of bacterial adhesion may prove to be an important criterion in the choice of antibiotics for particular clinical situations. However, the fact that in certain cases the degree of attachment is increased by sub-MIC levels of antibiotics indicates that these effects may also act against the host, and as such require an equally thorough investigation. The selection/...

selection of antibiotic-resistant strains of bacteria, which commonly follows the administration of low levels of antibiotics, is also a danger to be borne in mind when considering any possible clinical applications of these findings.

6 The biological properties of Corynebacterium parvum

Anaerobic corynebacteria were first recognised in 1897, when Roux, a veterinary surgeon, described Corynebacterium pyogenes, an organism he had isolated from purulent abscesses affecting cattle (Prévot, 1975). Since then, many strains of anaerobic corynebacteria have been distinguished. One of the major problems involved in study of these organisms has been the complicated taxonomy, related at least partly to the morphological similarities between strains. This difficulty was alleviated to some extent by Johnson and Cummins (1972), who divided the strains into four groups on the basis of serology, cell wall composition and estimates of DNA homology.

It was Torrey (1916) who first stressed the affinity of anaerobic corynebacteria for the reticuloendothelial system (RES), when he described the Bacillus lymphophilus species. Since then, anaerobic corynebacteria, in particular Corynebacterium parvum (C. parvum), have been shown to induce widespread effects on the lymphoid tissues. Injection of C. parvum intravenously into mice causes splenomegaly and hepatomegaly, with stimulation of the RES (Halpern et al., 1964). In addition, such injections promote humoral antibody formation (Neveu, Branellec and Biozzi, 1964), prevent the expression of contact sensitivity (Asherson and Allwood, 1971), depress the responsiveness to phytohaemagglutinin of spleen cells (Scott, 1972a), increase resistance to bacterial infections (Adlam, Broughton and Scott, 1972) and retard the growth of tumours (Halpern et al., 1966). C. parvum also leads in mice to transient anaemia, and there is evidence for increased phagocytosis of syngeneic red cells and autoantibody formation against red cells in C. parvum-treated mice (McBride, Jones and Weir, 1974).

There/...

There is evidence to suggest that activated macrophages may play a causal role in several of these phenomena. The adjuvant effect of C. parvum is thought to be at least partially mediated via activated macrophages, on whose surface the presentation of small amounts of antigen to potential antibody-forming cells is intensified. This has been demonstrated by Wiener (1975) who showed that peritoneal macrophages from C. parvum-treated mice are more efficient than normal macrophages in promoting antibody formation to sheep red blood cells in an in vitro system. Responsiveness to phytohaemagglutinin can be restored to spleen cells from C. parvum-treated mice by removal of macrophages (Scott, 1972b). Finally, evidence suggests that enhancement of tumour immunity by C. parvum may involve macrophage stimulation, since it is an effective anti-tumour agent in T-cell deprived mice (Woodruff, Dunbar and Ghaffar, 1973).

The underlying basis as to what actually occurs after introduction of C. parvum is not well understood. Most results have been interpreted to indicate the induction of comparatively non-specific, aggressor macrophages by C. parvum. However, Ojo, Haller and Wigzell (1978) have obtained data that indicate a relative selectivity in the aggressive patterns exhibited by such effector cells, and showed that the reactivity of C. parvum-induced peritoneal cells in the mouse displayed a complete concordance with the specificity pattern of mouse natural killer cells.

It is believed that C. parvum may activate macrophages in vivo or in vitro by both direct and immunologically mediated pathways (Bomford and Christie, 1975; Christie and Bomford, 1975). The exact mechanism by which these organisms stimulate macrophages is not clear, but

Ögmundsdóttir/...

Ögmundsdóttir and Weir (1976) have demonstrated a direct interaction between C. parvum and plasma membrane receptors of glass-adherent mouse peritoneal exudate cells, in vitro. These receptors were shown to have a specificity for sugar determinants in the cell wall of C. parvum. The interaction of neurotransmitters, hormones, drugs and toxins with plasma membrane receptors is generally recognised to act as a signal which initiates the cell stimulation, drug or toxin effects. In a similar vein it is possible that the binding of C. parvum to the plasma membrane of macrophages may be directly responsible for the wide range of activities shown by macrophages from C. parvum-treated animals (Ögmundsdóttir and Weir, 1976).

7 Infections with group B streptococci.

Beta-haemolytic streptococci of Lancefield group B were first implicated in human disease by Fry (1938), but their frequent association with serious neonatal infections was only recognised in the early 1970's (Franciosi, Knostman and Zimmerman, 1973). Although of less numerical importance, infections in adults do occur and may prove fatal (Bayer et al., 1976).

The reason for this apparent increase in the incidence of group B streptococcal infections among neonates and young infants remains obscure, but may represent a continuation of the shifts in the prevalence of aetiological agents associated with neonatal infection during the past 40 years (Baker, 1980). In the 1930's, the group A streptococcus was the most common blood culture isolate from neonates and post-partum women, but by the early 1950's had been replaced by Gram-negative enterobacteria. This was followed later that decade by widespread outbreaks of Staphylococcus aureus infections in nurseries. By 1960, coliform organisms were associated with the majority of serious neonatal infections, and the group B streptococcus emerged as a significant neonatal pathogen in the early 1970's.

7.1 Laboratory characteristics, serotyping and cell wall antigens of group B streptococci

Although group B streptococci (GBS) have a characteristic colonial morphology (Brown, 1920), more precise tests are required for the presumptive identification of this organism. Commonly used laboratory tests include/...

include bacitracin inhibition (94-98% of strains are resistant) (Facklam et al., 1974), hydrolysis of sodium hippurate broth (99% of strains are hippurate positive) (Facklam et al., 1974) and cyclic adenosine monophosphate (CAMP) testing, in which the CAMP factor (Christie, Atkins and Munch-Petersen, 1944) of GBS, in the presence of staphylococcal β-lysin, causes a synergistic haemolysis on sheep blood agar plates (98-100% of strains are positive) (Wilkinson, 1977). Strains which are non-haemolytic on blood agar may account for 2% (Wilkinson, 1978) to 36% (Roe et al., 1976) of isolates, and give positive results in the presumptive tests outlined above. They are virulent in man (Roe et al., 1976).

Serological grouping of isolates with hyperimmune antiserum containing group B-specific antibodies is required for definitive identification. Lancefield's early work with group B streptococcal antigens (Lancefield, 1934; Lancefield, 1938) led to the definition of two cell wall carbohydrate or polysaccharide antigens. The common group B-specific ("C") substance was present in all strains of the species, while the type-specific ("S") substance allowed further classification into four distinct serotypes, termed Ia, Ib, II and III. It has since been shown that all type I strains share, in addition, a cross-reactive antigenic determinant, now termed the Iabc minor polysaccharide (Lancefield, 1934; Lancefield et al., 1975). Finally, a fifth serotype, type Ic, has been identified (Wilkinson and Eagon, 1971), which shares the Ia antigen but has, in addition, a protein antigen designated the Ibc antigen. The Ibc antigen is shared by all type Ib strains and by up to 39% of type II strains (Wilkinson, 1978). A minority of strains possess/...

possess no type-specific antigens and are therefore untypable. These only represent 1-2% of isolates from patients with serious group B streptococcal infection (Wilkinson, 1978).

7.2 Asymptomatic colonisation in adults

It has been assumed in most studies that the female genital tract is the principal reservoir for the group B streptococcus in man (Eickhoff et al., 1964). Group B streptococci are frequent constituents of the genital flora of pregnant women, and the prevalence of colonisation is similar for each trimester of gestation (Franciosi et al., 1973; Gordon and Sbarra, 1976; Baker, 1977). Colonisation rates are not significantly different in pregnant and non-pregnant women from a given population (Baker, 1977). However, isolation of GBS from vaginal cultures of nonpregnant women is significantly greater in those who are sexually experienced (20% vs. 7.1%), those who are studied during the first half of the menstrual cycle (26.5% vs. 14.5%) and those who have an intrauterine device (50% vs. 18.6%) (Baker et al., 1977). Genital colonisation is also frequent in adult males (Franciosi et al., 1973), especially sexual partners of genitally colonised women. Group B streptococci are isolated from urethral cultures in up to 45% of men in this category, indicating that the organism is sexually transmitted (Franciosi et al., 1973). The presence of GBS in the adult genital tract does not produce symptoms of genital infection such as dysuria and urethral or vaginal discharge (Wallin and Forsgren, 1975).

7.3/...

7.3 Transmission of group B streptococci to neonates

The presence of GBS in the maternal genital tract at the time of birth is a significant determinant of infection in the neonate. Vertical transmission rates of 58-72% have been indicated among neonates born to women who have GBS isolated from vaginal cultures at delivery (Anthony and Okada, 1977). A smaller but substantial proportion (10-25%) of newborns of culture-negative mothers acquire GBS in the first week of life, and infants of carrier mothers sometimes acquire a different serotype of GBS (Anthony and Okada, 1977; Aber et al., 1976). Thus, almost one half of neonatal acquisitions in the first week appear to be unrelated to the mother and are presumed to be nosocomial in origin.

It is of interest that the estimated attack rate for invasive, early-onset group B streptococcal infection among colonised neonates is only 1-2% (Baker, 1980; Anthony and Okada, 1977). One possible reason for this difference between colonisation and disease rates is suggested by the observation that women harbouring GBS in the genital tract at delivery have significantly higher concentrations of serum antibody to their infecting serotype than do non-colonised women or those colonised with heterologous serotypes (Baker and Kasper, 1976). The offspring of colonised women are usually, therefore, passively immunised via transplacental antibody transfer. This would help to explain the very high attack rate for invasive group B streptococcal disease of the early onset type among small, premature infants (Franciosi et al., 1973; Anthony and Okada, 1977), since infants born before 34 weeks of gestation receive very low levels of maternal IgG.

7.4/...

7.4 Early-onset infection in neonates

The acute- or early-onset type of group B streptococcal infection, which appears within the first two days of life, has a mortality rate of about 55% (Anthony and Okada, 1977). It affects mainly premature and low birth weight neonates whose mothers may have had obstetric complications known to predispose their offspring to septicaemia (Baker et al., 1973; Anthony and Okada, 1977). These complications include premature onset of labour, rupture of membranes for more than 24 hours before delivery, perinatal fever and genital colonisation with GBS at delivery. However, the early-onset infection may also develop in full term infants in the absence of these obstetrical complications (Anthony and Okada, 1977). The first symptoms of early-onset infection often include respiratory distress, a common finding being unexpected apnoea accompanied by shock (Quirante, Ceballos and Cassady, 1974; Baker et al., 1973). Meningitis occurs in approximately 30% of early infections (Anthony and Okada, 1977) and pulmonary involvement, as indicated by both clinical and radiographic findings, is diagnosed in about 38% of early cases (Anthony and Okada, 1977). Although histopathological descriptions of the pulmonary disease have not been uniform, a common finding has been the presence of large numbers of streptococci in the membranes and interstitium of the lung (Franciosi et al., 1973; Ablow et al., 1976). The rapidly fatal course common in the pulmonary syndrome may be due partly to massive bacterial invasion from these sites (Anthony and Okada, 1977).

Clearly, the pathogenesis of these infections involves exposure to maternal GBS, either in utero (in the presence of ruptured or unruptured membranes),/...

membranes), or at birth. The serotypes of GBS isolated from the blood or cerebrospinal fluid of neonates with early-onset infection are identical with those isolated from genital cultures of their mothers (Baker, 1980). The distribution of serotypes among isolates from women at delivery and neonates with early-onset infection without meningitis are similar, but more than 80% of isolates from those infants with meningeal invasion belong to serotype III (Wilkinson, 1978).

7.5 Late-onset infection in infants

The late-onset type of infection, which affects infants more than seven days of age, has different characteristics from the early-onset syndrome. It is only rarely associated with obstetric complications, most commonly manifests itself clinically as meningitis (75% of cases), rarely produces pulmonary infection and has a much lower mortality rate (23%) (Baker et al., 1973; Anthony and Okada, 1977). In addition, the mothers are frequently not colonised with GBS. However, a significant number of the survivors of meningeal infection, whether associated with early- or late-onset disease, will have permanent neurologic sequelae (Barton, Feigin and Lins, 1973; Horn et al., 1974; Anthony and Okada, 1977). These may include speech, visual or hearing defects, diabetes insipidus, febrile seizures and retardation of motor development.

Although meningitis is the most common clinical manifestation of late-onset infection, other sites may become infected. Conjunctivitis, endocarditis, endophthalmitis, ethmoiditis, pericarditis, empyema, pyarthrosis and osteomyelitis have all been reported (Anthony and Okada, 1977),/...

1977), but the only one to occur with any frequency is bacteraemia in the febrile infant, either without focal infection, or with bone and/or joint infection (Baker, 1980).

It has been shown that GBS of serotype III predominate (91%) in late-onset meningitis, and that type III organisms also comprise 85% of the total isolates from infants of ten days old or more (Wilkinson, 1978). This may suggest that type III GBS possess virulence out of proportion to their general prevalence.

7.6 Group B streptococcal infections in adults

Prior to the introduction of antibiotics, most patients were young women in the post-partum or post-abortal period (Baker, 1980). Since the advent of penicillin, reported cases have been more varied in focus and source of infection, and included as many men as women. They have been shown to occur more commonly in the 'compromised host', that is, in patients with underlying illness, particularly genitourinary disorders, diabetes mellitus and malignancy (Bayer et al., 1976; Nicklas, 1978).

A number of adult infections due to GBS affects post-partum women, in most cases leading to endometritis and/or wound infection without bacteraemia (Baker, 1980; Bayer et al., 1976). Bacteraemic infections among post-partum women were rare before 1970, but may be increasing in frequency (Baker, 1980). The majority of these bacteraemic infections are clinically mild, although endocarditis and meningitis may uncommonly occur (Duma et al., 1969; Lerner et al., 1977).

The/...

The association between diabetes mellitus and group B streptococcal infection was first reported by Eickhoff et al. (1964), who noted group B streptococcal bacteraemia with cellulitis of affected extremities in seven diabetic patients suffering from peripheral vascular insufficiency. This association has been confirmed by others, including Bayer et al. (1976) who showed that of 24 adults with group B streptococcal bacteraemia, 45% were diabetic.

The range of adult infections caused by GBS is wide. It includes bacterial endocarditis, puerperal sepsis, meningitis, suppurative arthritis, urinary tract infections, pyelonephritis, abscess, skin and wound infections, bronchopneumonia, pulmonary empyema, pharyngitis and prostatitis (Nicklas, 1978). Serotype III organisms are most commonly isolated from adult patients (Bayer et al., 1976), although there may be an association of serotype II organisms with adult meningitis (Wilkinson, 1978).

7.7 Therapy and prevention of group B streptococcal disease

Group B streptococci are uniformly susceptible to penicillin G (Baker, Webb and Barrett, 1976), but both the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) are considerably higher for group B than for group A streptococci (Allen and Sprunt, 1978; Anthony and Concepcion, 1975). Susceptibility to ampicillin, semi-synthetic penicillins, chloramphenicol and cephalosporin is also uniform (Baker, Webb and Barrett, 1976). Resistance to erythromycin, lincomycin and clindamycin occurs in 1-2% of isolates (Baker, Webb and Barrett, 1976)/...

1976) and to tetracycline in 85% of isolates (Anthony and Concepcion, 1975). Resistance to the aminoglycosides is uniform, but the combination of ampicillin and gentamicin has synergistic activity and accelerates the killing of GBS in vitro (Schauf et al., 1976).

Penicillin G is the preferred drug for treatment of group B streptococcal infection in infants and adults. However, the dosage for treatment of infant infections is controversial (McCracken Jr. and Feldman, 1976), as there have been frequent reports of relapses of group B streptococcal infection in infants following penicillin therapy (Broughton et al., 1976; Truog, Davis and Ray, 1976; Walker, Santos and Quintero, 1976). It has been shown that there is a large discrepancy between the MIC and MBC of penicillin for GBS (McCracken and Feldman, 1976). A possible conclusion is that relapses of group B streptococcal infection could be due to the persistence of significant numbers of organisms that are inhibited but not killed by conventional dosages of penicillin. These organisms then begin to multiply when penicillin therapy is discontinued. However, such a discrepancy also exists for group A streptococci (Allen and Sprunt, 1978), though infections with this organism have been treated with conventional dosages of penicillin for decades, without evidence of relapse in normal hosts. It is therefore unlikely that the discrepancy between the MIC and MBC of penicillin for GBS can completely explain the difficulty in treating infections with the organism. The lower sensitivity of GBS to penicillin may be of greater clinical significance. In vitro measurements of susceptibility are performed with an inoculum of 10^4 - 10^5 colony forming units per ml, but/...

but some neonates suffering from meningitis have 10^7 - 10^8 colony forming units per ml of cerebrospinal fluid before initiation of chemotherapy (Feldman, 1976). If this larger inoculum is used for *in vitro* testing to penicillin G, the MIC increases to more than 128µg/ml (Feldman, 1976), a concentration of the antibiotic not achieved in the serum of infants treated with currently recommended doses (Feldman, 1976; McCracken and Feldman, 1976). The data suggest that the dosage of penicillin G or ampicillin used for the initial treatment of group B streptococcal meningitis should be increased to ensure efficacy (Baker, 1980).

Neonatal group B streptococcal disease continues to be a serious problem, in spite of antimicrobial therapy, and there is an urgent need to devise methods of prevention. The two approaches attempted to date have been chemoprophylaxis aimed at eradication of maternal or neonatal colonisation with GBS, or induction of type-specific serum antibody by passive or active immunisation. Neither of these methods has met with complete success. Antibiotic treatment of pregnant women, their husbands and neonates colonised with GBS often fails to eradicate carriage (Hall *et al.*, 1976; Paredes, Wong and Yow, 1976; Gardner *et al.*, 1979). Furthermore, it has been shown that colonisation status at delivery cannot be predicted accurately from either positive or negative cultures obtained during gestation (Anthony, Okada and Hobel, 1978). In addition, it has been estimated that for every infant who develops streptococcal sepsis, approximately 100 colonised but non-symptomatic infants can be identified. Thus, a chemoprophylactic regimen would have/...

have to be applied to many colonised but otherwise healthy women and/or their infants (Anthony and Okada, 1977)

There is much evidence to suggest that 'natural' immunity to GBS in the neonate is the result of passive immunisation via trans-placental passage of maternal IgG antibody (Baker and Kasper, 1976; Baker et al., 1977; Kasper et al., 1979). Thus, immunologic methods for the prevention of both early- and late-onset group B streptococcal disease seem feasible. The observation that native type III poly-saccharide can induce antibody formation in type III antibody-deficient adults (Kasper et al., 1979) may point the way to eventual prevention of infant group B streptococcal disease by immunoprophylaxis.

8 Aims of the study

Two separate lines of investigation were pursued:

1 To isolate and purify lectin-like molecules from various enterobacteria, and from the immunological adjuvant, *Corynebacterium parvum*. These experiments were designed to provide biochemical evidence for the presence of sugar-specific, bacterial adhesins, the identity of which had been proposed on the basis of *in vitro* attachment assays.

2 To study the mechanisms of attachment of group B streptococci, by means of an *in vitro* assay system. In view of preliminary reports it seemed possible that a bacterial lectin may be involved, and the investigation was aimed at an evaluation of this concept.

MATERIALS AND METHODS

Bacterial Strains

All strains of bacteria were obtained either from the National Collection of Type Cultures, Colindale, England, or from the Departmental stock cultures of the Bacteriology Department, University of Edinburgh Medical School.

Escherichia coli (*E. coli*)

NCTC 10418

NCTC 4428

Salmonella typhimurium (*S. typhimurium*)

LT2

Corynebacterium parvum (*C. parvum*)

NCTC 10390

Group B streptococcus (GBS)

Type III NCTC 11080

Type Ia 090R

Group A streptococcus

NCTC 10085

Yeast Cells

Saccharomyces cerevisiae was provided by the Microbiological Laboratories, Western General Hospital, Edinburgh.

Chemical/...

Chemical Reagents

All chemicals and biochemicals were of the purest grade available commercially, and were purchased from B.D.H. Chemicals Ltd., Poole, England, Sigma London Chemical Company Ltd., Poole, England, or Koch-Light Laboratories Ltd., Colnbrook, Bucks., England.

Growth of Bacteria

Strains of *E. coli* and *S. typhimurium* were incubated aerobically in static culture for 18h at 37°C in 300ml of Oxoid No. 2 nutrient broth. The same medium (6-10l) was inoculated with this starter culture (30ml/l) and incubated for 18h at 37°C. In some later experiments, the bacteria were incubated for 48h under the same conditions, to increase the number of fimbriae. For experiments requiring organisms grown on solid medium, Colombia base agar (Oxoid Ltd.) plates were employed. In particular, *E. coli* 4428 was passaged five times on agar, in order to produce a form of the organism devoid of fimbriae. This form was cultured on 20 large plates (14cm diameter) to produce a yield of the organism for extraction procedures.

C. parvum was grown in static, anaerobic culture for 48h at 37°C, in 300ml of horse digest broth, containing 3% ($^{w}/v$) glucose. The same medium (6l) was pre-reduced, before being inoculated with the starter culture (20ml/litre). The bulk culture was incubated anaerobically at 37°C for 48h.

All strains of streptococci were cultured in Todd-Hewitt broth, and were incubated aerobically at 37°C for 18h. For experiments in which/...

which group B streptococci were grown in sub-lethal concentrations of penicillin, benzyl penicillin was added to Todd-Hewitt broth to produce the required concentration of antibiotic. The bacteria were grown in this broth for 18h at 37°C.

All stock cultures were maintained on blood agar slopes in bijou bottles, and stored at 4°C.

Growth of Yeast

Saccharomyces cerevisiae was cultured in Sabouraud broth (Oxoid Ltd.), with aerobic incubation at 28°C, for 24h. The stock culture was maintained on a slope of solid Sabouraud medium, and stored at 4°C.

Agglutination Assay

The assay was performed as described by Eshdat et al. (1978). Cells of Saccharomyces cerevisiae (0.4g) were prepared by incubation in PBS with glutaraldehyde (1mg/ml) for 1h at 23°C, followed by two washes in PBS. This was followed by incubation for 30min with 10mg/ml glycine at 23°C, and two washes in PBS. The treated cells were stored at 4°C as a suspension (0.1mg/ml) in PBS containing 0.02% ($^{w}/v$) sodium azide. The assay was performed on a microscope slide by mixing 10µl of the solution or suspension under test with 5µl of PBS and 10µl of yeast cell suspension. Agglutination was monitored by the naked eye. Sugar solutions (5µl of 0.2M) could be added to the system, prior to the addition of yeast cells, to test the inhibitory effect of the sugars on the agglutinating activity.

Quantitative/...

Quantitative Analytical Methods

Protein estimations were performed according to the method of Lowry et al. (1951). Bovine serum albumin was employed as the standard. Total carbohydrate was determined by the phenol/sulphuric acid procedure of Dubois et al. (1956), with glucose as the standard.

Total phosphate was determined by a modification of the method of Chen et al. (1956). Samples were evaporated to dryness on a micro-Kjeldahl digestion rack, using both heaters. Digestion mixture (0.1ml concentrated sulphuric acid and 70% perchloric acid (3:2 v/v)) was added to each sample, and heated for 20min, using only the lower heater. The samples were allowed to cool, before adding 8ml freshly prepared colour reagent. The colour reagent was made by mixing 3M sulphuric acid (10ml), 2.5% ($^{w}/v$) ammonium molybdate (10ml), water (80ml) and ascorbic acid (1g). The contents of the tubes were mixed thoroughly, incubated at 37°C for 1h, and the absorbance measured at 820nm.

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE was performed in the presence of 1% ($^{w}/v$) sodium dodecyl sulphate (SDS) on 10% ($^{w}/v$) polyacrylamide slab gels, with a 10mm 4% ($^{w}/v$) stacking gel, as described by Poxton and Brown (1979). The discontinuous buffer system of Laemmli (1970) was used. Samples (100µl), containing a maximum of 200µg of protein in sample buffer, were heated for 3min in a boiling water bath, then loaded into the individual wells. The electrophoresis was performed at constant voltage,/...

voltage, initially at 60V until the sample had entered the separating gel, and thereafter at 150V, until the bromophenol blue tracking dye was about 2cm from the bottom of the gel.

For proteins, the gels were stained overnight with Coomassie Brilliant Blue R, and destained with the solutions described by Poxton and Sutherland (1976). The gel was left in each of the destaining solutions for approximately 45min.

Gels were stained for carbohydrate with periodic acid-Schiff's reagent (Segrest & Jackson, 1972), and for nucleic acid with methylene blue (Peacock & Dingman, 1967). Molecular weights were determined from calibration curves of the type shown in fig. 4.

Preparative PAGE

The gel was set up in the same way as for the qualitative gel, except that instead of having 14 small wells in the stacking gel, there was one large well, which could hold 1760µl of sample. 3.5mg of protein was dissolved in 1760µl of sample buffer, and applied to the gel. Electrophoresis was performed as above, and the gel was then fixed for 30min in a mixture of 10% ($^{w}/v$) trichloroacetic acid and 20% ($^{v}/v$)methanol. It was stained for 30min in a staining solution containing 0.25% ($^{w}/v$) Coomassie Blue R, 7.5% ($^{v}/v$) acetic acid and 50% ($^{v}/v$) methanol. Destaining, to reveal the protein bands, was performed in a solution containing 7% ($^{v}/v$) acetic acid and 10% ($^{v}/v$) methanol.

The relevant band was cut out of the gel with a scalpel, and placed/...

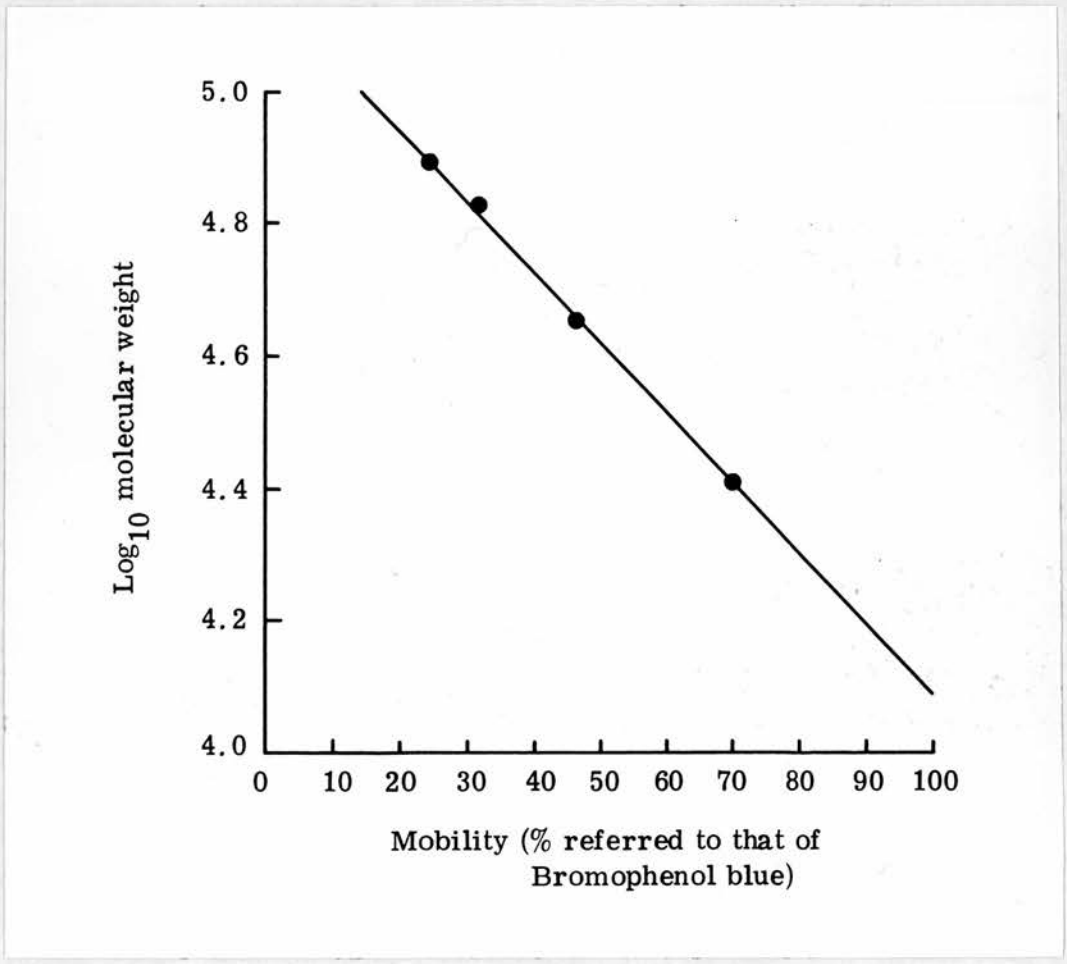


Fig. 4 Calibration curve for determination of molecular weights from polyacrylamide gels

placed into distilled water. This gel strip was cut into small pieces and placed into the electrophoresis tank (Fig. 5). The tank was filled with PAGE electrode buffer, and the protein electrophoresed out of the gel at 4mA (50V) overnight. The mixture of protein and Coomassie Blue which had migrated into the dialysis bag was pipetted off, and the protein recovered as a concentrated sample by the method of Tuszynski et al. (1977). The solution was made 10% ($^{w}/v$) in trichloroacetic acid, and allowed to stand at room temperature for 10min. It was then filtered through a 0.22µm Millipore filter, leaving particles of protein on top of the filter, and 0.15ml of chloroform, saturated with PAGE electrode buffer, was added to the filter. The protein was extracted by adding an equal volume of PAGE electrode buffer, shaking the mixture, centrifuging at 2000g for 10min, and removing the upper aqueous layer with a pipette.

SDS was removed, according to Tuszynski and Warren (1975), by dialysis against 0.1M sodium phosphate (pH 6.8) containing 1% ($^{v}/v$) mercaptoethanol. The protein-containing solution was diluted with 2ml of the dialysis buffer, then placed into a dialysis bag. Dialysis was performed against three changes of 150ml of buffer, and finally against 1500ml, over a total period of four days. The dialysis buffer was continuously stirred.

The preparation was completed by dialysis against 5mM Tris HCl buffer (pH 7.4), to remove any salts. The material was then lyophilised, and a sample checked for purity by qualitative PAGE.

Rocket/...

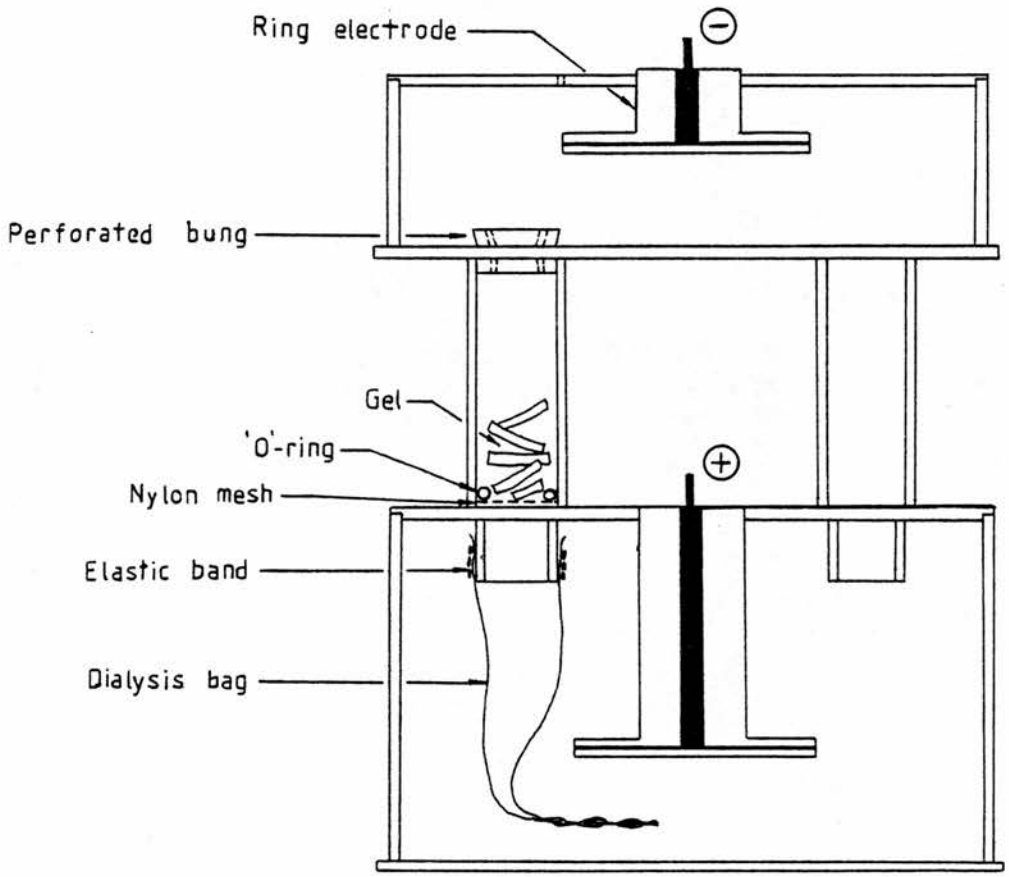


Fig. 5 Diagram of tank used to electrophorese protein out of a polyacrylamide gel strip

Rocket Immunoelectrophoresis

The technique was a modified version of that of Axelsen (1973). A piece of clean glass was covered with a thin film of agarose. Half of the glass plate was then masked out and 3ml of plain agarose, in a barbital-glycine/Tris buffer (pH 8.8), and containing 1% (^{v}/v) Triton X-100 (Weeke, 1973) was poured onto the exposed area. When the agarose had set, the masking plate was removed, and 18 circular wells were punched into the solid agarose. Three millilitres of the same agarose, but containing 250µl of serum, were poured over the remaining half of the plate. 10µl of sample was placed into each of the wells, and the gel was placed on the electrophoresis apparatus (Shandon Southern, Camberly, Surrey, England), with the antigen at the cathodic side. Electrophoresis was performed at 60V, overnight at 4°C, after which the gel was processed according to Weeke (1973). The gel was covered with a piece of filter paper, above which was a layer composed of several thicknesses of blotting paper. Several books were placed on top of the blotting paper to exert a slight pressure on the gel for 10min. The gel was then washed twice for 10min in 0.1M NaCl, and once in distilled water for 15min, before being re-pressed as before. Finally, the slide was dried with a hairdryer. It was stained for protein in a solution containing 5% (^{w}/v) Coomassie Brilliant Blue R-250, 45% (^{v}/v) ethanol and 10% (^{v}/v) glacial acetic acid, for 10min. Destaining was performed for two or three periods of 10min each, to reveal any peaks, in a destaining solution which contained 45% (^{v}/v) ethanol and 10% (^{v}/v) glacial acetic acid. The gel was left to dry naturally.

Ouchterlony/...

Ouchterlony Gel Diffusion

Ouchterlony gel diffusion slides were prepared by pouring a layer of plain agarose onto a clean microscope slide. After the agarose had set, six circular wells surrounding a central well were cut into the gel. The antigen was placed in the central well, and antisera were added to the six outer wells. The slide was left in a damp chamber for 2-4h, then examined for zones of precipitation between the antigen and the various antisera.

Extraction of Lectin-like Material from E. coli, S. typhimurium and C. parvum

The extraction was performed according to Eshdat et al. (1978). The bacteria were harvested by sedimentation at 12,000g for 20min at 4°C, suspended in 500ml cold 0.5% NaCl and recentrifuged as above. Depending on the mass of cells, the bacteria were suspended in 60-200ml PBS containing 850mM NaCl and 0.03mM phenylmethanesulphonyl fluoride. The suspension was homogenised over ice for 30min in a Sorvall Omnimixer (setting 5). The bacteria were collected by sedimentation at 10,000g for 20min, homogenised again under the same conditions, and reprecipitated as above. The combined supernate was centrifuged twice at 5000g for 10min to remove any remaining bacteria, and finally centrifuged at 48,000g for 3h to produce a pellet, which was suspended in 2-5ml PBS.

Affinity Chromatography of Crude, Lectin-containing Extract

Yeast mannan was linked to Sepharose 4B as described by Uy and Wold/...

Wold (1977). 1,4 butanediol diglycidyl ether (15ml) and 15ml 0.6M NaOH containing 30mg KBH_4 were added to 15g suction-dried, washed Sepharose 4B, and the mixture rotated mechanically for 10h at room temperature. The epoxide-activated Sepharose 4B was collected on a sintered glass funnel and washed with a large excess of distilled water. Yeast mannan (600mg) was dissolved in 12ml 0.1M NaOH, and 6g suction-dried epoxide-Sepharose 4B was suspended in this solution. The mixture was rotated mechanically at 37°C for 24h. The affinity adsorbent was collected by filtration, and washed with 500ml 0.1M borate buffer (pH 8), followed by a large volume of distilled water.

A 70mm x 15mm column was packed with the prepared mannan-Sepharose 4B, and equilibrated in PBS (pH 7.4) containing 0.2% ($^{w}/v$) sodium azide. The mannose binding activity of the column was checked with concanavalin A (con A) and the adsorbent shown to have a capacity for binding at least 200µg con A. The crude extract was centrifuged lightly to remove any solid material from the solution. Up to 2mg protein, in a maximum volume of 1ml, were washed onto the column with either PBS or 0.1M Tris-HCl buffer (pH 8) containing Dulbecco's 'B' solution (0.5ml/100ml). A minimum of 18 fractions, each of 1ml, were collected, or until no further protein was detectable in the eluate. The column was then washed with a solution of 0.1M α-methyl-D-mannoside in 0.9% ($^{w}/v$) NaCl, and a minimum of 18 fractions, each of 1ml, again collected. All fractions were screened for protein according to Lowry et al (1951). The fractions eluted with α-methyl-D-mannoside were dialysed against PBS overnight, and then against distilled water for a further 5h, in an attempt to remove the mannose moieties.

Production/...

Production of Standard Curve for Quantitative Determination of Growth of Group B Streptococci

The calibration curve was produced on the basis of culture turbidity, in a manner similar to that described by Zawaneh et al. (1979). An 18h culture of type III GBS was serially diluted tenfold in PBS, and the absorbance at 650nm of each of the ten dilutions was read on a Pye Unicam SP 600 spectrophotometer (Unicam Instruments Ltd., Cambridge, England).

To correlate the bacterial count with the spectrophotometer readings, tenfold dilutions, in sterile PBS, of the 18h culture were prepared, to a final dilution of 10^{-6}. Each dilution (0.1ml) was pipetted onto the surface of each of two blood agar plates, which had been preincubated for 2h at 37°C, to dry the agar surface. The bacteria were at once spread rapidly and widely with a fine wire loop. The plates were incubated at 37°C for 18h, and the viable count of the original culture calculated from the average colony count per plate. The absorbances at 650nm could then be related to the number of colony forming units/ml, and the calibration curve drawn.

Collection and Preparation of Buccal Cells (BEC)

BEC were gently scraped with a wooden spatula from the inside of the cheek of a healthy male donor, and suspended in 10ml Dulbecco's PBS. The suspension was placed in a thin-walled test-tube, and treated in a sonic water bath (Model 6441A, Dawe Inst. Ltd., Western/...

Western Ave., London) for 1.5min, to aid in the removal of background commensal organisms (Salit & Morton, 1981). The cells were then sedimented at 200g for 10min, and washed a further twice in Dulbecco's PBS. The washed pellet was suspended in 2ml Dulbecco's PBS, and a cell count performed in a Neubauer counting chamber (Weber and Sons, Lancing, England). The suspension was finally adjusted to a concentration of 2-6 x 10^4 per ml, by dilution with Dulbecco's PBS.

Adherence Assay

The assay technique was developed from those of several authors (Gibbons & van Houte, 1971; Fowler & Stamey, 1977; Zawaneh et al., 1979; Sobel et al., 1981). The absorbance at 650nm, of an 18h culture of GBS was adjusted to 0.43, which, from the previously constructed calibration curve, corresponded to 10^8 colony forming units per ml. The volume was noted. The bacteria were sedimented at 900g for 20min, washed once in Dulbecco's PBS, then resuspended to a concentration of 10^8 colony-forming units per ml in Dulbecco's PBS containing Dulbecco's 'B' solution (0.5ml per 100ml).

Samples of bacterial suspension (1ml) were mixed with 1ml volumes of BEC suspension in small, flat-bottomed, glass sample tubes (50 x 12mm, Samco G005, MacFarlane Robson Ltd., Thornliebank, Glasgow). The stoppered tubes were placed on a tube rotator (30 revolutions per min) and incubated at 37°C for 45min. At the end of the incubation, each mixture was filtered through a 10µm filter (Gelman Sciences Inc., Ann Arbor, Michigan, USA; 13mm diameter, polypropylene filter) held in a Millipore filter holder, mounted on the

end/...

end of a 10ml syringe. Each filter was washed with 30ml Dulbecco's PBS, then carefully removed from the filter holder and inverted onto a drop of Dulbecco's PBS on a clean microscope slide. The filters were lifted off the slide after approximately 2min. The slides were air-dried, fixed in methanol for 5min, and finally stained by Gram's method. The stained smears were mounted in DPX under coverslips.

Each mixture of bacteria and buccal cells was set up and assayed in duplicate, and the results averaged. Counting was carried out at a magnification of x 400, and for each individual experiment the number of bacteria adherent to each of 100 separate BEC was counted.

Statistical significance was tested by means of the Student's t test (Student, 1908).

Preparation of Sonicate from GBS

Type III GBS were grown for 18h in Todd-Hewitt broth, at 37°C. Bacteria were harvested by centrifugation at 20,000g for 10min, washed once in PBS, and suspended in 20ml PBS. The resulting suspension was treated in 5ml aliquots in a sonic water bath for 5min, and the cells were then sedimented at 20,000g (10min). The supernate was collected, dialysed against 5mM Tris-HCl buffer (pH 7.4) for 3.5h at 4°C and then lyophilised.

Modifications of Sonicate

Heat treatment of the sonicate was performed by treatment in an autoclave/...

autoclave at 121°C (15min). For periodate oxidation of the sonicate, a 2ml sample (140µg/ml protein in PBS) was made 0.1M in sodium metaperiodate, and left overnight at room temperature, in the dark. Excess periodate was destroyed by treatment with ethylene glycol, followed by lyophilisation. The sample was then re-dissolved in 2ml distilled water. A control sample was treated in the same way, except that no periodate was added.

Pretreatments of GBS

Bacterial cells were periodate-treated at room temperature in 4ml acetate buffer, pH 4.6, containing 10mg/ml sodium metaperiodate, for 5min. Control organisms were suspended in the same buffer, but containing no periodate. The bacteria were then washed once in Dulbecco's PBS, before being resuspended in Dulbecco's PBS + 'B' to the required concentration.

Bacteria were treated with neuraminidase from Clostridium perfringens, Type VI (Sigma, No. N 3001) at a concentration of 0.025 units/ml in 0.1M sodium acetate buffer (pH 5.0), according to Zawaneh et al. (1979). The treatment was performed for 1h at 37°C, and the bacteria were washed once in Dulbecco's PBS, prior to the adherence assay.

Trypsin treatment of bacteria was performed in 46mM Tris-HCl buffer (pH 7.4), supplemented with 11.5 mM $CaCl_2$ and containing trypsin (EC 3, 4, 21, 4 Sigma Chemical Co., Type III) at a concentration of 10,000 BAEE units per ml, as described by Saunders and Miller/...

Miller (1981). The incubation was carried out at 37°C for 1h, and the cells were washed twice in Dulbecco's PBS, before the attachment assay.

Bacteria were heat-treated either by heating in a 75°C water bath for 30min, or in an autoclave to 121°C for 15min.

Sugar treatment of bacteria was performed by suspending the cells in Dulbecco's PBS + 'B' containing the sugar, and rotating the suspension at 37°C for 0.5-1h. The bacteria were washed once in Dulbecco's PBS + 'B' prior to the adherence assay. The sugars tested were: 0.2M D-galactose; 0.2M L-fucose; 0.05 M lactose; 0.2M D-glucose; 0.2M α-methyl-D-mannoside; 0.2M maltose; 0.1M N-acetyl-D-galactosamine; 0.1M, 25mM, 12.5mM and 6.25mM N-acetyl-D-glucosamine.

Pretreatments of Buccal Cells

Washed BEC were treated with streptococcal sonicate by mixing the cells with the sonicate, dissolved in Dulbecco's PBS + 'B', on a tube rotator at 37°C for 1h. The BEC were then sedimented by centrifugation (200g, 10min).

BEC were treated with membrane lipoteichoic acid (0.7mg/ml) in the same manner as with the sonicate.

The technique for periodate oxidation of BEC was identical with that described for periodate treatment of GBS.

Gel Filtration of Sonicate

Gel filtration of the sonicate was performed on 270mm x 13mm columns/...

columns of both Sephadex G-75 and Sephadex G-100. The columns were equilibrated in PBS. Before application to the column bed, each freeze-dried sample of sonicate, containing a maximum of 2.5mg protein, was dissolved in 250µl PBS. Complete dissolution was ensured by treatment with a vortex mixer for 5s, and the solution was then spun in an MSE bench centrifuge (600g, 15min) to sediment any particulate matter. The clear supernate was gently removed by pipetting.

After equilibration of the column, the top of the column bed was exposed, and the clear solution of sonicate applied from a Gilson Pipetman P200 pipette. The sample was allowed to diffuse into the gel by gravity, and was then carefully washed on with two aliquots of 0.5ml PBS. A peristaltic pump (LKB 10200 Perpex Peristaltic Pump) maintained the rate of flow at 0.2ml/min, and 20 fractions, each of 2ml, were collected on a fraction collector (LKB 2070 Ultrorac).

Fractions were screened for absorbance at 260nm and at 280nm.

DEAE Cellulose Ion-Exchange Chromatography

DE32 ion-exchange cellulose (15g) (Whatman, H. Reeve Angel and Co., London, England) was precycled in 0.5M HCl and 0.5M NaOH, as recommended by the manufacturer, and finally washed extensively with distilled water. The slurry of exchanger in distilled water was degassed, then equilibrated in 0.05M Tris-HCl buffer (pH 7.8), containing 0.01M NaCl.

The/...

The precycled and equilibrated ion exchanger was briefly degassed again, before being packed into a 270mm x 13mm column. Final equilibration was performed by running equilibrating buffer through the column for 2.5h.

A 2ml sample, containing approximately 2.5mg protein, which had been dialysed against the equilibrating buffer, was applied to the column, and the column washed with 40ml of the same buffer. A flow rate of 0.2ml/min was maintained by a peristaltic pump. A salt gradient (0.01M NaCl to 1.0M NaCl) in 0.05M Tris-HCl buffer (pH 7.8) was applied to the column from a gradient mixer. Fractions, (40 x 1ml), were collected on a fraction collector. The absorbance (280nm) of the eluate was monitored continuously on an ultraviolet spectrophotometer (Pye Unicam SP6-550, Unicam Instruments, Cambridge, England) and a trace obtained on a pen recorder (Venture Servoscribe 1S, Belmont Instruments, Glasgow). Selected individual fractions were also screened (absorbance at 280nm), to ensure that the correct fractions were pooled.

Absorption of Sonicate with BEC

Sonicate was absorbed with BEC up to a maximum of three times. Sonicate, dissolved in Dulbecco's PBS + 'B', was mixed with washed epithelial cells and rotated for 1h at 37°C. The cells were sedimented (200g, 10min), and the sonicate cleared of any residual cells by centrifugation at 9000g for 30s (Beckman Microfuge B, Beckman Instruments Inc., California, USA).

Extraction/...

Extraction and Purification of Membrane Lipoteichoic Acid from Type III GBS

The extraction procedure was based on the method of Coley, Duckworth and Baddiley (1975). Bacteria were cultured in Todd-Hewitt broth (6l) and harvested at 16,000g (10min). The harvested organisms (17.9g wet weight) were ruptured in a French pressure cell (Aminco, Silver Springs, Maryland, USA). A 30% ($^{w}/v$) suspension of the bacteria in distilled water was subjected to a pressure of 6-7000lb/in^2, in the pressure cell. The resulting suspension was centrifuged (48,000g, 10min), and the supernate, containing the cell membranes, was collected. The layer of cell walls was washed off the top of the pellet of unbroken cells, the latter being resuspended in distilled water and re-pressed. This procedure was repeated three times, until most of the cells were ruptured. The resulting suspension of cell membranes (100ml) was freeze-dried, and the lyophilised material suspended in 300ml chloroform-methanol (2:1 $^{v}/v$) mixture. This mixture was stirred at 4°C for 7h, then filtered and the membranes resuspended in a further 300ml chloroform-methanol mixture. After being stirred overnight, the suspension was again filtered, and the membranes washed in chloroform-methanol mixture, to ensure complete removal of lipids. Traces of solvents were removed by drying the residue in air.

The defatted membranes (1.78g) were suspended in 65ml distilled water, and stirred vigorously with an equal volume of 80% ($^{w}/v$) aqueous phenol for 40min at 4°C. The resulting emulsion was centrifuged (16,000g, 30min), and the upper aqueous layer collected, then dialysed/...

dialysed against running water overnight. The dialysate was mixed with an equal volume of 0.2M acetate buffer (pH 5.0), containing 10^{-3}M $MgCl_2$. RNAse and DNAse were added, and the mixture incubated at 37°C, under toluene, for 48h.

The volume of the incubation mixture was reduced to 52ml by rotary evaporation, and the aqueous phenol extraction procedure repeated in order to remove the enzymes and protein not removed in the first extraction. After centrifugation, the upper aqueous layer was dialysed against running water overnight and freeze-dried.

The freeze-dried powder (138mg) was dissolved in 2.7ml distilled water. All insoluble material was removed by centrifugation (600g, 10min), and the clear supernate applied to a 600mm x 16mm column of Sepharose 6B. The material was eluted with an upward flow (12ml/h) of 0.2M ammonium acetate, containing 0.01% (^{w}/v) sodium azide, the column having been previously equilibrated in this buffer. The gel filtration was performed at 4°C, and 50 fractions, each of 3ml, were collected. Every second fraction was analysed for carbohydrate, total phosphorus, and nucleic acid content.

Electron Microscopy

Broth cultures of bacteria were fixed for 10min in 0.25% (^{v}/v) formaldehyde, centrifuged for 30min at 600g and washed once in 1% (^{w}/v) ammonium acetate. The cells were then resuspended in 1ml 1% (^{w}/v) ammonium acetate. Equal volumes of specimen and 2% (^{w}/v) phosphotungstic acid (buffered to pH 7.0) were mixed on the surface of/...

of a clean glass slide, and a drop of the suspension transferred by means of a fine platinum loop onto the surface of a colloidon membrane supported on a copper electron microscope grid. After 30s, excess fluid was removed by allowing a piece of filter paper to come into contact with the edge of the specimen drop, and the preparation was allowed to dry overnight.

Cultures which had been grown on nutrient agar were suspended by gently adding 7.5ml 1% ($^{w}/v$) ammonium acetate to the surface of the plate. The plate was allowed to stand for 5min, and was then agitated gently for 1min. The resulting suspension was transferred to a test tube, fixed with 0.25% ($^{v}/v$) formaldehyde, centrifuged and resuspended in 1ml 1% ($^{w}/v$) ammonium acetate. Grids were then prepared as described above.

All grids were examined in an Hitachi HU12A electron microscope.

Bacterial Counts

Total bacterial counts were performed in a Thoma type counting chamber (Hawksley, England). The bacterial count was evaluated from the following formula:

$$\frac{\text{No. of bacteria}}{\text{No. of squares counted}} \times 4 \times 10^{6} \times \text{initial dilution factor} = \text{cells/ml}$$

Surface viable counts were made by the spreading method, in which agar plates were dried at 37°C for 2h with the lids ajar, and were then inoculated with 0.1ml aliquots of tenfold dilutions of the/...

the bacterial suspension. The inoculum was at once spread widely with a fine wire loop, and the viable count calculated from the average colony count per plate.

Scanning Spectrophotometry

Scanning spectrophotometry was performed on a Unicam SP8000 Ultraviolet Recording Spectrophotometer (Unicam Instruments, Cambridge, England).

Antibiotic Sensitivity of GBS

Type III GBS was seeded onto both blood agar and horse blood agar plates. The following antibiotic discs were placed: benzyl penicillin; erythromycin; cephaloridine; chloramphenicol; tetracycline; bacitracin; ampicillin.

Determination of Minimal Inhibitory Concentration (MIC) of Benzyl Penicillin for Type III GBS (NCTC 11080)

The tube dilution test was employed, as described by Gould (1975). The test medium was Oxoid Sensitivity Test Broth containing 0.0024% ($^{w}/v$) bromothymol green.

Sterile distilled water (4ml) was injected into a vial containing 600mg 'Crystapen' (Benzyl-penicillin (sodium B.P.)), to produce a stock solution of 150mg/ml. This stock solution was diluted in sterile distilled water to a concentration of 2.5µg/ml. The/...

The final dilution, to a concentration of 0.25µg/ml, was made in sensitivity test broth. Doubling dilutions in sensitivity test broth were prepared from the 0.25µg/ml penicillin-containing broth, to give a range of nine concentrations of penicillin, from 0.25µg/ml to 9.8×10^{-4}µg/ml. This set of dilutions was prepared in duplicate, and for each set there was also a growth control (containing no antibiotic) and a media control (which was not inoculated). One set of tubes was inoculated with GBS, and the other set with the Oxford Staphylococcus. The MIC of benzyl penicillin for the latter is known to be 0.03µg/ml. In each case the inoculum was one drop of a $^{1}/_{250}$ dilution of an overnight broth culture. The tubes were incubated at 37°C for 24h, and growth of the organisms was monitored by both turbidity, and a change in colour of the medium from green to yellow.

A loopful of culture from each of the tubes inoculated with GBS was plated out on blood agar, and incubated at 37°C for 24h, in order to indicate the minimum bactericidal concentration.

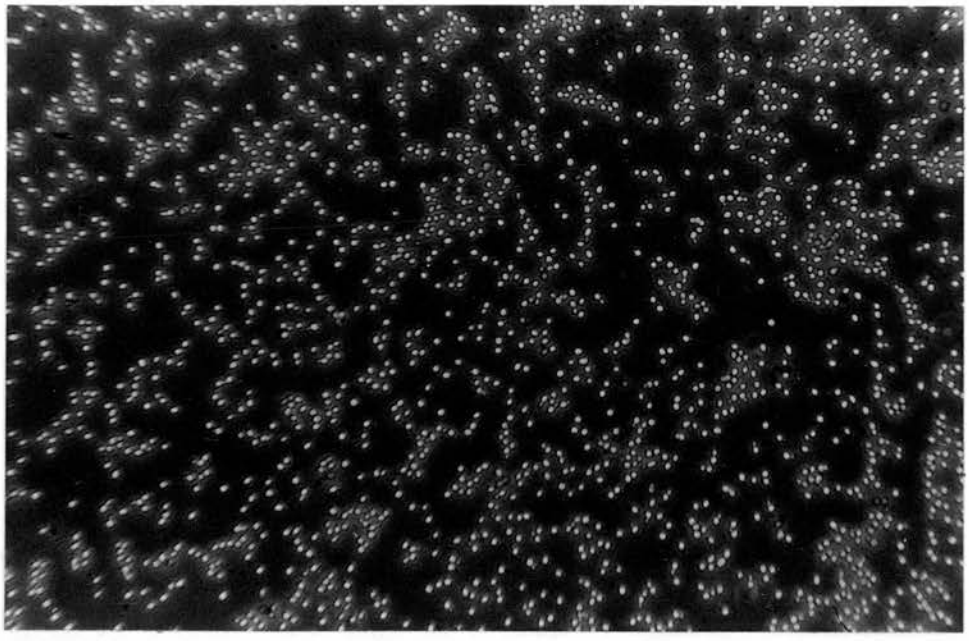
RESULTS

1 Preparation and Purification of Lectin-like Molecules from Bacterial Cell Surfaces

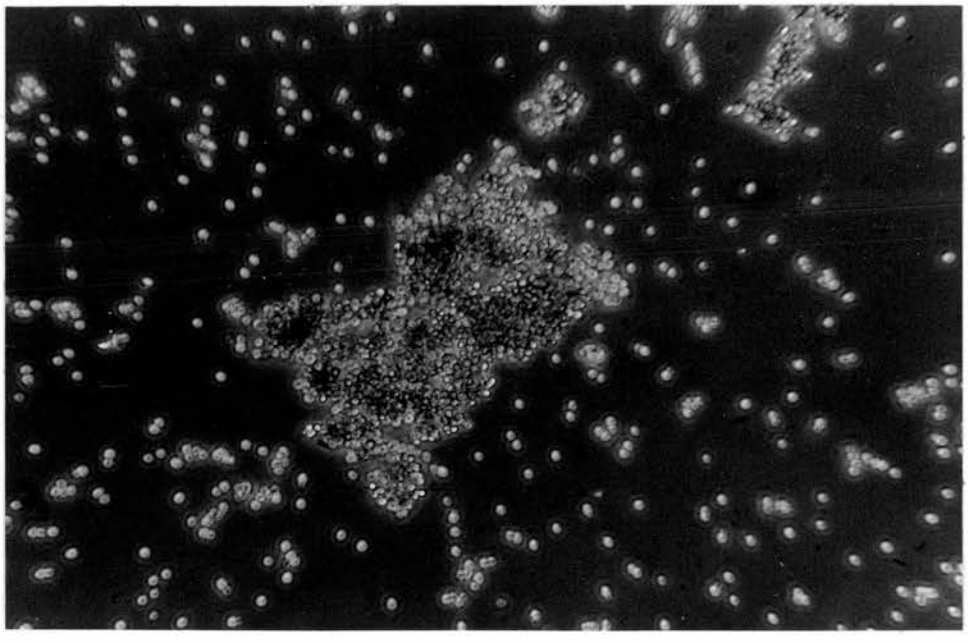
Cell surface extracts were prepared from various strains of E. coli, from C. parvum and from S. typhimurium, by the homogenisation procedure described by Eshdat et al. (1978). These extracts were subjected to affinity chromatography on yeast mannan-linked Sepharose 4B, to purify any mannosephilic lectin-like molecules present in the resulting mixture of proteins. The fractions eluted from the column were tested for their yeast agglutinating power (Fig. 6) and analysed by SDS-PAGE.

1.1 Extraction from E. coli 10418

Whole cells of E. coli 10418 strongly agglutinate cells of Saccharomyces cerevisiae, but 0.1M α-methyl-D-mannoside (αMM) inhibits the agglutination reaction. Extraction from 4.6g (wet weight) of cells of this organism, which had been incubated for 18h, yielded 640µg protein. Affinity chromatography of this material, produced the elution profile shown in fig. 7. The first 20 fractions were eluted with 0.1M Tris-HCl buffer (pH 8) containing Dulbecco's 'B', and fractions 21-40 were eluted with 0.1M αMM in 0.9% ($^{w}/v$) NaCl. Despite dialysis of the fractions containing αMM, no agglutination of yeast cells could be produced with the material eluted with the mannoside. However, most of the fractions eluted with Tris buffer caused agglutination of yeast cells, fraction 9 producing the strongest reaction. SDS-PAGE (Fig. 8) of selected fractions indicated the presence of a major/...



a



b

Fig. 6 Agglutination of yeast cells by bacterial extracts containing lectin-like molecules. Photomicrograph x 400.

a) Negative control b) Positive agglutination

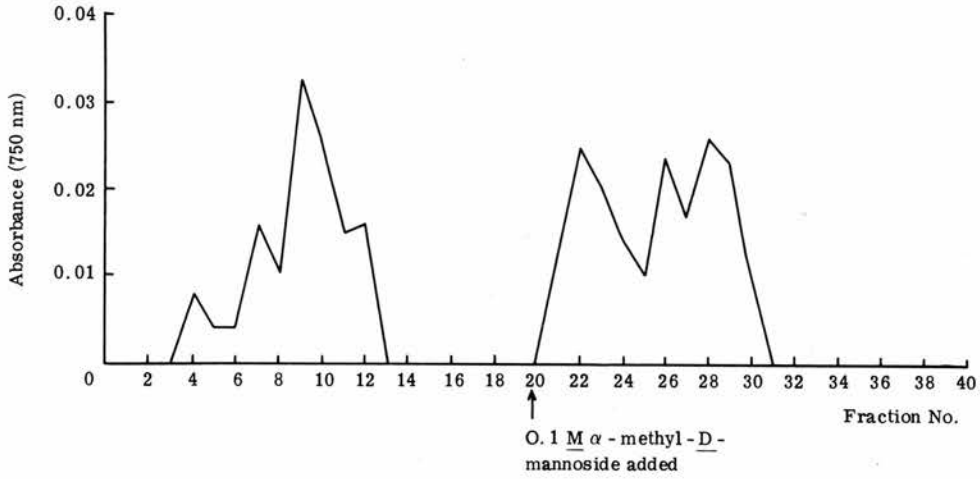


Fig. 7 Affinity chromatography of E. coli 10418 extract on mannan-Sepharose 4B. The extract was applied to the column in a 0.1M Tris-HCl buffer (pH 8), containing Dulbecco's 'B' (0.5ml/100ml). The arrow indicates the point from which the column was eluted with 0.1M α-methyl-D-mannoside in 0.9% (W/v) NaCl. Fractions were screened by the Lowry protein assay, and the results expressed as absorbance (750nm) of the assay mixtures.

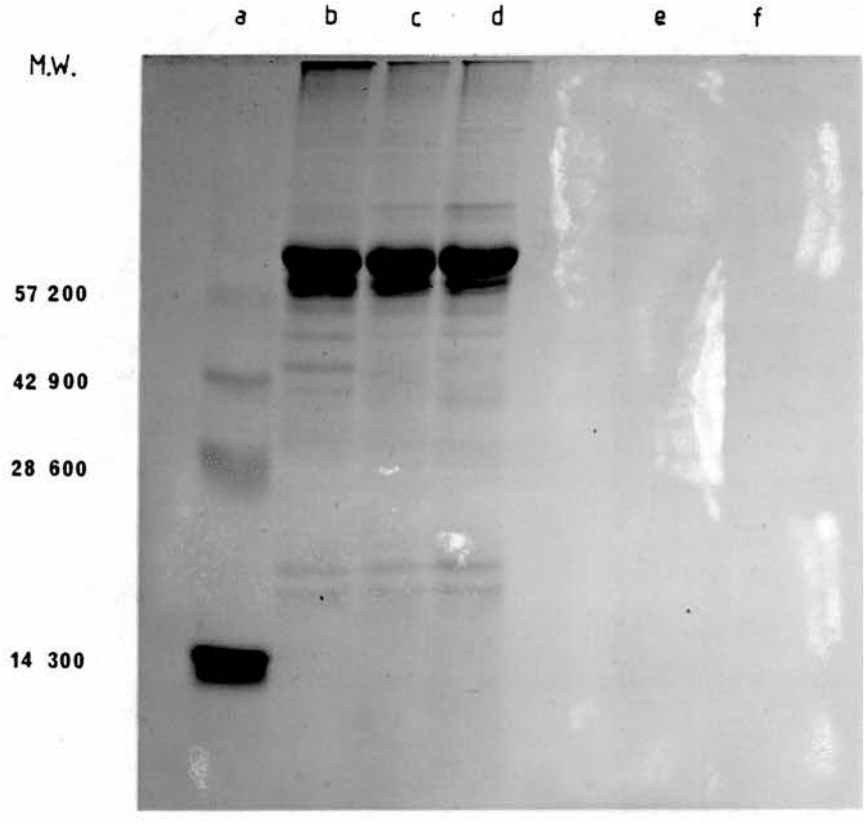


Fig. 8 SDS-PAGE of selected fractions from affinity chromatography of E. coli 10418 extract: (a) molecular weight marker; (b) fractions 7 + 8; (c) fraction 9; (d) fractions 10 + 11; (e) fractions 21 - 25 combined; (f) fractions 26 - 30 combined.

major band, with a molecular weight of approximately 60,000, in those samples which produced agglutination of yeast cells. This band was accompanied by one of a slightly lower molecular weight (approximately 58,000), giving an appearance on the gel of a 'doublet'. Two bands of much lower molecular weight (approximately 23,700 and 21,500) were also invariably present. The fractions eluted with αMM did not show any deeply staining bands. Further purification of the 60,000 molecular weight band was later performed by preparative SDS-PAGE (see results section 1.5).

1.2 Extraction of 'lectin-like' material from E. coli 4428, in the fimbriate and non-fimbriate phases

E. coli 4428, when grown in nutrient broth at 37°C under static culture conditions, produced strong, mannose-sensitive agglutination of yeast cells. This same strain was passaged five times on nutrient agar, to produce bacteria which would not agglutinate yeast cells. The latter reverted quickly to the yeast agglutinating form, if re-inoculated into nutrient broth. Cells of these isogenic variants were examined under the electron microscope, and it was shown (Figs. 9 and 10) that whereas the organisms which had been grown in nutrient broth possessed many fimbriae, those passaged on agar plates possessed no fimbriae.

Cells of both isogenic variants were grown separately on nutrient agar in sets of 20 large petri dishes. In each case, the extraction procedure yielded approximately 300μg of protein from 6.0g of cells.

SDS-PAGE/...

Fig. 9 Electron micrograph of E. coli 4428 in the fimbriate phase (x 8000). Negatively stained with 1% ($^{W}/v$) phosphotungstic acid.

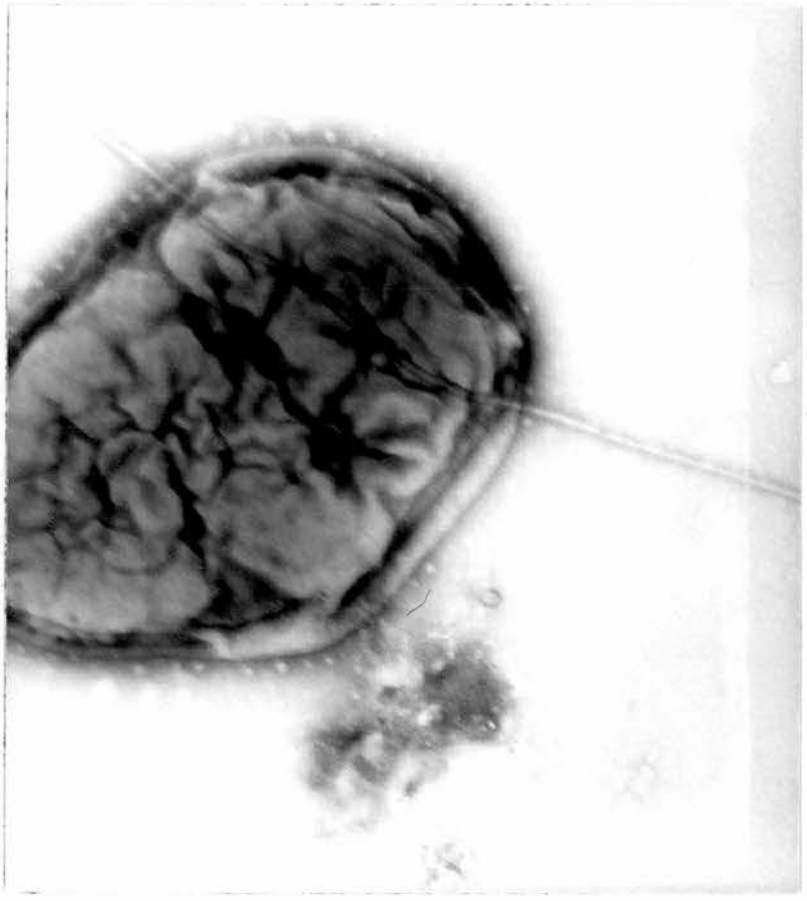


Fig. 10 Electron micrograph of E. coli 4428 in the non-fimbriate phase (x 8000). Negatively stained with 1% ($^{W}/v$) phosphotungstic acid.

SDS-PAGE of the two extracts showed that their protein profiles were identical. Affinity chromatography of the two samples produced, in both cases, fractions which caused agglutination of yeast cells, though none of the fractions eluted with αMM showed agglutinating activity. It was thus made apparent that although the variant which is devoid of fimbriae will not agglutinate yeast cells, mannose-specific lectin molecules are, nevertheless, present in the cell wall.

1.3 Extraction of lectin-like material from C. parvum

The total wet weight of bacteria harvested from 6l of medium was 41g, and extraction of these cells produced 14mg protein. SDS-PAGE showed the preparation to be heterogeneous (Fig. 11). Affinity chromatography on mannan-Sepharose 4B of 2mg of extract resulted in the elution profile shown in fig. 12. Table 2 shows the fractions and combined fractions used for SDS-PAGE analysis.

Table 2 Data relating to samples run on SDS-PAGE

Fraction	Approximate mass of protein (μg)	Agglutination assay
3	230	Negative
4+5	240	Negative
6+7	210	Positive
15+16	100	Very strong
27	50	Very weak

Table/...

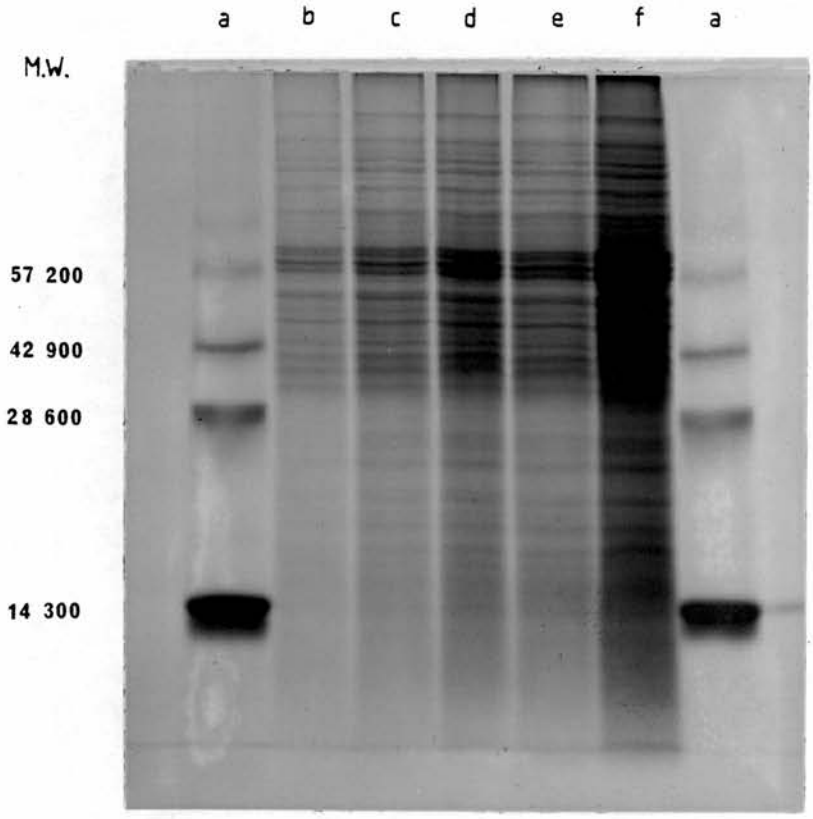


Fig. 11 SDS-PAGE of crude extract from C. parvum: (a) molecular weight marker; (b) 30µg extract; (c) 45µg extract; (d) and (e) 100µg extract; (f) 200µg extract.

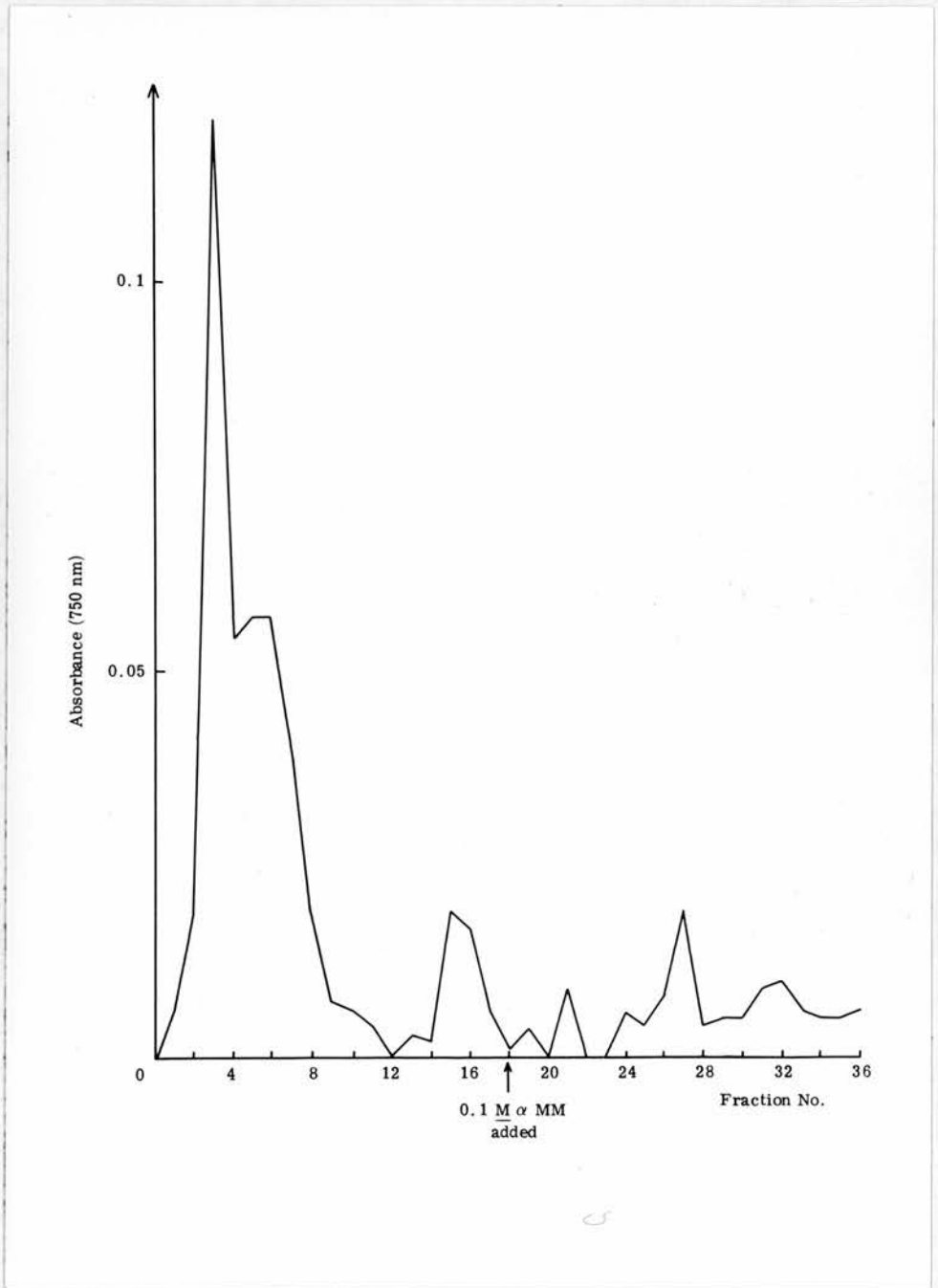


Fig. 12 Affinity chromatography of C. parvum extract on mannan-Sepharose 4B. The extract was applied to the column in 0.01M PBS (pH 7.4). The arrow indicates the point from which the column was eluted with 0.1M α-methyl-D-mannoside in 0.9% (w/v) NaCl. Fractions were screened by the Lowry protein assay, and the results expressed as the absorbance (750nm) of the assay mixtures.

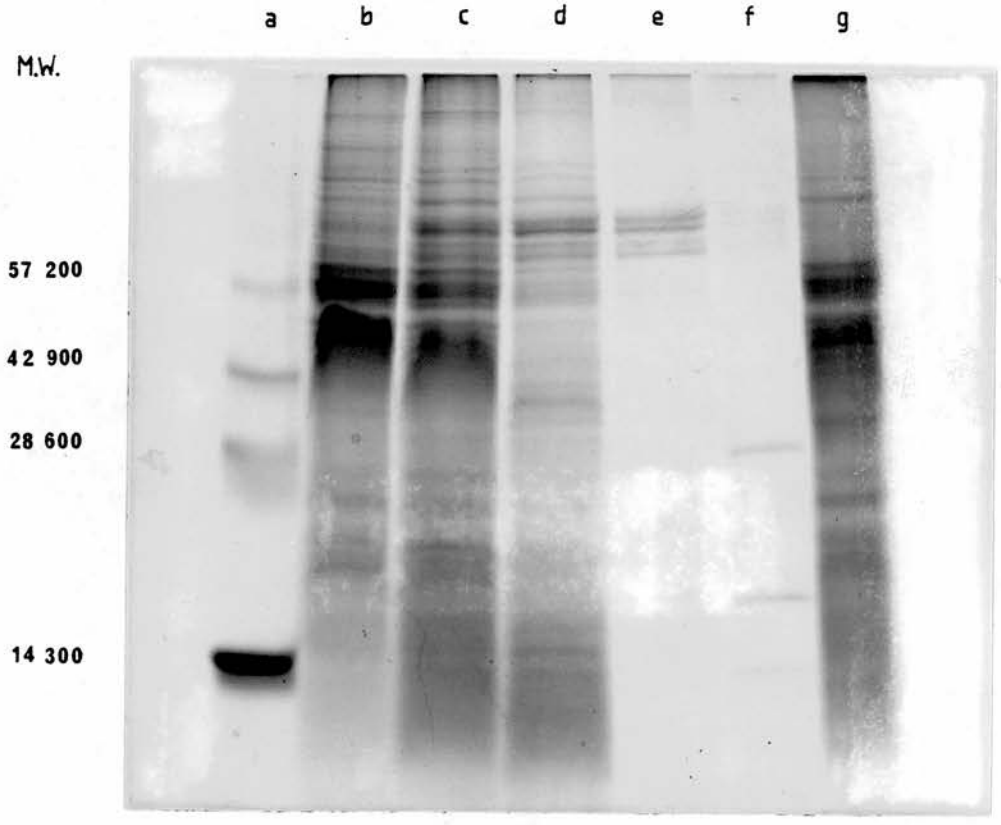


Fig. 13 SDS-PAGE of selected fractions from affinity chromatography of C. parvum extract: (a) molecular weight marker; (b) fraction 3; (c) fractions 4 + 5; (d) fractions 6 + 7; (e) fractions 15 + 16; (f) fraction 27; (g) crude extract.

Table 2 shows that the main agglutinating activity was retained in fractions 15 and 16. SDS-PAGE gave an indication of the protein bands associated with agglutination of the yeast cells (Fig. 13). Repeated PAGE analysis resulted in the finding that agglutinating activity was confined to two major doublet protein bands, with molecular weights of approximately 76,000 and 69,000 respectively, along with possibly a very faint third band of approximately 58,000 molecular weight. Also of interest was the finding that in fractions eluted with αMM, three further bands of approximate molecular weights 12,000, 16,000 and 30,000 appeared consistently, although they were not apparently associated with strong agglutination of yeast cells.

1.4 Extraction of lectin-like material from Salmonella typhimurium

The extraction process yielded 560µg protein, from a bacterial mass of 1.5g. Affinity chromatography of 480µg of the material produced the elution profile shown in fig. 14. As indicated on the graph (Fig. 14), certain fractions were associated with particularly strong agglutination, and these fractions were combined for SDS-PAGE (Fig. 15), but unfortunately the concentration of protein was too low to give a reliable indication of the bands associated with agglutinating activity. Fractions eluted with αMM did not cause significant agglutination of yeast cells, but a small amount of high molecular weight material was evident on PAGE. The protein profile of the crude extract was much simpler than profiles of extracts from other organisms, with a heavily staining band of molecular weight 60,000.

1.5/...

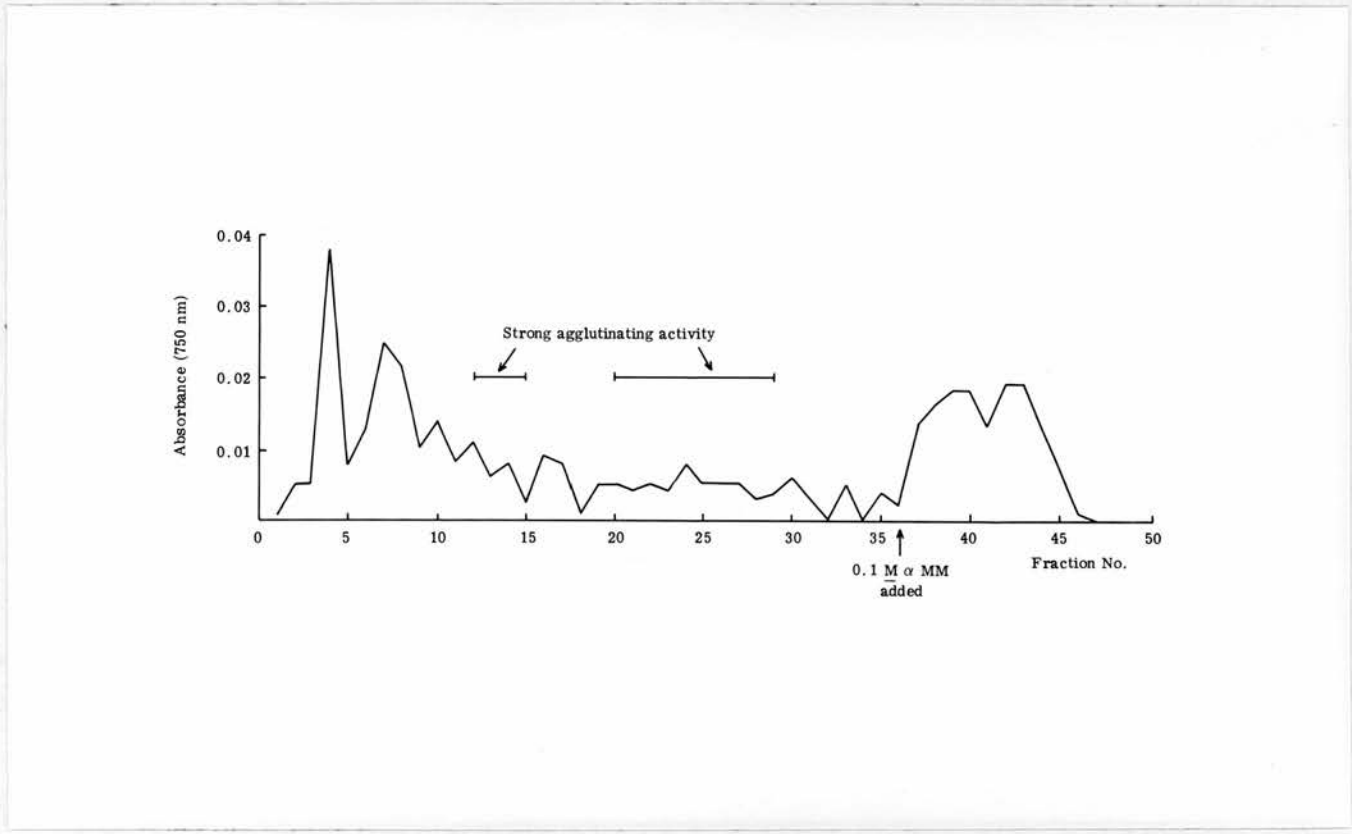


Fig. 14 Affinity chromatography of S. typhimurium extract on mannan-Sepharose 4B. The extract was applied to the column in a 0.1M Tris-HCl buffer (pH 8), containing Dulbecco's 'B' (0.5ml/100ml). The arrow indicates the point from which the column was eluted with 0.1M α-methyl-D-mannoside in 0.9% (w/v) NaCl. Fractions were screened by the Lowry protein assay, and the results expressed as the absorbance (750nm) of the assay mixtures.

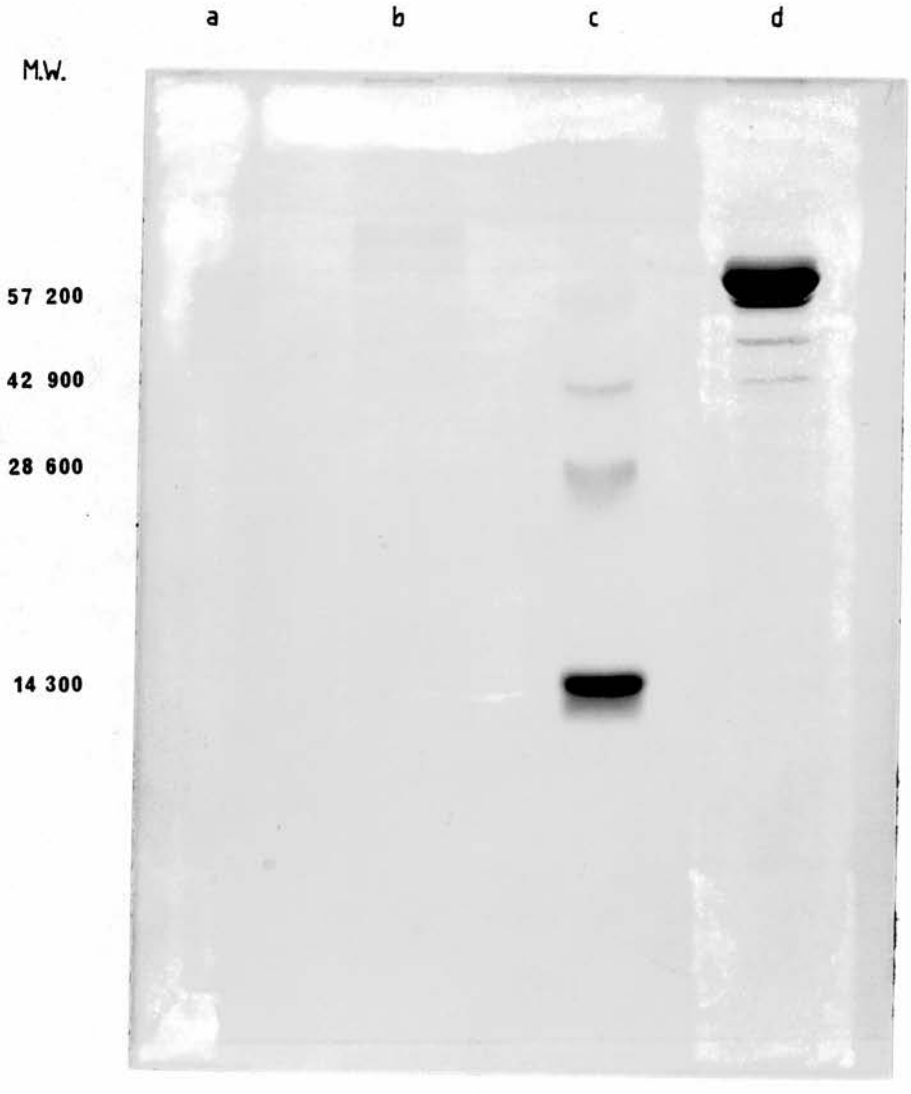


Fig. 15 SDS-PAGE of selected fractions from affinity chromatography of S. typhimurium extract: (a) combined fractions causing strong agglutination of yeast cells; (b) fractions 37 - 46; (c) molecular weight marker; (d) crude extract.

1.5 Isolation of lectin from E. coli 10418 by preparative polyacrylamide gel electrophoresis

A bulk culture (6l) of this organism was grown for 48h in static culture at 37°C. The cells (6.5g wet weight) were extracted by the normal procedure, to yield 11.6mg protein. After dialysis against 5mM Tris-HCl buffer (pH 7.4) to remove the excess salt, samples of the extract were analysed by SDS-PAGE (Fig. 16). On the basis of the results described in section 1.1, the band with a molecular weight of 60,000 was selected for purification. A preparative 10% polyacrylamide gel was set up, and 3.5mg of protein electrophoresed as described in Materials and Methods. After the relevant band had been cut from the gel, the protein electrophoresed out of the polyacrylamide, and the Coomassie blue and SDS removed, the final sample, containing the protein, had a total volume of 2ml. To check the purity of the sample, 150µl was removed for analysis by SDS-PAGE. The gel showed the presence of only one band, with a molecular weight corresponding to 60,000 (Fig. 17). The remainder of the sample was therefore lyophilised. In preliminary experiments, Dr. John Stewart (Immunology Laboratory, Department of Bacteriology, University of Edinburgh Medical School) has shown that this purified material blocks the binding of E. coli 10418 to mouse peritoneal exudate macrophages in a dose-response fashion. The crude extract has also been shown to block this binding. However, preincubation of macrophages with the material prior to attachment assays with Staphylococcus albus, causes an increase in the degree of bacterial binding.

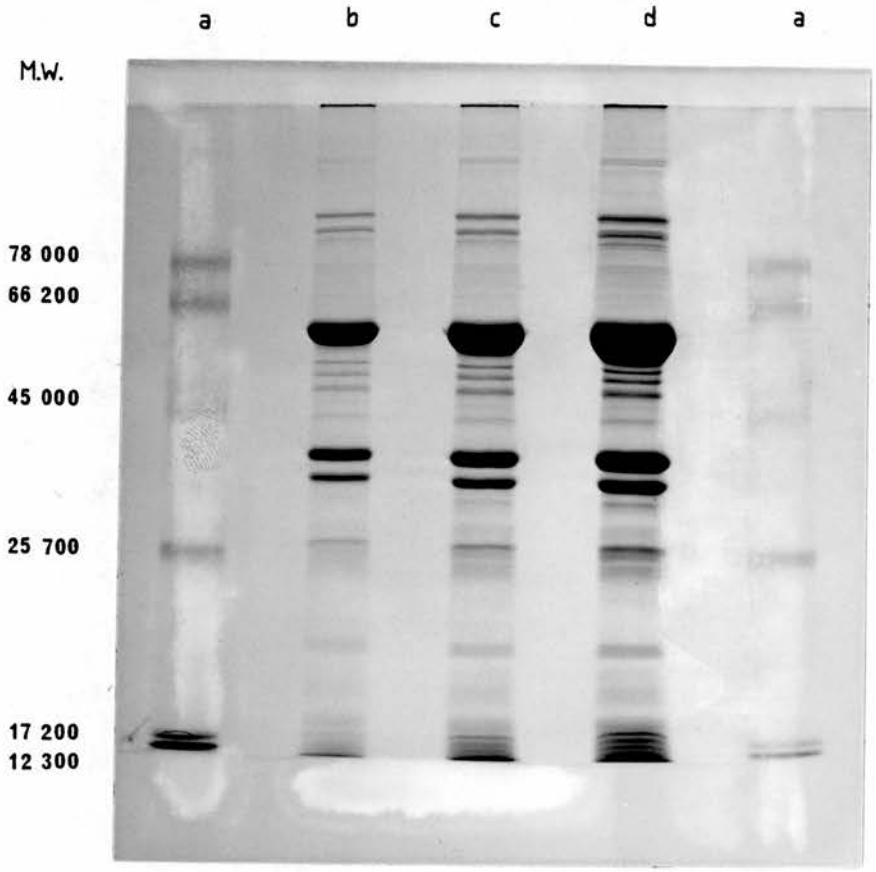


Fig. 16 SDS-PAGE of crude extract from E. coli 10418: (a) molecular weight marker; (b) 50µg extract; (c) 100µg extract; (d) 200µg extract

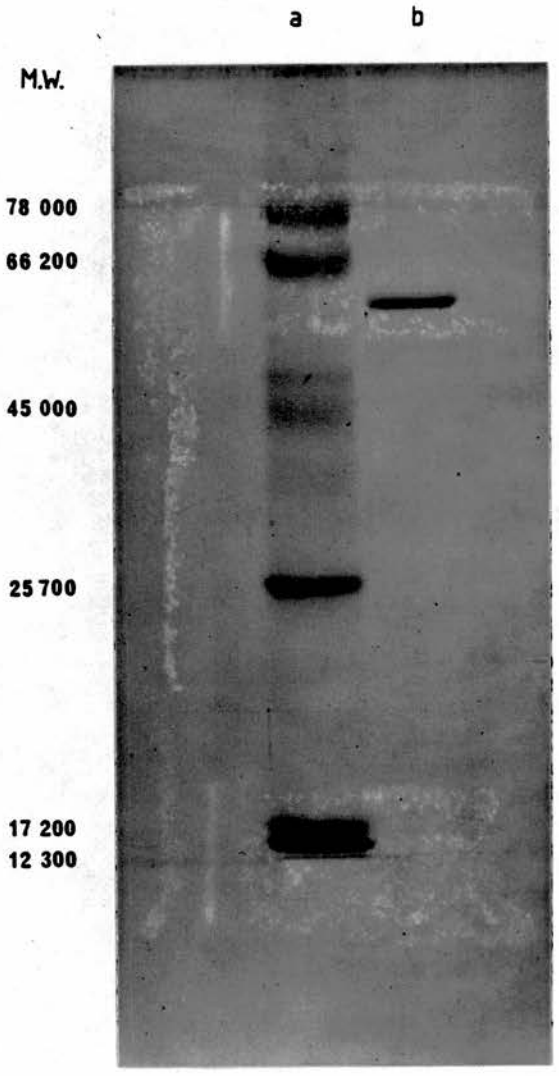


Fig. 17 SDS-PAGE of purified material (60,000 molecular weight band) from E. coli 10418: (a) molecular weight marker; (b) purified protein

2 Calibration Curve for Quantitative Determination of Growth of Type III GBS

The bacterial counts calculated from the 10^{-5}, 10^{-6} and 10^{-7} dilutions were averaged, to give an accurate figure for the viable count of the original culture. The absorbance (650nm) of the original culture was 0.89, and the absorbances of successive doubling dilutions were read, down to a final dilution of $^{1}/512$. These figures were correlated with the number of colony forming units per ml in each successive dilution, and the values plotted against one another, to produce the standard curve (Fig. 18), which relates absorbance (650nm) of the culture to its bacterial density.

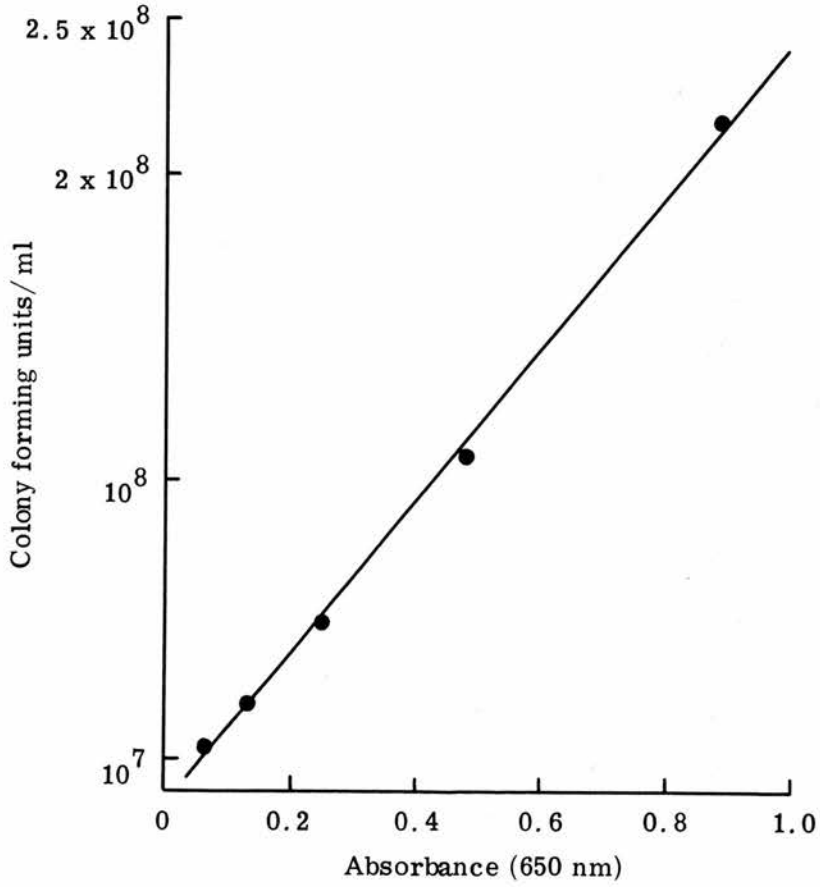


Fig. 18 Calibration curve for quantitative determination of growth of type III GBS

3 General Features of the Binding of GBS to BEC

Preliminary experiments were performed to check that GBS would attach to BEC, and to determine the optimum incubation period.

3.1 Attachment of group A streptococci and GBS to BEC

Group A streptococci have been shown to bind well to BEC (Beachey, 1975), and were therefore used as a positive control. As shown in table 3, there was no significant difference ($P > 0.1$) in the binding to BEC of group A streptococci (10085) and type III GBS.

Table 3 Adherence of group A streptococci and type III GBS to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	2.3 ± 0.6
Type III GBS	13.9 ± 2.5
Group A streptococcus	15.7 ± 3.2

3.2 Time course of the binding reaction between type III GBS and BEC

Incubation of mixtures of GBS and BEC for various periods of time from 5min to 90min indicated that attachment of the organism to the epithelial cells reached a maximum after 45min (Fig. 19). In all assays, an incubation period of 45min was employed.

3.3/...

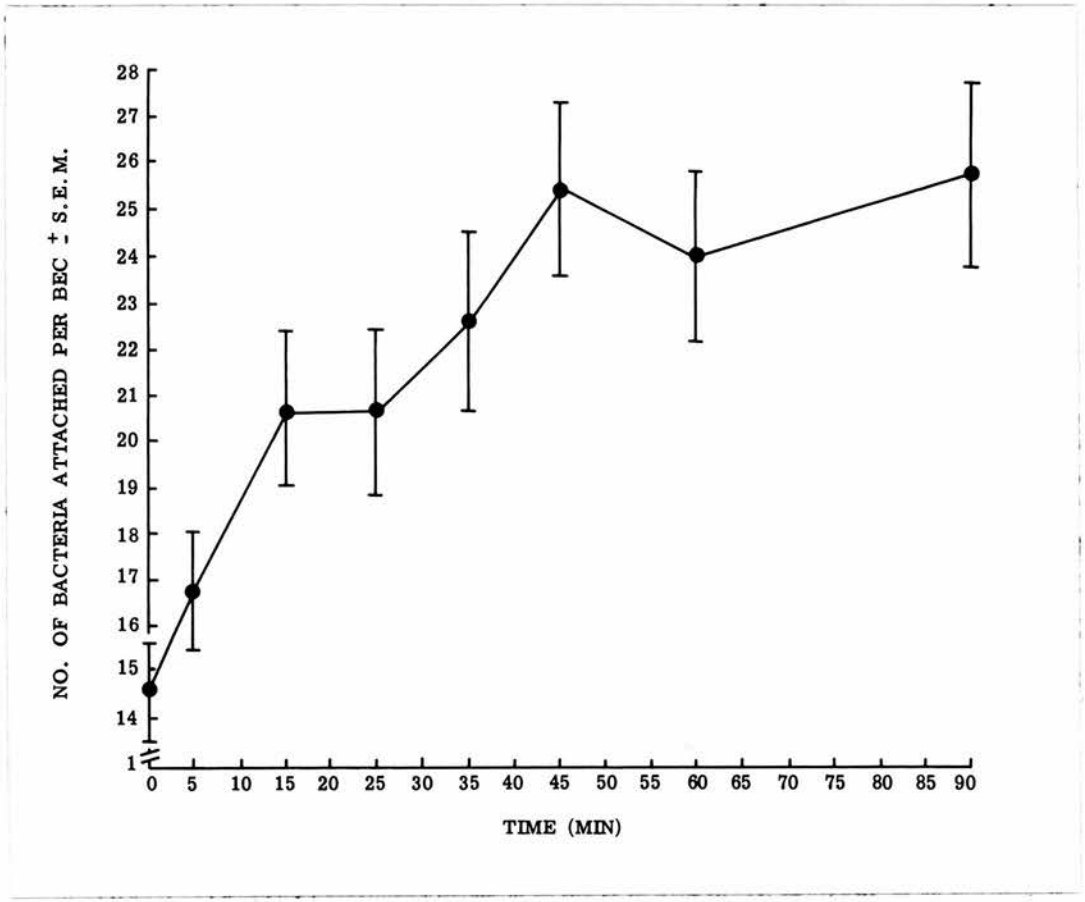


Fig. 19 Time-course of the binding of type III GBS to buccal epithelial cells

3.3 Attachment of type III and type Ia GBS to BEC

Type III GBS attached to the BEC in greater numbers than did the type Ia (Table 4). The difference, however, was not statistically significant ($P > 0.05$).

Table 4 Adherence of GBS types Ia and III to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	28.0 ± 4.4
GBS type Ia	41.9 ± 6.7
GBS type III	48.1 ± 4.0

4 Attachment-Inhibition Studies with Sonicate Prepared From Type III GBS

Various characteristics of the bacterial adhesins which mediate binding of GBS to BEC were studied in experiments which examined the capacity of the sonicate to block bacterial attachment.

4.1 Preparation of the sonicate

In a typical experiment, 5mg of protein were extracted from 8.9g (wet weight) of washed bacteria. The extraction procedure was a very mild one and had no real effect on either the chamber count or the viable count of the organisms subjected to the treatment (Table 5).

Table 5 Effect of sonication on the viable and chamber counts of type III GBS

	Viable Count*	Chamber Count*
Sonicated organisms	3.32×10^{10}	4×10^{10}
Unsonicated organisms	3.43×10^{10}	3.56×10^{10}

*Number of organisms per ml

SDS-PAGE of the crude sonicate showed that it was a heterogeneous preparation (Fig. 20), although some preparations were more homogeneous than others. Preparation of the sonicate at 4°C did not reduce this heterogeneity (Fig. 21).

Although/...

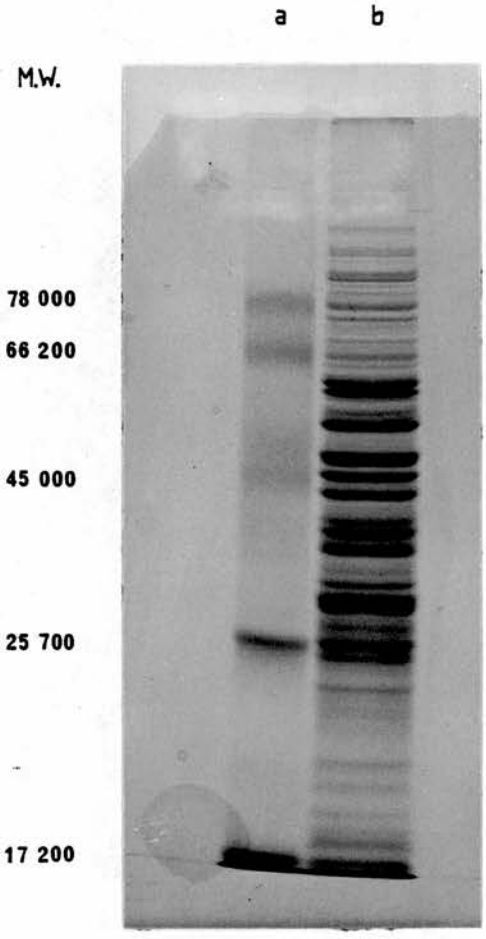


Fig. 20 SDS-PAGE of sonicate from type III GBS: (a) molecular weight marker; (b) 100µg sonicate

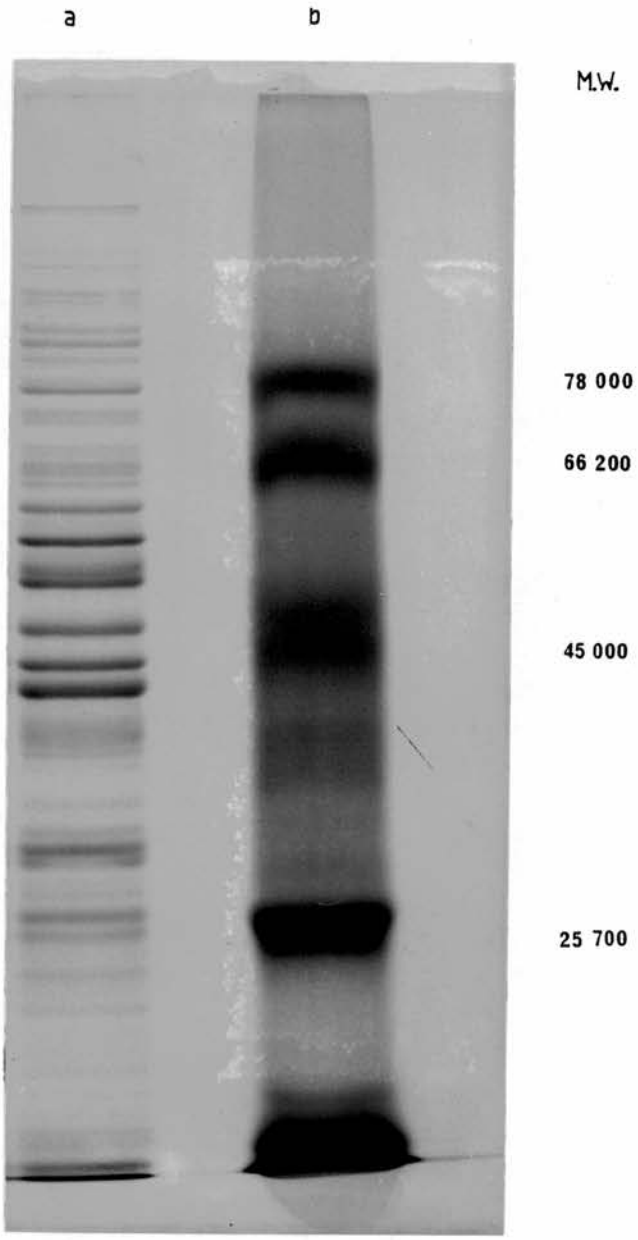


Fig. 21 SDS-PAGE of sonicate prepared at 4°C from type III GBS:
(a) 115µg sonicate; (b) molecular weight marker

Although the viability of the sonicated organisms was unchanged, the results in table 6 show that subsequent attachment of sonicated GBS to BEC was significantly reduced ($P < 0.001$). This indicates that there has been some interference with the adhesive mechanisms of the bacteria.

Table 6 Effect of sonication of type III GBS on their subsequent attachment to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	10.8 ± 2.4
Type III GBS	26.0 ± 3.8
Sonicated type III GBS	17.1 ± 2.4

4.2 Dose-response inhibition by sonicate of type III GBS attachment

Pre-incubation of BEC with the sonicate reduced the subsequent binding of type III GBS to the treated BEC, in a dose-response fashion. A dose-response curve is shown in fig. 22.

4.3 Effect of type III GBS sonicate on attachment of type Ia GBS to BEC

Pretreatment of BEC with sonicate prepared from type III GBS significantly/...

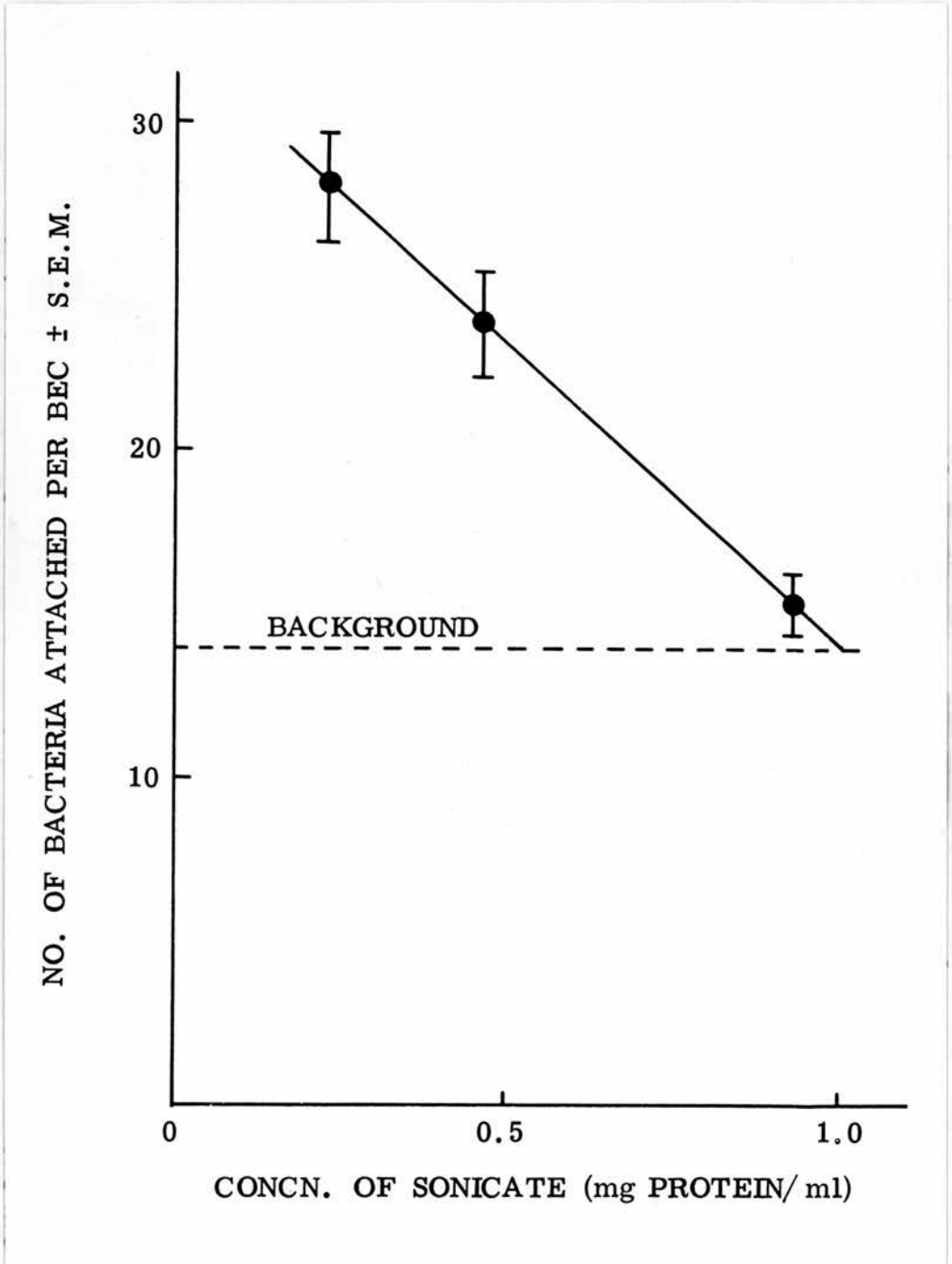


Fig. 22 Dose-response inhibition of binding of type III GBS to buccal epithelial cells by sonicate prepared from type III GBS

significantly reduced ($P < 0.01$) attachment of type Ia GBS to the BEC (Table 7).

Table 7 Effect of pre-incubation of BEC with type III GBS sonicate, on subsequent attachment of type Ia GBS

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	12.6 ± 2.0
BEC + type Ia GBS	22.7 ± 3.0
Sonicate treated BEC + type Ia GBS	17.2 ± 2.1

4.4 Effect of type III GBS sonicate on attachment of group A streptococci to BEC

Pretreatment of BEC with sonicate prepared from type III GBS caused an increase in the number of group A streptococci which attached to the treated cells, although this increase was not significant ($P < 0.05$). There was no inhibition of binding (Table 8).

Table 8/...

Table 8 Effect of pre-incubation of BEC with type III GBS sonicate, on subsequent attachment of group A streptococci

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	8.6 ± 2.0
BEC + group A streptococcus	13.0 ± 2.3
Sonicate treated BEC + group A streptococcus	17.1 ± 3.6

4.5 Effect of heat treatment and periodate oxidation of sonicate on its blocking capacity

As shown in table 9, treatment of the sonicate at 121°C for 15min significantly reduced ($P < 0.001$) its ability to block the binding of type III GBS to BEC, but periodate oxidation of the sonicate affected its blocking capacity to a much lower degree. Since there was no significant difference ($P > 0.1$) between the activities of the periodate-treated sonicate and the periodate control sonicate, it seems unlikely that the periodate oxidation per se is affecting the activity of the material.

Table 9/...

Table 9 Effect of heat and periodate oxidation on the capacity of type III GBS sonicate to block binding of type III GBS to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	2.6 ± 1.4
Untreated BEC + GBS	8.5 ± 1.6
Sonicate-treated BEC + GBS	3.2 ± 0.5
Heated* sonicate-treated BEC + GBS	6.7 ± 0.9
Periodate oxidised sonicate-treated BEC + GBS	5.0 ± 0.8
No periodate control sonicate-treated BEC + GBS	4.2 ± 1.1

*121°C for 15min

4.6 Gel filtration of sonicate

Gel filtration of three separate preparations of sonicate was performed on Sephadex G-75. Fraction were screened for absorbance at 280nm, and in each case the elution profile consisted of two discrete peaks (Fig. 23). Gel filtration on Sephadex G-100 did not produce any further resolution. SDS-PAGE of fractions pooled from each individual peak revealed that whereas the peak I material contained largely the same protein bands as the crude sonicate, the peak II material showed no protein bands at all (Fig. 26). Staining of gels for/...

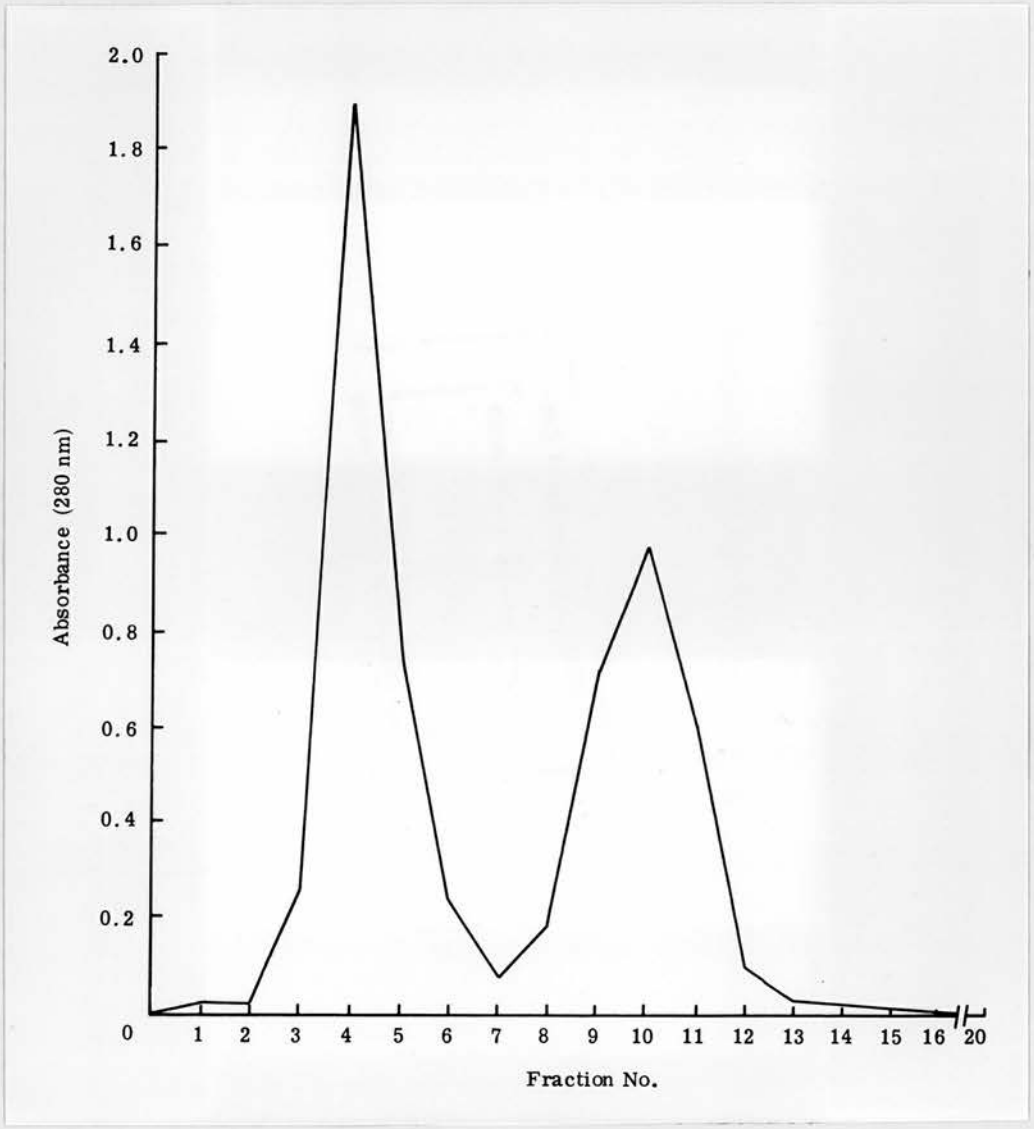


Fig. 23 Sephadex G-75 gel filtration of type III GBS sonicate. The sonicate (2.5mg) was dissolved in 250µl of 0.01M PBS (pH 7.4) and applied to a column (270mm x 13mm) of Sephadex G-75. Fractions of 2ml were collected, and screened for protein by their absorbance (280nm).

Peak I : fractions 3-6
Peak II : fractions 8-12

for nucleic acid and for carbohydrate did not reveal the identity of the material in peak II.

Scanning ultraviolet spectrophotometry indicated that the maximum absorbance of both the crude sonicate and the peak I material was about 260nm, while the peak II material had a maximum absorbance at 250nm (Fig. 24).

The relative amount of protein to carbohydrate was shown to be similar for the crude sonicate, the peak I material and the peak II material (Table 10).

Table 10 Relative proportions of protein and carbohydrate in crude and fractionated sonicate

Sample	Protein concentration*	Carbohydrate concentration*	Protein concentration / Carbohydrate concentration
Crude sonicate	140	25	5.6
Peak I	600	117.5	5.11
Peak II	625	92.5	6.76

*All concentrations expressed as µg/ml

4.7 Inhibition of binding of type III GBS to BEC, by fractions from gel filtration of sonicate

Pre-incubation of BEC with the material from both peaks I and II of /...

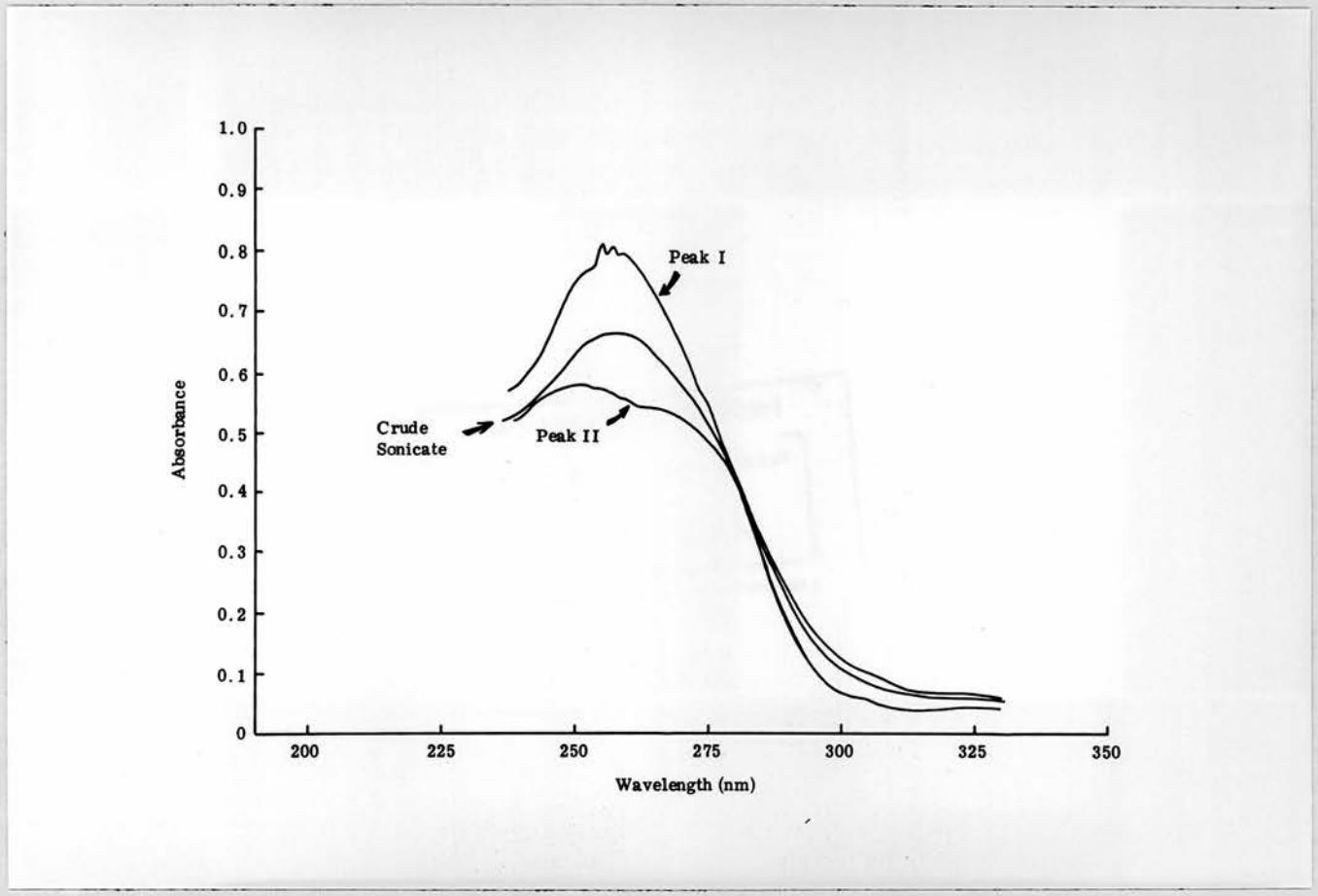


Fig. 24 Scanning ultraviolet spectrophotometry of crude sonicate, and peaks I and II of gel-filtered sonicate

of the gel-filtered sonicate caused a highly significant reduction ($P < 0.001$ in both cases) in subsequent attachment of type III GBS (Table 11).

Table 11 Inhibition of binding of type III GBS to BEC with material from peaks I and II of the gel-filtered sonicate

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Peak I	
Background	9.8 ± 1.5
BEC + GBS	21.6 ± 3.7
Peak I-treated BEC + GBS	12.0 ± 2.1
Peak II	
Background	8.0 ± 1.8
BEC + GBS	13.6 ± 1.8
Peak II-treated BEC + GBS	9.0 ± 0.9

4.8 DEAE cellulose ion-exchange chromatography of sonicate

Further fractionation of the peak I material from gel filtration of the sonicate was attempted by ion-exchange chromatography. After binding to the DEAE cellulose, the material was eluted with a gradient of 0.01M to 1.0M NaCl. As shown in fig.25, the protein left the column/...

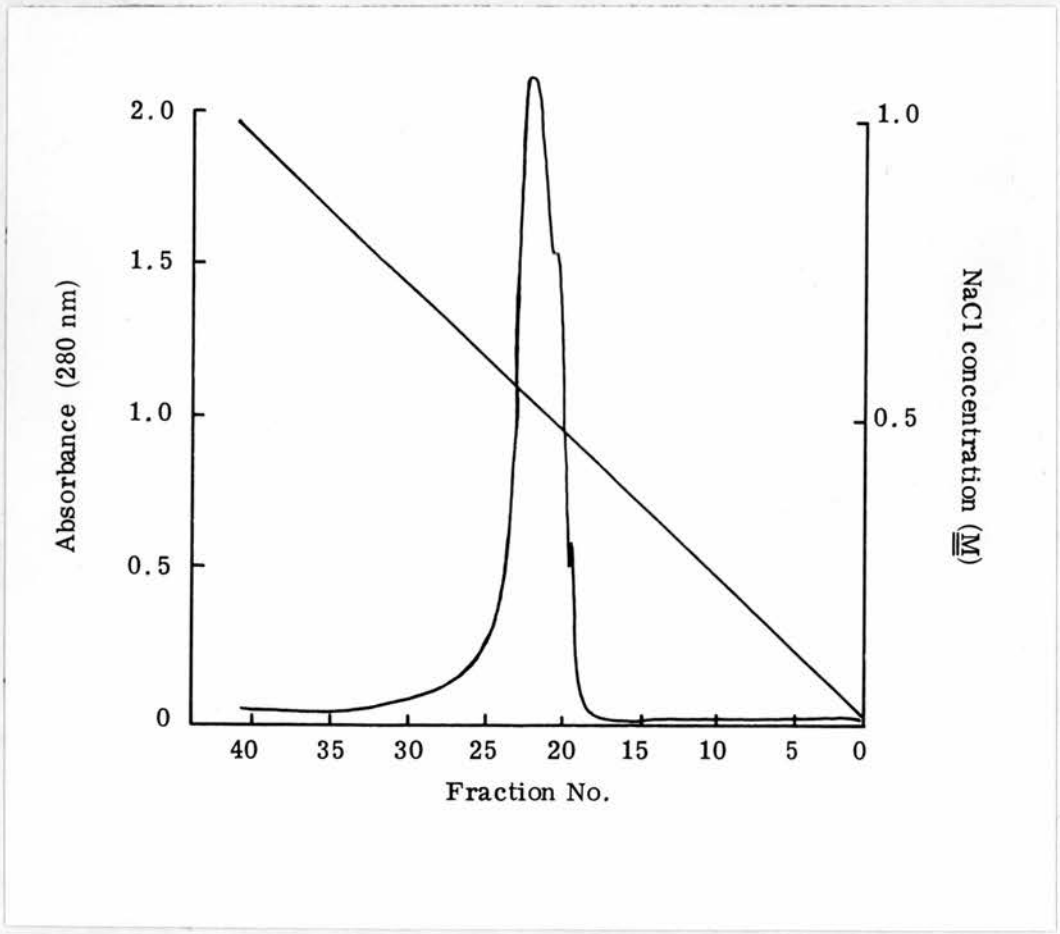


Fig. 25 DEAE cellulose ion-exchange chromatography of peak I material. The sample was applied to the column in 2ml 0.05M Tris-HCl buffer (pH 7.8), containing 0.01M NaCl. The column was washed with 40ml of the same buffer. A salt gradient (0.01M NaCl to 1.0M NaCl), in 0.05M Tris-HCl buffer (pH 7.8), was then applied to the column, and 40 fractions, each of 1ml, were collected. The effluent was continuously screened for protein by its absorbance at 280nm.

column as a single peak. The fractions comprising the peak were combined, and a sample was subjected to SDS-PAGE (Fig. 26). The preparation was thus shown to be still very heterogeneous, although there were slightly fewer bands, and some of the bands were shown to have increased in density. Pre-incubation of BEC with the DEAE cellulose-purified sonicate caused a significant reduction ($P = 0.02$) in the subsequent attachment of type III GBS to the BEC (Table 12).

Table 12 Inhibition of binding of type III GBS to BEC with DEAE cellulose-purified sonicate

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	9.3 ± 2.0
BEC + GBS	16.8 ± 3.4
Sonicate-treated BEC + GBS	11.6 ± 2.8

4.9 Absorption with BEC of the purified sonicate

In an attempt to determine which of the protein bands, identified by SDS-PAGE, were involved with inhibition of bacterial attachment, the DEAE cellulose-purified sonicate was absorbed up to three times with BEC, and the absorbed samples analysed by SDS-PAGE. The results gained from such experiments are purely qualitative, but all showed a marked reduction,/...

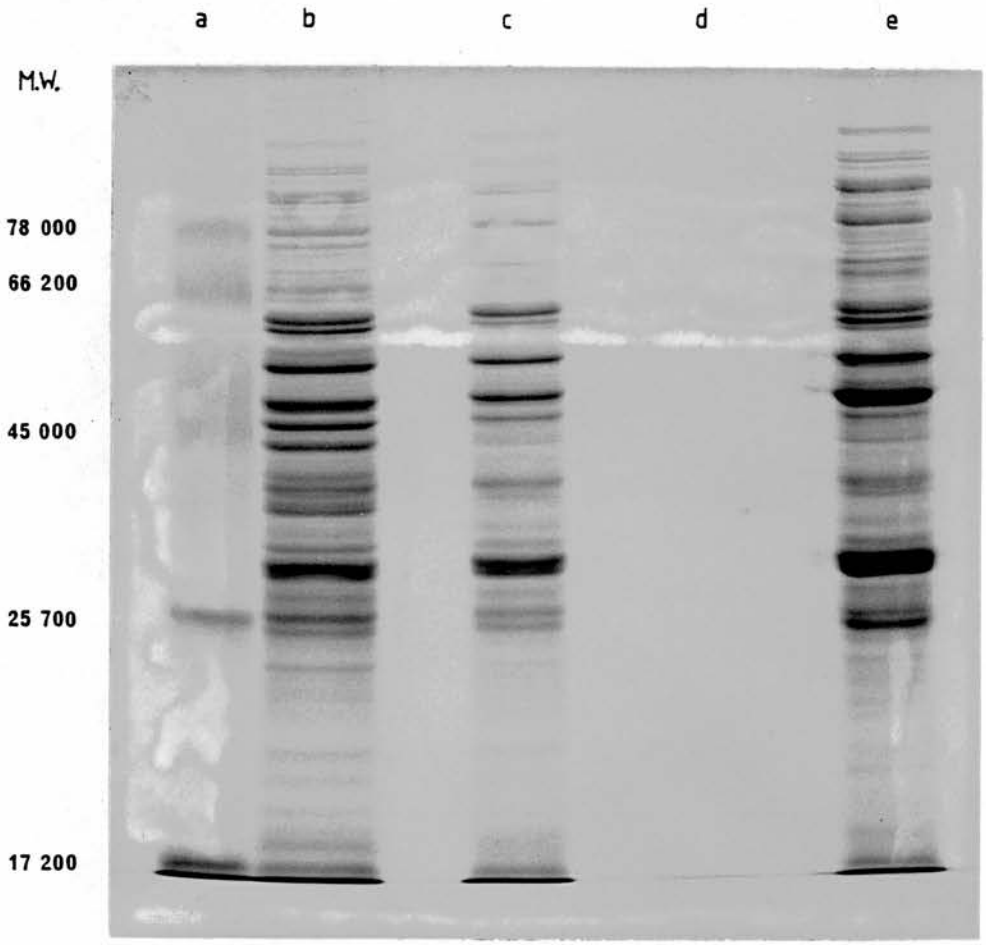


Fig. 26 SDS-PAGE of fractions from gel filtration and ion-exchange chromatography of sonicate: (a) molecular weight marker; (b) crude sonicate; (c) peak I material; (d) peak II material; (e) DEAE cellulose-purified sonicate

reduction, even after a single absorption, in the dense band of molecular weight 47,300 (Fig. 27). After absorption three times, a number of bands were reduced in density (Fig. 27).

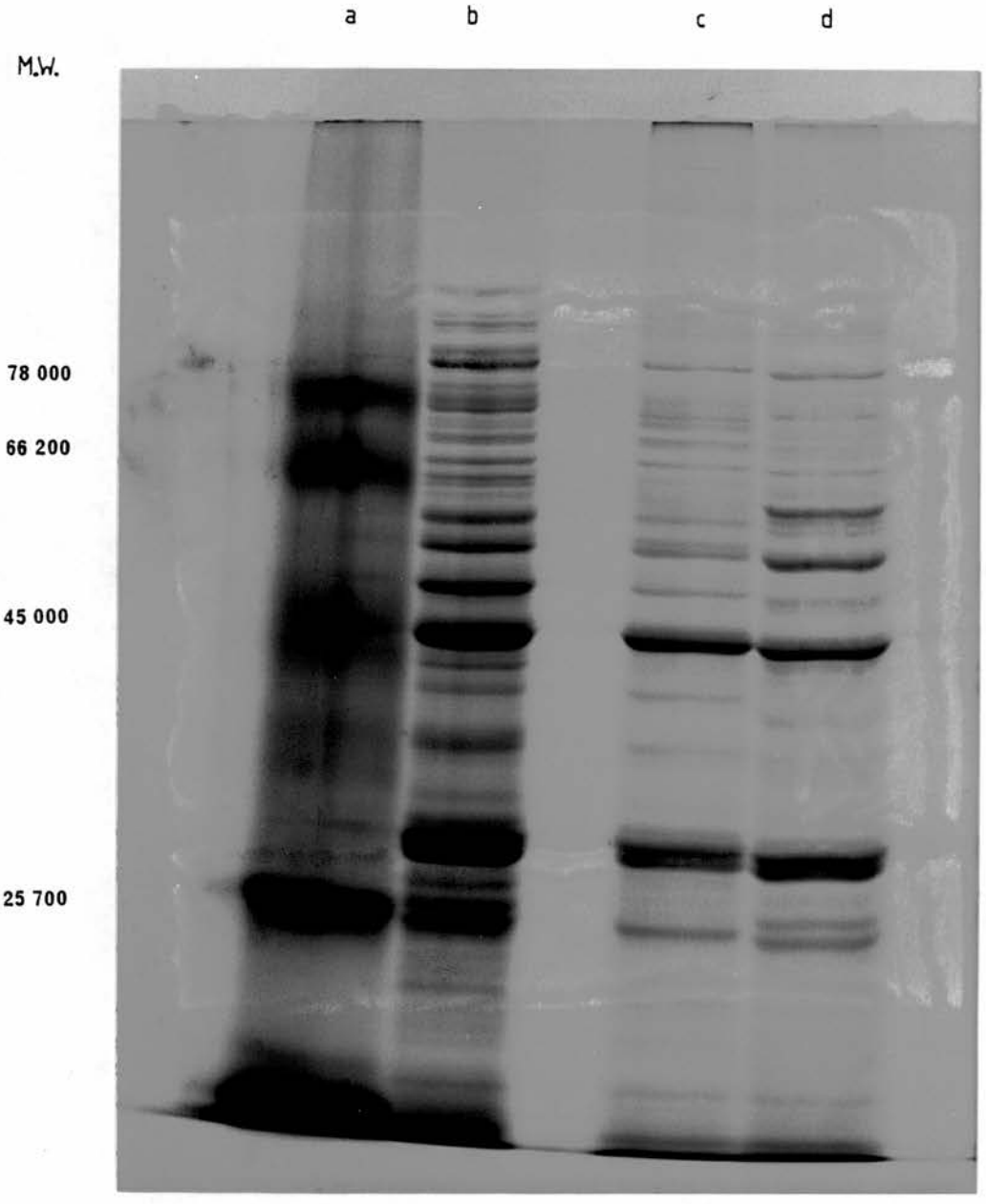


Fig. 27 SDS-PAGE of samples of sonicate after absorption with BEC: (a) molecular weight marker; (b) unabsorbed sonicate; (c) sonicate absorbed three times; (d) sonicate absorbed once

5 Purification of Membrane Lipoteichoic Acid From Type III GBS

Membrane lipoteichoic acid (LTA) was prepared from type III GBS for use as a potential inhibitor of attachment of the organism to BEC.

5.1 Extraction and purification

The phenol extract of the membranes was subjected to gel filtration on Sepharose 6B, as outlined in 'Materials and Methods'. Screening of the fractions for total phosphate, carbohydrate and nucleic acid produced the elution profile shown in fig. 28. Rocket immunoelectrophoresis of selected fractions, against antiserum raised in rabbits to type III group B streptococci (kindly donated by Dr. C.G. Cumming, Department of Oral Medicine and Pathology, Edinburgh University) showed antigenic activity in fractions 21 to 33 (see fig. 29). These fractions were pooled and dialysed against 2l distilled water, at 4°C, to remove the ammonium acetate. The material was lyophilised, the final weight of freeze dried extract being 18mg. Analysis of the purified phenol extract (performed by Dr. C.G. Cumming) has shown that its chemical composition is consistent with that expected for a lipoteichoic acid.

5.2 Type-specific antigenicity of membrane lipoteichoic acid

The purified phenol extract was tested in an Ouchterlony diffusion slide experiment with specific antisera raised against GBS types Ia, Ib, Ic, II and III. Precipitation appeared only when the antigen was reacted with type III antiserum, thus indicating that the phenol extract is a type-specific antigen.

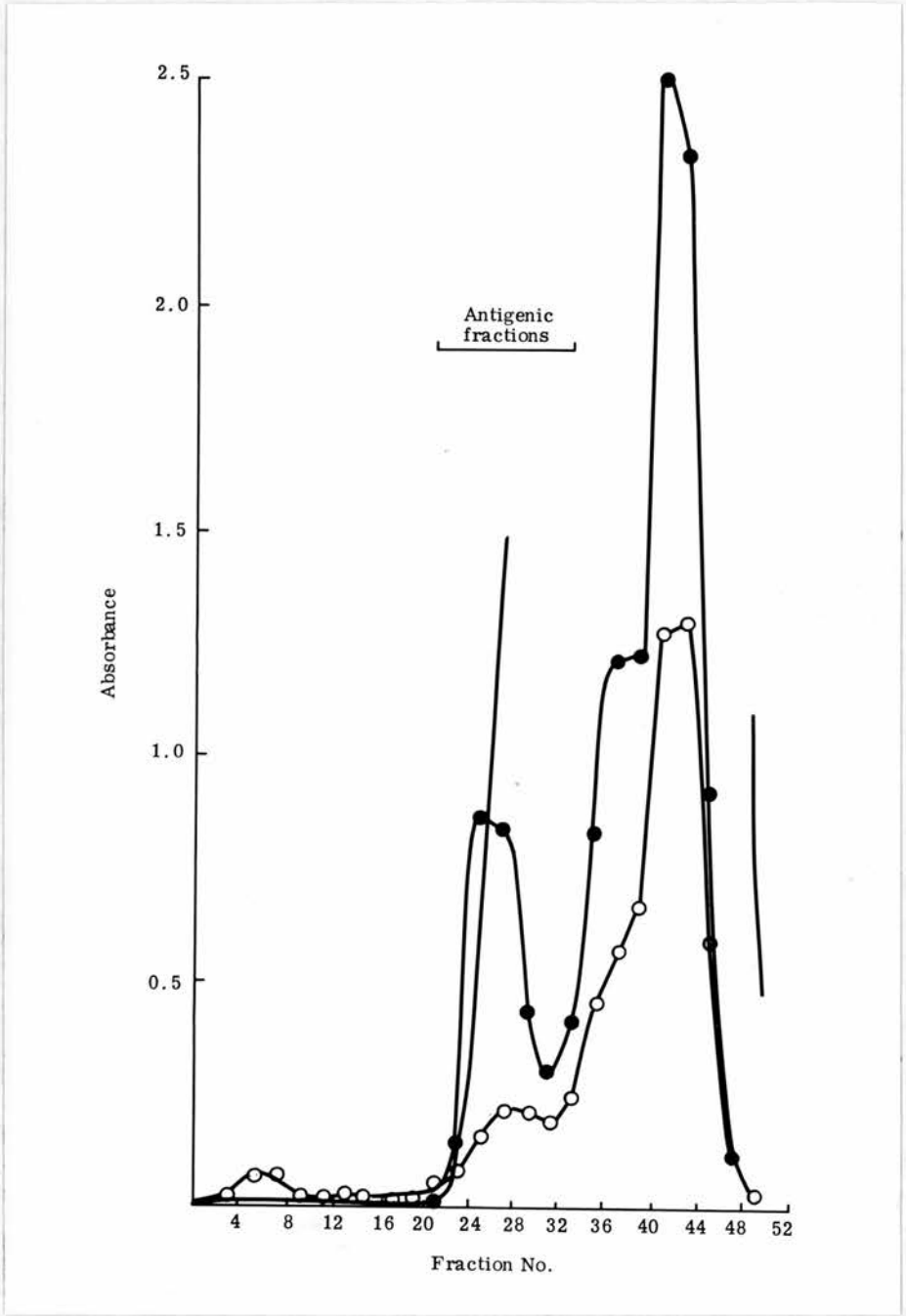


Fig. 28 Gel filtration, on Sepharose 6B, of phenol extract of type III GBS cell membranes. The lyophilised extract (138mg) was dissolved in 2.7ml distilled water and applied to a column (600mm x 16mm) of Sepharose 6B. The material was eluted with 0.2M ammonium acetate buffer. Fractions of 3ml were collected.
●——● total phosphate; o——o carbohydrate;
——— nucleic acid.

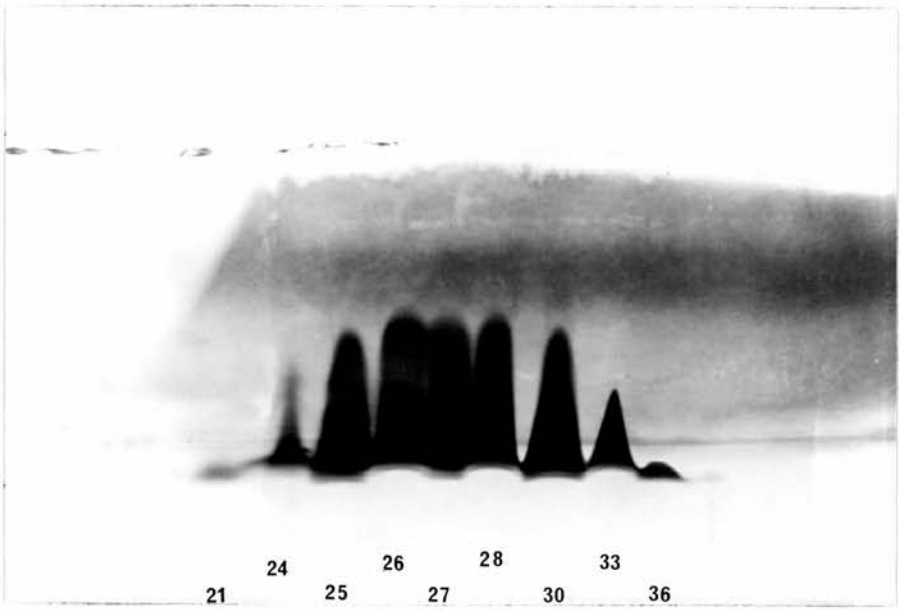


Fig. 29 Rocket immunoelectrophoresis of fractions from gel filtration of phenol extract of type III GBS cell membranes, against type III GBS antiserum

6 Pretreatment of BEC Prior to GBS Adherence Assays

6.1 Periodate oxidation of BEC

Periodate oxidation of the BEC prior to the adherence assay caused a highly significant reduction ($P < 0.001$) in the number of bacteria which subsequently attached to the epithelial cells. The background count was not affected by the periodate treatment (Table 13).

Table 13 Effect of periodate treatment of BEC on subsequent adherence of type III GBS

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	10.8 ± 1.9
Periodate-treated BEC background	10.1 ± 1.6
BEC + GBS	19.3 ± 2.8
Periodate-treated BEC + GBS	13.0 ± 1.9

6.2 Pre-incubation with membrane lipoteichoic acid

Pre-incubation of BEC with membrane lipoteichoic acid (0.7mg/ml) purified from type III GBS did not cause a significant reduction ($P > 0.1$) in subsequent attachment of type III GBS (Table 14).

Table 14/...

Table 14 Effect of pretreatment of BEC with type III GBS membrane LTA, on subsequent adherence of type III GBS

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	22.2 ± 2.6
BEC + GBS	33.0 ± 3.2
LTA-treated BEC + GBS	28.9 ± 3.8

6.3 Pre-incubation with sonicate

See results in section 4.

7 Pretreatment of Type III GBS Prior to Adherence Assays

Bacteria were subjected to several types of treatment, prior to the adherence assay.

7.1 Trypsinisation of type III GBS

Trypsin treatment of the GBS caused a significant reduction ($P < 0.01$) in their subsequent attachment to BEC (Table 15).

Table 15 Effect of trypsin treatment of type III GBS on their adherence to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	24.5 ± 3.2
BEC + GBS	40.8 ± 5.9
BEC + trypsinised GBS	30.8 ± 3.6

7.2 Periodate oxidation of type III GBS

There was no reduction in attachment of GBS to BEC after periodate oxidation of the bacteria (Table 16).

7.3 Heat treatments of type III GBS

Mild/...

Mild heat treatment (75°C, 30min) of the bacteria did not reduce their attachment to BEC, despite the fact that subculture of the heated organisms onto blood agar showed them to be non-viable. Stronger heat treatment (121°C, 15min) however, reduced the degree of attachment to virtually zero, giving a highly significant ($P < 0.001$) degree of inhibition (Table 16).

Table 16 Effects of periodate oxidation and heat treatments of type III GBS on their attachment to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Periodate oxidation	
Background	19.9 ± 3.4
BEC + GBS	26.5 ± 3.2
BEC + periodate oxidised GBS	30.0 ± 4.4
Mild heat treatment	
Background	21.4 ± 3.2
BEC + GBS	30.5 ± 3.4
BEC + heated GBS	35.4 ± 3.5
121°C for 15min	
Background	19.2 ± 2.6
BEC + GBS	30.5 ± 3.4
BEC + heated GBS	20.0 ± 2.0

7.4/...

7.4 Neuraminidase treatment of type III GBS

After treatment with neuraminidase (0.025units/ml, 1h), the attachment of type III GBS to BEC was significantly increased ($P < 0.001$), when compared with untreated bacteria (Table 17).

Table 17 Effect of neuraminidase treatment of type III GBS, on their subsequent attachment to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	9.8 ± 1.8
BEC + GBS	17.5 ± 2.7
BEC + neuraminidase treated GBS	26.5 ± 3.7

7.5 Sugar inhibition studies

Of all the sugars with which the bacteria were pre-incubated (see 'Materials and Methods'), only N-acetyl-D-glucosamine caused a significant reduction ($P < 0.001$) in attachment of the organism. Inhibition of attachment by N-acetyl-D-glucosamine was a dose response effect, as shown in tables 18 and 19, and in fig. 30.

Table 18/...

Table 18 Effect of various concentrations of N-acetyl-D-glucosamine on attachment of type III GBS to BEC

Experiment	Mean number of bacteria attached per epithelial cell ± 2 SEM
Background	19.2 ± 3.0
25mM N-acetyl-D-glucosamine	20.9 ± 2.9
12.5mM N-acetyl-D-glucosamine	25.4 ± 3.4
6.25mM N-acetyl-D-glucosamine	31.3 ± 3.7
No sugar	32.0 ± 4.1

Table 19 Data plotted in figure 30

Concentration of N-acetyl-D-glucosamine	% inhibition of binding ± 2 SEM	n*
25mM	86.5 ± 0.8	3
12.5mM	53.4 ± 1.4	4
6.25mM	3.0 ± 0.7	4
No sugar	0	3

*n = number of smears counted

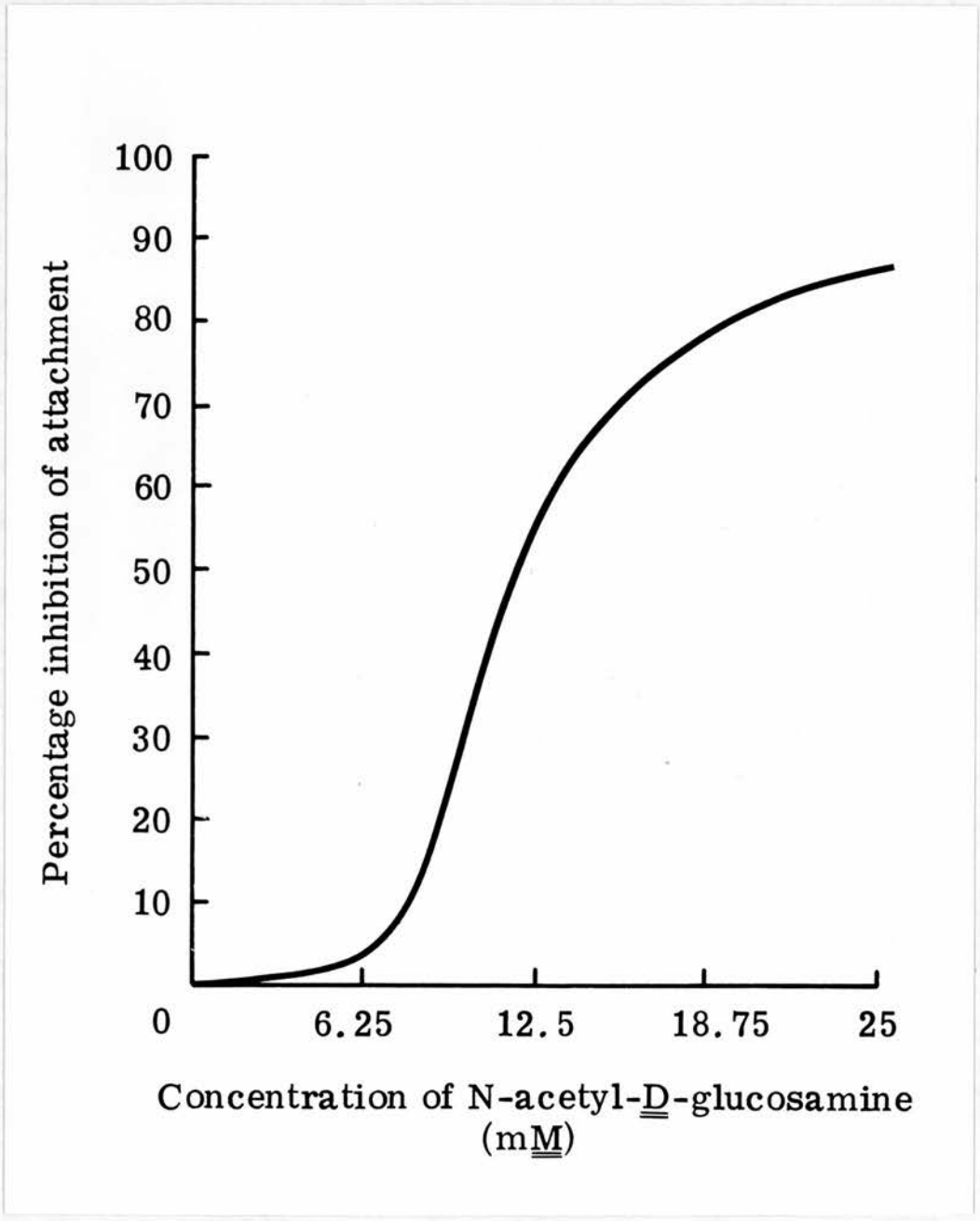


Fig. 30 Dose-response inhibition of type III GBS attachment to BEC by N-acetyl-D-glucosamine

8 Effect of Subminimal Inhibitory Concentrations of Penicillin on Adherence of Type III GBS to BEC

8.1 Antibiotic sensitivity of type III GBS (NCTC 11080)

The disc sensitivity test indicated that this strain of GBS was sensitive to a wide range of antibiotics, including penicillin G, erythromycin, cephaloridine, chloramphenicol, tetracycline and ampicillin.

8.2 Determination of minimal inhibitory concentration (MIC) of penicillin for type III GBS (NCTC 11080)

In both the tubes inoculated with GBS and those inoculated with Oxford staphylococcus, the growth controls grew well, and there was no growth in the media controls.

Examination of the tubes inoculated with Oxford staphylococcus showed a definite cut-off point between tube 4 (0.031µg/ml penicillin), in which there was no growth, and tube 5 (0.016µg/ml penicillin), which showed much growth. The MIC of penicillin for the Oxford staphylococcus is known to be 0.03µg/ml, thus indicating that the dilutions of penicillin were prepared correctly.

The cut-off point was less obvious for the GBS. Tube 4 (0.031µg/ml penicillin) was the lowest concentration of penicillin in which there was no growth, therefore 0.03µg/ml was taken, by definition, as the MIC. However, there was a gradual increase in growth from tube 5 (0.016µg/ml penicillin)/...

penicillin) to tube 9 (9.8×10^{-4}µg/ml penicillin), only tube 9 showing a completely yellow colouration of the broth. Thus, over a range of concentrations of penicillin from 0.016µg/ml to 9.8×10^{-4}µg/ml, there was a gradual increase in growth of the organism. A loopful of culture from some of the tubes inoculated with GBS was plated out on blood agar and incubated overnight. The resulting colony counts were as follows:

Tube No.	Growth
2	1 small colony
3	1 colony
4	2 colonies
5	6 colonies
6	Growth increased in this direction, but good in all cases
8	
9	

8.3 Effect of subminimal inhibitory concentrations of penicillin in the growth medium of type III GBS, on subsequent attachment of the organism to BEC

The effects of penicillin on the attachment of GBS to BEC depended very much on the dose of antibiotic employed. Typical curves are shown in figs. 31 and 32. The very low concentrations of penicillin tended to cause a reduction in binding, but as the concentration increased towards the MIC, the degree of attachment returned to its former level, or even increased. It proved difficult, however, to achieve/...

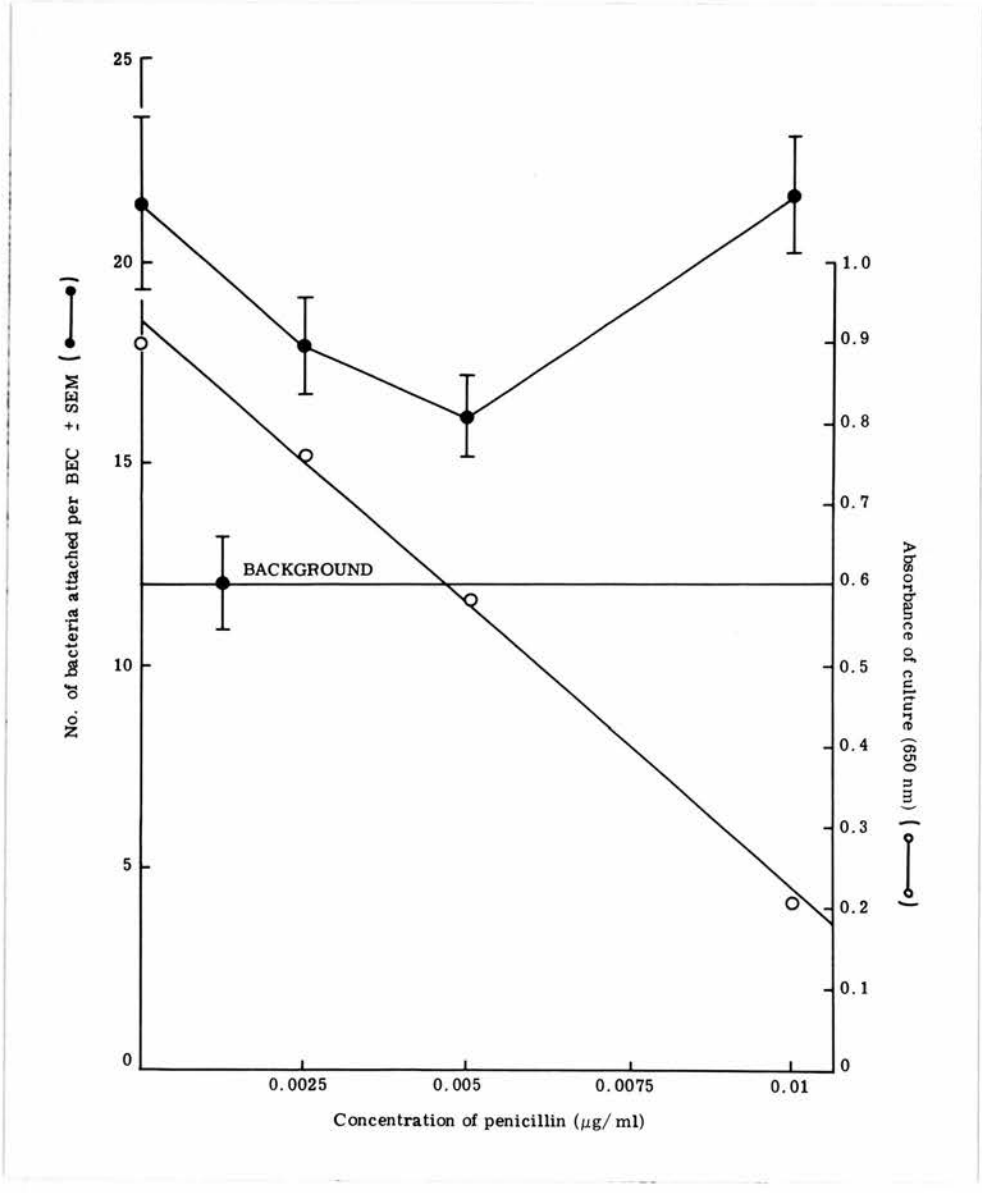


Fig. 31 Effect of subminimal inhibitory concentrations of penicillin on attachment of type III GBS to BEC

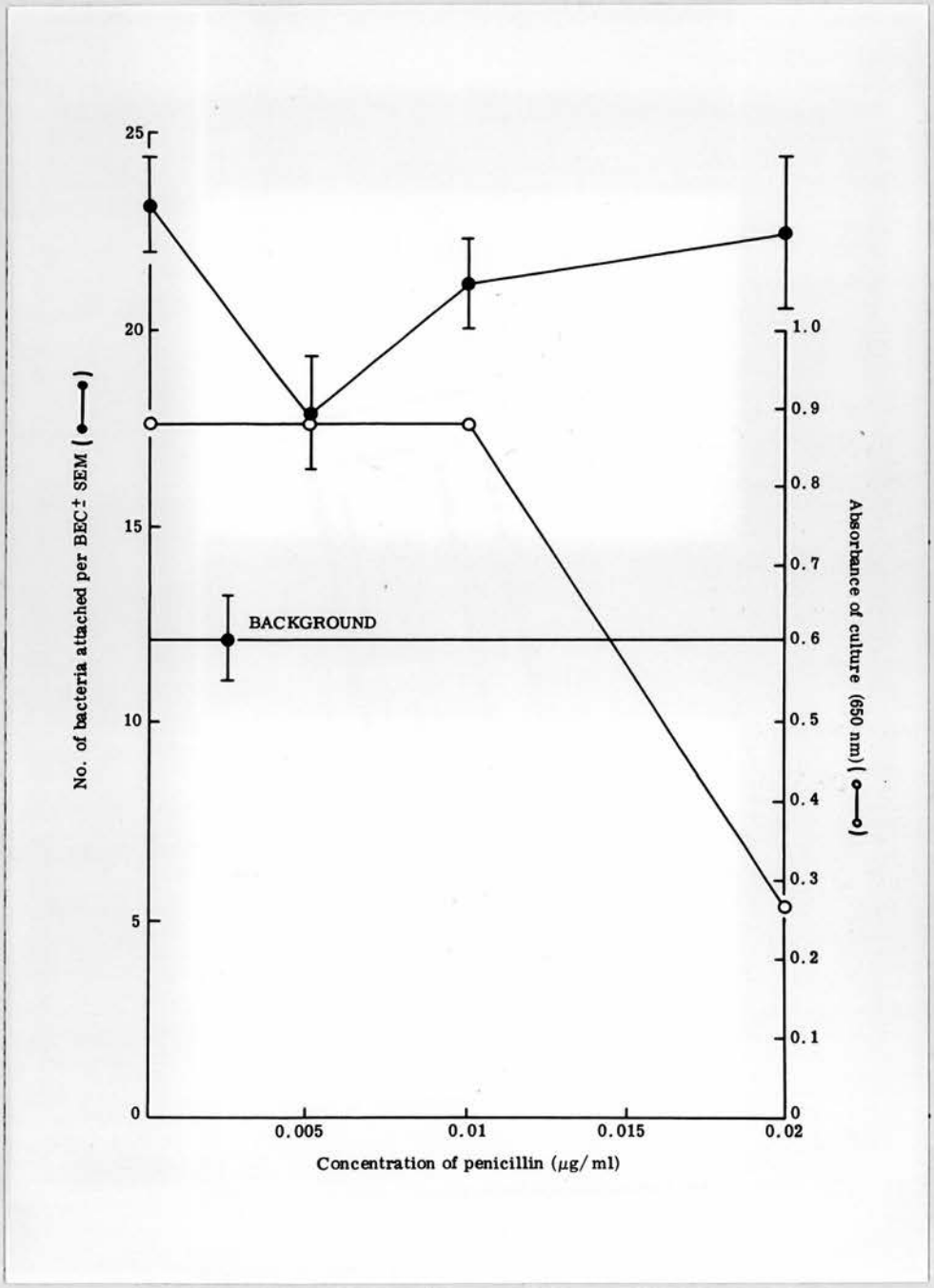


Fig. 32 Effect of subminimal inhibitory concentrations of penicillin on attachment of type III GBS to BEC

achieve a reproducible effect with a particular concentration of penicillin. This was also true of the turbidity of the overnight cultures, in which a particular concentration of penicillin did not always produce the same reduction in growth of the organism. However, although the results could not be accurately standardised, it appears that the subminimal inhibitory concentrations of penicillin in the broth did have some effect on attachment of the organism to BEC.

DISCUSSION

The work described in this thesis can be divided into two main sections. Preliminary experiments were concerned with the isolation of lectin-like material from Salmonella typhimurium, various strains of Escherichia coli, and Corynebacterium parvum. These organisms had already been shown to possess adhesins that recognise sugar molecules (Duguid, Anderson and Campbell, 1966; Ofek, Mirelman and Sharon, 1977; Knott, Ögmundsdóttir and Weir, unpublished observations), but only a limited amount of work had been performed to purify the bacterial cell wall components responsible for lectin-like attachment to eukaryotic cell surfaces.

The second part of the investigation involved a study of the adherence of group B streptococci (GBS) to buccal epithelial cells (BEC). In particular, experiments were designed to show whether or not a lectin type of interaction would explain the features of the binding reaction.

Accordingly, the results will be discussed in two separate sections: 1) mannosephilic lectins of E. coli, S. typhimurium and C. parvum, and 2) features of the binding of group B streptococci to buccal epithelial cells.

1 Mannosephilic lectins of E. coli, S. typhimurium and C. parvum

The mannose-specific agglutination of erythrocytes (Duguid and Gillies, 1957; Old, 1972) and yeast cells (Mirelman, Altmann and Eshdat, 1980) by various enterobacteria, including strains of E. coli and S. typhimurium, is believed to be due to type I fimbriae. Type I fimbriae have been purified from both E. coli (Salit and Gotschlich, 1977) and S. typhimurium (Korhonen et al., 1980c). These purified preparations produced mannose-sensitive haemagglutination patterns characteristic of the whole bacteria, thus proving that type I fimbriae can act as haemagglutinins. However, the preparative procedure adopted in the present investigations was based upon that of Eshdat et al. (1978), and has been applied to various strains of E. coli, S. typhimurium and C. parvum. The extraction procedure was followed in all cases by affinity chromatography of the material on mannan-linked Sepharose 4B. Similar affinity chromatography techniques have been used to purify many lectins (Uy and Wold, 1977; Townsend and Stahl, 1981; Gilboa-Garber et al., 1972). In the present experiments, it was found that the mannose-specific lectin molecules present in the crude bacterial extracts did not bind firmly to the column, and that the majority of the material capable of agglutinating yeast cells came off in the buffer effluent, prior to the addition of α-methyl-D-mannoside (αMM). The reasons for this are unclear, but may relate to factors such as the composition and pH of the buffer, the flow rate through the column, or the temperature. Concanavalin A binds to the affinity column used in the present experiments at pH 8, in the presence of Mn^{2+} and Ca^{2+} ions/...

ions (Uy and Wold, 1977). These conditions were reproduced, in an attempt to increase binding to the column, but without success. However, this difficulty was overcome to a large extent by collecting many fractions of low volume, and screening each of them individually for agglutinating activity. Selected fractions could then be subjected to SDS-PAGE, and the protein bands associated with yeast agglutination thus visualised. Separation of the components responsible for agglutination was found to be possible in this way, because although they were not firmly bound to the column, their progress through the affinity adsorbent was retarded. This effect was presumably mediated through a degree of interaction between the lectins and the mannan residues. Thus, bands of high molecular weight, which would be expected to appear at the void volume of a plain Sepharose 4B column, were eluted from the mannan-linked column much later, and therefore separated from the other components of the extract. This effect will be obvious when the results for the individual experiments are discussed.

1.1 Preparation of lectin-like material from E. coli 10418

E. coli 10418 caused strong, mannose-sensitive agglutination of yeast cells. Affinity chromatography of the extract from this organism showed that some protein was eluted with αMM, but this did not show up on SDS-PAGE, and the material did not cause yeast cell agglutination. However, most of the fractions eluted with plain buffer caused some degree of agglutination. The strongest activity appeared to be associated with a major band, of molecular weight 60,000, and a minor band, of/...

of molecular weight 58,000, which appeared together as a doublet (Fig. 8). As described earlier, despite their high molecular weights, these bands were eluted long after the void volume, and are likely to have interacted with the mannan linked to the Sepharose, which thus slowed their progress through the column.

This yield of purified extract produced by this technique was very low, and the preparations were not completely homogeneous. In order to prepare a pure sample of the material with a molecular weight of 60,000, preparative polyacrylamide gel electrophoresis was employed. SDS-PAGE of the crude extract (Fig. 16) revealed that the 60,000 molecular weight band was present in a high concentration, though many other protein bands were also evident. This crude extract caused agglutination of yeast cells, indicating that it contained lectin-like material. After the preparative gel had been run, and the relevant material electrophoresed out and dialysed, a purity check was made. Only one band was revealed by SDS-PAGE (Fig. 17), therefore the preparation was deemed to be homogeneous.

There is evidence to suggest that the mannose-binding ability of E. coli may play a role in attachment of the bacteria to phagocytic cells. Bar-Shavit et al. (1980) showed that attachment of E. coli to macrophages was inhibited by methyl-α-D-mannoside and mannan, but not by other sugars tested. The material purified from E. coli 10418, which is likely to be a mannose-specific lectin type of molecule, was therefore tested for its ability to inhibit binding of bacteria to mouse/...

mouse peritoneal exudate macrophages. Pretreatment of the macrophages with the extract would be expected to inhibit binding of the homologous strain, if it is indeed an adhesin. Experiments performed by Dr. J. Stewart (Immunology Laboratory, Bacteriology Department, University of Edinburgh) have supported this hypothesis, and demonstrated that inhibition by the purified material of binding of E. coli 10418 to mouse peritoneal macrophages follows a dose-response pattern. It is assumed, therefore, that the higher the concentration of inhibitor used, the more mannose residues on the phagocyte membrane are capped. It is not known where on the bacterial surface this adhesin resides. It may be fimbrial or associated with the cell wall. The crude, unpurified extract also blocks binding of E. coli 10418 to macrophages, but its activity is lower. Pretreatment of macrophages with the purified E. coli 10418 preparation prior to attachment assays with Staphylococcus albus causes increased attachment of bacteria to the monolayer. This finding indicates that the inhibitory activity has a degree of specificity, since attachment of Staph. albus, which does not bind to mannose residues, is not inhibited. The reason for enhancement of the binding of this organism is not clear, but may occur because Staph. albus attaches to a portion of the bound extract, exposed at the surface of the monolayer.

1.2 Preparation of lectin-like material from E. coli 4428 in the fimbriate and non-fimbriate phases

One of the most important questions relating to the lectin-like material extracted by the method described, is its position on the bacterial/...

bacterial cell. Eshdat et al. (1978), who first described the technique, showed that both the amino acid composition and molecular weight of the lectin isolated by this means from E. coli 7343 were different from those of E. coli K12 type I fimbriae isolated by Salit and Gotschlich (1977). It therefore seemed likely that the two materials were different molecular species. In view of these findings, an experiment was designed to show whether lectin-like material could be extracted from non-fimbriate cells of E. coli. If this could be done, then it would seem reasonable to suppose that lectin molecules were also present on the cell wall of the organism.

E. coli 4428 was chosen for these experiments, because in the past it has been shown that serial culture of this strain on agar plates produces a non-fimbriate form of the organism. After five sub-cultures on nutrient agar plates, the whole bacteria would not agglutinate yeast cells, and were shown by electron microscopy to be devoid of fimbriae. This was in contrast to cultures of E. coli 4428 grown statically in broth, which produced strong agglutination of yeast cells, and were heavily fimbriate. Extraction from these isogenic variants, followed by affinity chromatography, revealed the presence of yeast-agglutinating activity in fractions purified from both the non-fimbriate and the fimbriate organisms. These data suggest that at least some lectin-like material is present on the cell wall, but that it is inactive in causing agglutination of yeast cells. The importance of fimbriae in mannose-sensitive haemagglutination mechanisms has been discussed by Duguid, (Duguid, Anderson and Campbell, 1966; Duguid, Clegg and Wilson, 1979). These/...

These results further underline the important role played by bacterial appendages in the mediation of bacterial attachment to other cells. Even though lectin-like molecules are apparently expressed at the surface of the non-fimbriate bacteria, there is no evidence of agglutinating activity. Fimbriae are important in adherence mechanisms, because their small diameter enables them to penetrate the high potential energy barrier found at short distances between two bodies. The bulk of the bacterial cell is far too large to be able to do so. However, this physico-chemical explanation is somewhat oversimplified. Although the presence of mannose-sensitive haemagglutinin in a culture is invariably associated with type I fimbriae on the bacteria, some mannose-resistant haemagglutinating strains do not possess fimbriae (Duguid, Clegg and Wilson, 1979). In such cases, other factors must be playing a role. Nevertheless, this does not diminish the importance of fimbriae, and studies with other organisms, including Actinomyces naeslundii (Ellen et al., 1978), Vibrio cholerae (Tweedy et al., 1968), Neisseria gonorrhoeae (Swanson et al., 1971) and Bacteroides melaninogenicus (Okuda and Takazoe, 1974) have also strongly implicated fimbrial appendages as agents of haemagglutination and attachment to other eukaryotic cells.

1.3 Preparation of lectin-like material from S. typhimurium LT2

Cells of S. typhimurium LT2 were extracted in the same manner, and the results compared with those gained from the E. coli experiments. Strong yeast agglutinating activity was present in a number of the affinity chromatography fractions (Fig. 14). SDS-PAGE analysis of the fractions/...

fractions associated with agglutinating activity was not successful, because there was insufficient protein to show clearly on the gel. However, a photograph of the gel has been included (Fig. 15) because it illustrates that the crude extract was less heterogeneous than those prepared from E. coli, and contained a dense protein band of molecular weight 60,000. This band is very similar to that shown in the extract from E. coli 10418, which was purified and shown to block binding of the homologous strain to macrophages. No relationship has been demonstrated between these two proteins, but it would be useful to study them for cross-reactivity, and to examine the capacity of a purified sample of the S. typhimurium 60,000 molecular weight band to inhibit bacterial binding to macrophages.

1.4 Preparation of lectin-like material from C. parvum

Interest in the binding mechanism of C. parvum was stimulated by the finding that pretreatment of the organism with D-mannose caused a marked reduction in its subsequent attachment to mouse peritoneal exudate macrophage monolayers (Knott, Ögmundsdóttir and Weir, unpublished). Similar pretreatment of the organisms with D-glucose or D-galactose did not affect the binding. Previous work had shown that pretreatment of the macrophage monolayers with a variety of sugars including glucose and galactose, but excluding mannose inhibited binding of C. parvum (Ögmundsdóttir and Weir, 1976). These data therefore suggest the presence of two separate interactions between C. parvum and macrophages. The first involves a lectin (or lectins) in the macrophage membrane, which recognises carbohydrate residues, particularly glucose and galactose, in bacterial cell walls. The second type of interaction involves a lectin/...

lectin in the bacterial cell wall, which recognises mannose residues on the macrophage plasma membrane. This second mechanism bears many parallels with the mannose-sensitive attachment of *E. coli* to epithelial cells (Ofek, Mirelman and Sharon, 1977) and to phagocytes (Bar-Shavit *et al.*, 1980). It was therefore decided to attempt the extraction of a mannosephilic lectin from *C. parvum*, by the method employed previously for *E. coli* and *S. typhimurium*.

The crude extract was very heterogeneous, and did not agglutinate yeast cells. However, fractionation of the material on mannan-Sepharose 4B produced a highly active agglutinin component. This active component was eluted from the column as a second minor peak, prior to the addition of αMM. Its purity was shown by SDS-PAGE to be far greater than that of the crude extract (Fig. 13). Some of the other fractions caused weaker agglutination, and these were shown by SDS-PAGE to be more heterogeneous than the highly active material. It is possible that the presence of contaminating proteins interferes with the binding between active agglutinin molecules and the mannan residues on yeast cells. This hypothesis would also help to explain the lower activity of the crude *E. coli* 10418 extract, compared with the purified preparation, in blocking attachment of the homologous strain to mouse peritoneal macrophages, as discussed earlier (Section 1.1).

The results of SDS-PAGE analysis of the various fractions from affinity chromatography of the *C. parvum* extract indicate that yeast agglutinating activity is related to two or three protein bands, each of which appear as 'doublets'. The two major 'doublet' bands have molecular/...

molecular weights of approximately 76,000 and 69,000 respectively, while the third 'doublet', which shows up only very faintly, has a molecular weight of about 58,000. Repetition of the affinity chromatography gave a very similar result, and the active preparation could not be purified to the level of a single band on SDS-PAGE. A possible explanation is that the lectin activity is expressed by distinct iso-lectins. Iso-lectins are multiple molecular forms of the same lectin, which differ from one another in electrophoretic mobility. They have been demonstrated in a number of seed extracts, for example from Pisum sativum (Entlicher, Koštíř and Kocourek, 1970).

Very weak, indefinite agglutination of yeast cells was produced by the material eluted with αMM from the affinity column. Nevertheless, an interesting finding was the presence, on SDS-PAGE, of three lower molecular weight bands in the αMM effluent (Fig. 13). These bands, with apparent molecular weights of 12,000, 16,000 and 30,000 may represent subunits of the active lectin molecules. Most lectins are composed of subunits (Sharon and Lis, 1972), and these subunits themselves may be composed of complementary polypeptide fragments which assemble to form the intact subunit (Wang et al., 1971). The valency of these subunits will vary, but for a lectin to cause agglutination of cells it must possess at least two binding sites. It is therefore possible that the three lower molecular weight bands represent subunits of the intact lectin, but have valencies which are too low to induce agglutination of yeast cells. However, no chemical analyses have been performed, and it is equally possible that they represent different molecules altogether. Another explanation for the lack of agglutinating activity/...

activity may have been the presence of contaminating αMM, which had not been removed, even after extensive dialysis. Beachey, Eisenstein and Ofek (1981) have indicated that in their studies of mannose-binding activity of E. coli strains, all binding experiments were performed at 4°C with 0.02% sodium azide. Under those conditions, the binding of radiolabelled mannose was completely reversed by adding an excess of unlabelled mannose. In contrast, the affinity chromatography procedure described in this thesis was performed at room temperature. This may be a possible reason for the failure of dialysis to remove αMM from the lectins after affinity chromatography.

The avidity of these three low molecular weight proteins for the mannan-Sepharose was obviously greater than that of the active agglutinin, since they bound firmly to the column and were not eluted until the addition of αMM. The reason for this is unclear.

The position of the lectin molecules on whole cells of C. parvum is not known. Preliminary electron micrographs (data not shown) indicated that the cell surface of C. parvum is not completely smooth, and there was slight evidence for short, fine, hair-like projections from some of the cells. However, these were not fimbriae in the accepted sense, being very ill-defined. Honda and Yanagawa (1974) have shown that fimbriae mediate the agglutination of trypsinised sheep erythrocytes by Corynebacterium renale. The same workers (Yanagawa and Honda, 1976) demonstrated that most (91-100%) cells of Corynebacterium kutscheri, C. diphtheriae and C. pseudodiphtheriticum possessed fimbriae, while only 10-37% of cells of C. equi, C. hoagi, C. xerosis, C. pyogenes and C. murisepticum/...

C. murisepticum possessed sparsely-arranged fimbriae. In C. bovis, C. striatum and C. pseudotuberculosis, fimbriae were present on very few (0.5 to 3%) cells. Thus, fimbriae have been observed on several species of the genus Corynebacterium. More extensive electron microscopic examination of C. parvum, grown under varying cultural conditions, would be required to show whether fimbriae also play a role in attachment of C. parvum to macrophages.

These results provide further evidence for the role of mannosephilic bacterial lectins in the attachment of bacteria to macrophages. The isolation of a lectin-like molecule from cells of C. parvum has extended the observation that attachment of this organism to mouse macrophages can be blocked by pretreatment of the bacteria with D-mannose (Knott, Ögmundsdóttir and Weir, unpublished), and provides a molecular basis for the finding. It is possible that this type of interaction may help to explain some of the adjuvant and anti-tumour properties of C. parvum, which are thought to be mediated through macrophages (Weir and Ögmundsdóttir, 1980).

2 Features of the binding of group B streptococci to buccal epithelial cells

2.1 Choice of assay system

One of the most difficult aspects of microbial attachment studies lies in designing an assay system that allows accurate and reliable quantitation of adherence of the test organism to a collector surface. Ideally, all such experiments should be performed *in vivo*, an example being the adult rabbit intestinal ligated loop assay employed by Evans *et al.* (1975; 1978) for their studies of colonisation factor antigen in enterotoxigenic strains of *E. coli*. However, it is often difficult to control the experimental conditions in animal models, and they are expensive to maintain. Many studies of bacterial adherence have therefore relied on simpler, *in vitro* methods. A brief discussion of these systems will follow, because it will help to explain the choice of assay described in this thesis.

In principle, the target cells, to which the bacteria will attach, can be held either stationary, as a monolayer on a coverslip, or be left free in suspension. The monolayer system is favoured for experiments with tissue culture cells (Hackney *et al.*, 1980; Varian and Cooke, 1980) or macrophages (Bar-Shavit *et al.*, 1980), and has the advantage that it is easy to wash away unattached organisms after the incubation procedure. In preliminary experiments (data not shown) attempts were made to attach group B streptococci to neutrophil monolayers, but they proved unsuccessful because of the very low degree of binding detected.

Various/...

Various types of epithelial cells have been employed as collector surfaces for *in vitro* attachment assays, performed in suspension. These include vaginal epithelial cells (Mårdh and Weström, 1976), pharyngeal cells (Andersson *et al.*, 1981), uroepithelial cells (Svanborg-Edén and Hansson, 1978) and buccal epithelial cells (Beachey, 1975). Buccal cells have been used widely in studies of attachment mechanisms of many different organisms. They were chosen for the present study because they are readily obtainable as required, with minimal discomfort to the donor. There are some problems associated with the use of buccal cells. For example, the background commensal flora varies from day to day, and in certain instances, colonisation may be high. Some workers (Salit and Morton, 1981) have advocated removal of the natural flora by treatment of the cells in an ultrasonic water bath for up to five minutes. In the present study, however, only a short period of treatment in the ultrasonic bath was applied, because pilot studies showed that after a five-minute treatment, the cells did not stain properly; the nuclei, in particular, were very pale. Another characteristic of the assay was the wide variation in the number of bacteria that attached to individual cells in a given population of buccal epithelial cells. In addition, the receptivity of the buccal cells for the GBS varied to some extent from day to day. Similar findings have been reported by other workers for vaginal and uroepithelial cells (Parsons *et al.*, 1979; Svanborg-Edén, Eriksson and Hanson, 1977). The explanations for these phenomena are not clear, but may reflect stages in the maturation of individual cells, and/or the hormonal status of the donor. Botta (1979) has shown that adhesion of GBS to human vaginal epithelial cells fluctuates/...

fluctuates during the menstrual cycle, which implicates hormonal alterations in host cell receptivity. However, the problems posed by these factors were overcome by ensuring that a large sample of epithelial cells was examined in each smear, and by performing separate background counts and positive control experiments for each individual assay.

The assay technique proved straightforward in practice, although the counting procedure was time-consuming. An alternative approach would have been to label the bacteria with a radioactive isotope, and to measure the amount of radioactive label bound to the epithelial cells after the non-adherent bacteria had been washed away (Parsons et al., 1979). One drawback of such radioassays, however, is that one cannot check that all non-adherent bacteria have been washed away, an error which is immediately apparent if stained smears are being examined.

The number of bacteria used in the assay was adjusted by a turbidimetric method. The relationship between absorbance of the culture at 650nm and the number of colony forming units per ml was shown to be linear. This method was used instead of a direct chamber count because the chains of streptococci, although relatively short in an 18h culture, were difficult to quantify accurately by a microscopical technique.

2.2 General features of the binding of GBS to BEC

The binding of type III GBS to BEC was compared with binding of group A streptococci to BEC, in order to check that GBS would attach to/...

to BEC. It is recognised that tissue tropisms exist, and it may have been that GBS would not adhere to these particular cells. However, by comparing their attachment with that of group A streptococci, which have been previously shown to adhere to BEC (Beachey, 1975), it was evident that no statistically significant difference existed between the degree of binding of the two organisms. It therefore seemed feasible to use BEC as the target cells in later experiments.

The optimum period of incubation was shown to be 45min, a finding that agrees with those of other workers employing similar assays (Mårdh and Weström, 1976; Yamazaki, Ebisu and Okada, 1981). Prolonged incubation of assay mixtures does not increase the degree of bacterial attachment, possibly because at such high bacteria:epithelial cell ratios the receptor sites are saturated in a short space of time. Accordingly, assay mixtures were always incubated for 45min.

Type III strains of GBS were shown to adhere in greater numbers than type Ia GBS to BEC, although the difference was not statistically significant. Botta (1979) showed that type III strains of GBS possessed the greatest ability to attach to vaginal and oral epithelial cells, followed by types Ia and II. Many clinical observations indicate that type III organisms possess a striking degree of virulence (Wilkinson, 1978), and it is tempting to speculate that the ability of GBS to attach to epithelial surfaces may be a virulence factor. In view of these data, type III GBS were used in all experiments performed to study the general nature of attachment to BEC, because by working with a strain that adhered well, any effects on the adherence following experimental modifications were made more apparent.

2.3/...

2.3 Adherence-inhibition studies with type III group B streptococcal sonicate

One approach to the identification of bacterial cell surface molecules involved in attachment of micro-organisms to epithelial surfaces, is to study inhibition of adherence by a range of bacterial extracts. If adherence of an organism is reduced by pretreatment of the target cell with a particular extract, then it is very possible that the extract contains adhesin components. This type of investigation was employed in the present studies. Various bacterial extracts were prepared from type III GBS. These included a detergent extract (de Cueninck, 1979), an ethylenediaminetetraacetic acid (EDTA) extract (Poxton, 1979) and a sonicate, from whole cells, and an aqueous phenol extract of cell membranes (Coley, Duckworth and Baddiley, 1975). The detergent extraction procedure was not successful, because of the very low yield of material produced, and the difficulty of removing the detergent afterwards. Similarly, it was not clear whether all the EDTA could be removed by dialysis. In view of the effects that these chemicals themselves may have on attachment, the only whole-cell extract used for inhibition experiments was the sonicate. The effect, on subsequent group B streptococcal attachment, of BEC pretreatment with the aqueous phenol extract of the membranes will be discussed later (Section 2.5).

Preparation of the sonicate was straightforward. A suspension of washed, whole cells of type III GBS was subjected to ultrasonic oscillations in an ultrasonic water bath, to remove a layer of material from the surface of the bacteria. This procedure was very mild, and did not/...

not affect either the viability or the structural integrity of the cells, as shown by bacterial counts performed both before and after sonication. The sonicate itself, which was defined as the supernate retained after sedimentation of the sonicated cells, was therefore likely to contain mainly cell surface components, and not intracellular material.

Despite the fact that the cells were largely undamaged by ultrasonic treatment, subsequent attachment of sonicated GBS to BEC was significantly reduced. This may have been due to depletion of adhesin, which had been shed during the extraction procedure. Such an explanation has been proposed for loss of the ability of group A streptococci to adhere to BEC after penicillin-stimulated release of lipoteichoic acid (LTA), LTA being in that case the adhesin (Alkan and Beachey, 1978). A lack of adhesin may not, however, be the only explanation. The microarchitecture of bacterial cell surfaces is very complex, and cell wall projections such as fimbriae (Duguid et al., 1955) or fibrillae (Beachey and Ofek, 1976) often play an important role in adherence phenomena. It is therefore possible that the reduction in adherence of the sonicated organisms is caused, at least partly, by disruption of the surface ultrastructure. The presence of fimbrial projections from the cell surface has been demonstrated by electron microscopy of thin sections of type Ia GBS (de Cueninck, 1979). However, such structures were absent, or present in a diminished form, on other types of GBS examined. It would be valuable to study the sonicate under an electron microscope, to see whether it contains any such appendages. Whatever the case, it is apparent that the adhesive mechanism of the bacteria is disrupted/...

disrupted in some way by the sonication procedure.

SDS-PAGE of the sonicate revealed the presence of many protein bands, after staining the gel with Coomassie blue (Fig. 20). It was believed that this heterogeneity may have been caused partly by breakdown of proteins during the preparation procedure, which was routinely performed at room temperature. In an attempt to produce a more pure material, a sample of sonicate was prepared at 4°C, with the aim of reducing any breakdown. However, this procedure proved fruitless, producing an equally heterogeneous sonicate.

Pretreatment of BEC with this crude, heterogeneous sonicate prepared from type III GBS inhibited, in a dose-dependent fashion, binding of the homologous strain (Fig. 22). It therefore seemed feasible to suggest that a component (or components) of the sonicate was attaching to receptors for GBS on the host cell, and thereby blocking subsequent binding of whole organisms. Nevertheless, this result could also have been caused by non-specific adsorption of protein onto the BEC, resulting in a steric hindrance effect. This type of non-specific interaction has been suggested to explain inhibition of gonococcal attachment by lipopolysaccharide (LPS) (Tramont et al., 1981), since LPS prepared from many organisms will block adherence of N. gonorrhoeae.

Specificity of the interaction was therefore investigated by studying the effect of pretreatment of BEC with type III GBS sonicate prior to attachment assays with type Ia GBS and group A streptococci.

Adherence/...

Adherence of type Ia GBS was significantly reduced by the sonicate, but group A streptococcal adherence was not inhibited. Although this study of the specificity was very limited, it does at least indicate that the blocking of group B streptococcal attachment by the sonicate is unlikely to be a random, non-specific effect.

Although the sonicate was largely proteinaceous, it also contained a carbohydrate component, the ratio of protein:carbohydrate being approximately 6:1. It is possible that some of the carbohydrate is derived from glycoproteins in the mixture, and is not therefore present as free sugar. The ability of the sonicate to inhibit attachment of type III GBS to BEC, after it had been subjected to heat treatment or to periodate oxidation, was tested. Heating the sonicate reduced, very significantly, its capacity to block adhesion, which indicates that the component responsible is thermo-labile and may be a protein. Periodate treatment affected the activity of the sonicate to a much lower degree, and if compared with the periodate-control sonicate, to which no periodate was added, had no significant effect at all. Sodium metaperiodate oxidation cleaves the C-C bond between vicinal hydroxyl groups of sugars. Its lack of effect on the activity of the sonicate would therefore imply that the carbohydrate component does not contain the major adherence-inhibiting agent. This result will be discussed further at a later stage, in relation to the effects of modifications of whole cells on their subsequent attachment to BEC (Section 2.4).

In/...

In view of the heterogeneity of the sonicate, it was felt necessary to purify the preparation, so that the results of the inhibition experiments could be related more exactly to the individual components of the mixture. Gel filtration of the sonicate, on both Sephadex G-75 and Sephadex G-100, consistently produced two peaks when the fractions were screened for protein by absorbance at 280nm (Fig. 23). This purification procedure was not, however, successful in terms of separating the various proteins in the mixture. SDS-PAGE revealed that although the protein profile of the peak I material was slightly less complex than that of the crude extract, it still contained largely the same protein bands. On the basis of the elution pattern from the Sephadex columns, however, a totally unexpected finding was the complete absence of any protein bands on SDS-PAGE of the peak II sample. Resolution of the sonicate into these two peaks by gel filtration was achieved on three separate occasions, with different preparations, therefore peak II is unlikely to represent an artefact. Various hypotheses can be proposed to explain this finding. In view of the fact that its progress through the gel filtration column is slow, peak II is likely to contain low molecular weight material. If this is the case, then its electrophoretic mobility during SDS-PAGE may be very high, causing it to run off the gel, ahead of the other samples. These small molecules could represent fragments of intact proteins in peak II.

Another possible explanation would be that peak II contains components of the mixture that had some physico-chemical affinity for the/...

the Sephadex gel, and which were retained to an extent by molecular interaction. If these components were very large proteins, they may have been unable to enter the 10% ($^{w}/v$) polyacrylamide gel. This seems unlikely, however, since there was no evidence of Coomassie blue-staining material at the top edge of the separating gel.

Discussions with biochemists suggested that peak II may contain nucleic acid, which had leaked out of the bacterial cells during the sonication procedure. This suggestion was followed up by a number of experiments aimed at revealing the chemical nature of the peak II material.

Samples of crude sonicate, and material from peaks I and II were diluted to give an identical absorbance at 280nm. They were then examined by scanning ultraviolet spectrophotometry to see whether there was a shift to a lower wavelength of the absorbance for peak II material, which would indicate a higher content of nucleic acid. The interesting finding was that all the samples demonstrated greatest absorbance at about 260nm, although the maximum absorbance for peak II was slightly less than 260nm (Fig. 24). Such data did not aid the identification of the peak II material. The accepted value for absorbance by nucleic acid is 259nm, and the result of the spectrophotometry may indicate that all the samples are contaminated with nucleic acid. Although not attempted at the time, treatment of the samples with ribonuclease and deoxyribonuclease prior to the scanning spectrophotometry may have indicated whether this was the case. Another consideration which should not be discounted is the fact that some proteins demonstrate a maximum absorbance at wavelengths below 280nm. In particular, it has been established that denaturation or proteolysis of proteins may result in conformational/...

conformational changes associated with the appearance of absorption maxima at wavelengths of less than 280nm (Glazer and Smith, 1961). However, when one considers the complex mixture of substances present in a sonicate, including not only proteins, but also carbohydrates and possibly lipids and nucleic acid as well, it is hardly surprising that the maximum absorbance is not at 280nm.

A polyacrylamide gel, on which a sample of the peak II material had been electrophoresed, was stained with methylene blue, to reveal the presence of any nucleic acid, but no bands were evident. A similar result was obtained with a gel stained for carbohydrate. Indeed, when the ratios of protein concentration:carbohydrate concentration were evaluated for the crude sonicate and for the material in peaks I and II, the values did not differ markedly, the highest ratio being found for peak II.

Despite the elusive chemical nature of the peak II material, its biological activity was marked. Pretreatment of buccal cells with both peak I and peak II material caused highly significant inhibition of attachment. It was stated earlier that the peak II sample may contain low molecular weight constituents that represent fragments of the inhibitor in peak I. This hypothesis would also help to explain the inhibition of attachment by peak II material, which must be binding, in some way, to receptors on the BEC.

In view of these unsuccessful attempts to purify the sonicate by gel filtration, the peak I material was re-fractionated by ion-exchange/...

exchange chromatography. An anion exchanger, DEAE-cellulose, was chosen for this procedure, because it was considered likely that a proteinaceous adhesin would possess an overall negative charge, and thus bind to the positively-charged cellulose. In practice, this was shown to be true, and the sonicate bound strongly to the column. Upon application of a salt gradient, the bound material was eluted from the ion-exchanger as a single peak (Fig. 25). It was evident from the elution profile, however, that application of a shallower salt gradient may have enabled resolution into three peaks, since there were two slight 'steps' on the ascending part of the curve. Nevertheless, it was still possible that partial purification had been achieved, because some of the components of the mixture may not have bound to the DEAE-cellulose in the first place. Accordingly, those fractions comprising the material eluted by the salt gradient from the column were pooled, and subjected to SDS-PAGE (Fig. 26). This revealed that there had been only a slight degree of purification, but that certain bands had increased in relative density of staining with Coomassie blue. The bands that became denser probably represented those proteins with the greatest affinity for the positively-charged cellulose, and which therefore bound more readily to the column during the 'washing-on' procedure. The ion-exchange-purified sonicate retained its ability to inhibit attachment of the homologous strain of GBS to BEC.

. Absorption of samples of the partially-purified sonicate with BEC, followed by SDS-PAGE, indicated a consistent reduction in the density/...

density of particular protein bands. The most striking effect was on the dense band with a molecular weight of 47,300, which was almost totally absorbed after a single treatment with BEC (Fig. 27). Conversely, certain components were completely unaffected after three absorptions. These results indicate further that the inhibition of bacterial attachment by the sonicate is probably due to specific binding of particular proteins to the epithelial cells, as opposed to random adsorption of protein onto the BEC.

2.4 Effects of modifications of whole bacterial cells and epithelial cells on subsequent group B streptococcal attachment

Despite the fact that attempts to purify the sonicate were unsuccessful, the results gained from studying its effects on bacterial attachment gave valuable insight into the nature of the binding mechanism of GBS. As stated previously, the component of the sonicate responsible for its ability to inhibit binding appeared to be heat-labile, but unaffected by periodate oxidation, a result commensurate with the involvement of a protein. This theory was further substantiated by the finding that heating (121°C, 15min) whole cells of GBS almost totally abolished their ability to adhere to BEC. Conversely, periodate oxidation of whole cells of GBS caused no reduction in their subsequent attachment to BEC. Thus, the data obtained by both modifying the sonicate and by treating whole cells, indicate the involvement of a bacterial protein rather than a carbohydrate in the adherence mechanism. An interesting finding was that milder heat treatment (75°C,/...

(75°C, 30min) of GBS did not affect attachment to BEC. Evidence that the binding mechanism is mediated by a lectin-like molecule on the bacterial surface will be discussed later (Section 2.6), but it should be mentioned at this point that some lectins are remarkably thermostable, compared with other proteins. This property was exploited by Gilboa-Garber, Mizrahi and Garber (1972) in their technique for isolating a galactose-binding lectin from Pseudomonas aeruginosa. Partial purification of the crude, bacterial lectin-containing extract was achieved by heating it to 70°C for 15min, which denatured most of the proteins except the lectins (Gilboa-Garber, Mizrahi and Garber, 1972). Thus, the adhesin on the surface of GBS may be a fairly thermostable protein, a characteristic that would correlate well with its proposed lectin-like activity, as discussed later.

It should be noted that both of these heat treatments killed the bacteria. Since the organisms subjected to the lower temperature adhered equally as well as unheated bacteria, it is apparent that viability of GBS is not a necessary prerequisite for adherence to epithelial cells. This finding agrees with that of Zawaneh et al. (1979), who showed that killing GBS with ultra-violet light or penicillin did not affect their adherence to human vaginal cells.

Another finding that indicates the possibility of a cell-surface protein being the adhesin responsible for group B streptococcal attachment to BEC, is the significant reduction in the degree of binding of trypsin-treated GBS, compared with untreated organisms. Such results must/...

must be interpreted with caution, however, because the proteolytic activity of the trypsin may not be directed at the adhesin itself, but at a different protein that is merely stabilising or anchoring the adhesin in position. If the adhesin resides in a 'fibrillar' layer, similar to that seen for group A streptococci (Beachey and Ofek, 1976), then the trypsin may be non-specifically stripping off this outer coat, and thereby reducing the adherence.

Pretreatment of GBS with another enzyme, neuraminidase, caused a highly significant increase in the number of organisms that attached to BEC. This effect is of particular interest in view of the report of Milligan et al. (1978), which relates high production of neuraminidase by certain strains of GBS to an enhanced pathogenic potential. This enhancement of pathogenicity by neuraminidase could be explained, at least partly, by the subsequent increase in attachment of such strains to epithelial surfaces. The adhesin on the bacterial cell wall is probably masked to some extent by sialic acid. After removal of the sialic acid residues by neuraminidase, the adhesin will be exposed, and therefore free to interact with the receptor on the epithelial cell. Removal of sialic acid from the bacterial surface is also likely to reduce the overall negative charge of the cells, which may be another factor that helps to produce the observed increase in attachment. Zawaneh et al. (1979) found that binding of GBS to human vaginal cells was similarly increased by treatment with neuraminidase.

One of the most striking pieces of evidence for the proposal of a lectin-like interaction between GBS and BEC was that treatment of BEC/...

BEC (as opposed to GBS, as discussed earlier) with sodium metaperiodate caused a highly significant reduction in subsequent adherence of the organism. This finding suggests that sugar residues on the epithelial cell surface may play a role as receptors for GBS. Ofek, Mirelman and Sharon (1977) showed a similar effect on attachment of a mannose-sensitive strain of E. coli to BEC. The periodate oxidation procedure will have structurally altered any sugar residues expressed at the surface of the epithelial cells. This will interfere with any interactions between a lectin-like adhesin on the bacteria and a sugar on the BEC. The probable involvement of bacterial lectins in adhesion of micro-organisms to host cell surfaces has been well documented for a number of bacteria, including such diverse organisms as E. coli (Ofek, Mirelman and Sharon, 1977), Eikenella corrodens (Yamazaki et al., 1981), Aeromonas hydrophila (Atkinson and Trust, 1980) and Fusobacterium nucleatum (Mongiello and Falkler, 1979). However, no specific mechanism has been demonstrated previously for GBS.

2.5 The role of group B streptococcal lipoteichoic acid in adherence of GBS to BEC

There is much evidence to support the role of membrane lipoteichoic acid (LTA) in the attachment of group A streptococci to mucosal cells (Beachey, 1975). It has been reported that pretreatment of pharyngeal epithelial cells with LTA purified from group A streptococci also inhibits subsequent attachment of group B streptococci to the cells (Botta, 1981). The specificity of this inhibition is questionable, however,/...

however, because the same pretreatment also reduces adherence of Staphylococcus aureus to the pharyngeal cells (Botta, 1981). Lipoteichoic acids are amphipathic molecules which can bind spontaneously to many types of mammalian membranes (Wicken and Knox, 1975). It is quite possible, therefore, that the inhibition of attachment of GBS and Staphylococcus aureus to epithelial cells by LTA purified from group A streptococci is largely due to steric hindrance. To overcome this objection, membrane LTA was prepared from the strain of type III GBS used throughout this study. Aqueous phenol extraction of membranes of the organism, followed by gel filtration on Sepharose 6B, allowed the preparation of a polymer which reacted with antiserum to type III GBS and which had the chemical composition expected of a lipoteichoic acid. The reaction with antiserum raised against whole cells indicates that the LTA is expressed at the cell surface, and could therefore mediate adhesion of the organism to epithelial surfaces. Pretreatment of BEC with this material did not, however, significantly reduce adherence of the homologous strain. This indicates that the LTA of GBS does not bind in the same way as group A streptococcal LTA to epithelial cells. It seems unlikely, therefore, that LTA-mediated binding of type III GBS to BEC is a major adherence mechanism. This result correlates well with the finding of Zawaneh et al. (1979), who showed that treatment of GBS with high concentrations of penicillin did not inhibit subsequent adherence of the bacteria to vaginal epithelial cells. Penicillin treatment stimulates bacteria to lose their LTA, and in the case of group A streptococci there is a parallel loss of the organisms' ability to bind to BEC (Alkan and Beachey, 1978). Such/...

Such an effect on attachment is not evident for GBS, which suggests that LTA does not play a prominent role in their adherence to epithelial cells.

It was also discovered, during the course of the LTA preparation, that the purified polymer would only react with antiserum against type III GBS, that is, against the homologous type. Specific antisera to types Ia, Ib, Ic and II did not produce bands of precipitation against the LTA, when tested in an Ouchterlony diffusion experiment. This incidental finding, that the membrane antigen is type-specific, has provided an interesting piece of confirmatory evidence for the lack of involvement of LTA in adhesion of GBS. Zawaneh et al. (1979) reported that no inhibition of attachment of GBS to vaginal epithelial cells was produced by either anti-group or anti-type antisera. This suggested that neither group-specific nor type-specific antigenic determinants were involved in the adherence mechanism. The report in this thesis, that the membrane LTA component of type III GBS is a type-specific antigen, and that it causes no significant reduction of bacterial binding to BEC, thus correlates well with the results of Zawaneh et al. (1979). This further reduces the likelihood that LTA is an important adhesin for GBS.

2.6 Lectin-mediated adherence of GBS to BEC

Evidence gained from several sources thus indicated that the LTA mechanism proposed for adherence of group A streptococci was not responsible/...

responsible for binding of GBS to epithelial surfaces. Attention was therefore focussed on the lectin type of interaction mentioned earlier. The findings that the bacterial adhesin was heat-sensitive and that the receptor on the BEC was denatured by periodate oxidation both lent credence to the hypothesis that binding of GBS to BEC was lectin-mediated. Similarly, Zawaneh et al. (1979) published data showing that the receptor for GBS on vaginal epithelial cells was heat-stable, but that the bacterial adhesin was heat-sensitive. In both systems, therefore, it was possible that the epithelial cell receptor was a carbohydrate.

Sugar inhibition studies, in which GBS were pretreated with a variety of simple sugars prior to the adherence assay, were therefore performed. Adherence of the bacteria was unaffected by pretreatment with D-galactose, D-glucose, α-methyl-D-mannoside, L-fucose, lactose maltose and N-acetyl-D-galactosamine. The only sugar that could be shown to inhibit binding of GBS to BEC was N-acetyl-D-glucosamine. Over a range of concentrations from 0mM to 25mM, inhibition by this sugar followed a dose-response pattern. A graph relating the percentage inhibition of attachment, to the sugar concentrations used (Fig. 30), was sigmoidal in form, not unlike the dose-response curve presented by Beachey (1975) for inhibition by LTA of the attachment of group A streptococci to mucosal cells. These data suggest that type III GBS may possess a surface lectin which recognises and binds to N-acetyl-D-glucosamine residues, or to a sugar with a very similar structure.

Wheat/...

Wheat germ agglutinin (WGA) is an example of a lectin that is specific for N-acetyl-D-glucosamine (Burger and Goldberg, 1967). Stanley and Carver (1978) have shown that Chinese hamster ovary cells have binding sites with high and low affinities for WGA. It therefore seems plausible to suggest that a bacterial lectin with the same sugar specificity could mediate adhesion of an organism to mammalian cells.

Levy (1979) reported that addition of WGA to suspensions of mouse fibroblasts (L-cells) blocked the subsequent attachment of ^{14}C-labelled Chlamydia psittaci to the L-cell surface. This blocking action of WGA was antagonised by N-acetyl-D-glucosamine, but not by other sugars. Levy (1979) also showed that WGA similarly blocked attachment of Chlamydia trachomatis to L-cells, and that the results were similar when other established cell lines of human, simian and murine origin were employed as the target cells. Thus, attachment of these two strains of chlamydiae to host cells of diverse origin, was shown to involve an N-acetyl-D-glucosamine entity, to which the ligand on the micro-organism attached. A similar mechanism is proposed for the binding of GBS to BEC. The use of WGA as an inhibitor of group B streptococcal binding has not been investigated, but could possibly provide extra evidence for the validity of this theory.

Wheat germ agglutinin is not a blood-group-specific haemagglutinin. It will agglutinate all types of human erythrocytes, as well as a variety of normal and neoplastic animal cells (Goldstein and Hayes, 1978)./...

1978). Sugar inhibition of rabbit erythrocyte agglutination by WGA showed that of the sugars found in glycoproteins, only N-acetyl-D-glucosamine would inhibit WGA (Allen, Neuberger and Sharon, 1973). It would be of interest to study the haemagglutinating activity of both whole cells of GBS and the sonicate, to see whether either of these contained agglutinating activity that could be blocked by N-acetyl-D-glucosamine.

The involvement of a lectin in group B streptococcal attachment is also suggested by the data of Elbein et al. (1981). These workers showed that GBS adhere to canine kidney (MDCK) epithelial cells once the mammalian cells have been infected with influenza virus, but that the bacteria do not attach to uninfected kidney cells. Further studies have shown that GBS recognise and adhere to influenza virus glycoproteins that have been synthesised in infected MDCK cells and inserted into the cell membrane (Elbein et al., 1981). The influenza virus glycoproteins have been partially characterised (Collins and Knight, 1978). Two types of oligosaccharide are found in these glycoproteins. One type, called the high mannose type, contains only mannose and N-acetyl-glucosamine, while the second, or 'complex', type contains mannose, N-acetyl-glucosamine, galactose and sialic acid. Details of the anomeric configuration and branching of these glycoproteins are not known. It may be significant, however, in view of the data presented in this thesis, that these glycoproteins contain N-acetyl-glucosamine. If, as suggested, GBS possess a lectin-like adhesin which recognises N-acetyl-D-glucosamine, then the necessity for/...

for infection of MDCK cells with influenza virus to allow group B streptococcal attachment may possibly be explained by the subsequent availability of N-acetyl-glucosamine residues expressed by the viral glycoproteins.

2.7 Effect of sub-minimal inhibitory concentrations of penicillin on adherence of GBS to BEC

A study of the effect of sub-minimal inhibitory concentrations of penicillin on adherence of GBS to BEC was prompted by recent reports of modifications of bacterial adherence mechanisms by sublethal concentrations of antibiotics (Alkan and Beachey, 1978; Eisenstein, Ofek and Beachey, 1979; Eisenstein, Beachey and Ofek, 1980). In the case of GBS, such effects may be of particular importance, because there are significant problems associated with antibiotic regimens in the treatment of group B streptococcal disease. Treatment, with penicillin, of colonised adults often fails to eradicate carriage of the organism (Gardner et al., 1979), and there have been many reports of relapses of group B streptococcal infection in infants, following penicillin therapy (Truog, Davis and Ray, 1976). Various theories have been proposed to explain these clinical findings, as outlined in the 'Introduction', but one avenue which has not been explored is the possibility that penicillin can alter the adherence of GBS to epithelial cells.

The minimal inhibitory concentration (MIC) of penicillin, determined for the strain of type III GBS used in these experiments, fell within the range of MIC's quoted by Baker, Webb and Barrett (1976) for/...

for 244 clinical isolates of GBS. However, in the tube dilution assay used to determine the MIC, there was no sharp cut-off point between the tube containing the MIC of penicillin (0.03µg/ml) and tubes containing lower concentrations of penicillin. Instead, there was a gradual increase in the degree of bacterial growth over a range of penicillin concentrations from 0.016µg/ml to 9.8×10^{-4}µg/ml. The concentrations of penicillin that were subsequently tested for their effect on bacterial attachment all fell within this range. They were, therefore, below the MIC, but were presumably having some effect on growth of the bacteria in view of their reduction of the turbidity of an overnight culture.

The preliminary results, presented in this thesis, indicate that growth of GBS in broth containing very low sub-MIC levels of penicillin may result in reduced attachment of the organisms to BEC. Similar suppression of binding activity has been reported for E. coli, following growth in broth containing subinhibitory concentrations of streptomycin (Eisenstein, Beachey and Ofek, 1980), ampicillin (Sandberg, Stenqvist and Svanborg-Edén, 1979) and penicillin (Ofek et al., 1979). This suppression, however, is not evident at concentrations of penicillin greater than 0.005µg/ml, and as the penicillin concentration increases towards the MIC, binding of GBS increases again to the original level. The reasons for this unusual dose-response effect are unclear, and further studies with different antitiotics may help to clarify the situation. The activity of penicillin was investigated initially, because it is the antibiotic used most widely for treatment of group B streptococcal disease. Eisenstein, Beachey and Ofek (1980) observed that/...

that a variety of antibiotics which interfere with protein synthesis caused suppression of the mannose-specific ligand of various strains of E. coli. If, as previous data suggest, attachment of GBS is mediated by a surface protein, it would be interesting to investigate the effect on adherence of growth of the organisms in the presence of an antibiotic that inhibits protein synthesis.

2.8 Concluding remarks

The main aim of this investigation into group B streptococcal adherence has been to define the type of mechanism involved, particularly with respect to the bacterial adhesin. One of the possible clinical applications of data obtained from bacterial attachment studies is the potential ability to prevent colonisation of the host, and subsequent disease, by interfering with adherence of pathogenic micro-organisms to tissue surfaces. An example of direct interference is the finding that injection of a mannose-sensitive strain of E. coli, in the presence of α-methyl-mannoside, into the urinary tract of mice resulted in a considerable decrease in the number of bacteriuric mice, when compared with bacteria injected in the absence of the sugar (Aronson et al., 1979). Having established the specificity of the streptococcal lectin for N-acetyl-D-glucosamine, this principle, of blocking the bacterial adhesin with receptor analogues, could possibly be applied to GBS. However, such protection would be very local and transient.

A more potent means of prevention may prove to be immunisation of susceptible hosts with purified preparations of the identified adhesin./...

adhesin. A vaccine has been prepared from purified gonococcal pili, for example, and administered to human volunteers (Tramont et al., 1981). Immunisation with this particular vaccine raised antibody capable of blocking adherence of homologous and heterologous strains of gonococci to buccal epithelial cells. Similar vaccines could be prepared from other bacterial adhesins, once they have been purified. It was to this end that effort was put into purifying the group B streptococcal sonicate. No evidence is available to show that the inhibitory component of the sonicate is the N-acetyl-D-glucosamine-specific lectin, whose presence at the bacterial cell surface is proposed on the basis of sugar-inhibition studies. However, the possibility exists that the inhibitory component is the lectin. In that event, affinity chromatography of the sonicate may provide a suitable means of obtaining a pure preparation. Hydrolysis of chitin produces oligomers which can be bound to Sepharose, thus producing an excellent affinity adsorbent for N-acetyl-glucosamine-specific lectins (Uy and Wold, 1977). A column packed with such an affinity adsorbent would be ideal for purification of the group B streptococcal adhesin proposed in this thesis.

It must be stressed that all the data presented in this thesis, concerning adherence of GBS, is based upon a single type of assay. As indicated in the 'Introduction', individual assay systems sometimes highlight one particular facet of the adherence mechanisms of an organism. The studies on adhesion of V. cholerae to rabbit intestinal brush border preparations (Jones and Freter, 1976) and to intact slices/...

slices of rabbit ileum (Freter and Jones, 1976) are a prime example, where the organism utilises different mechanisms to attach to the different tissue surfaces. It would therefore be of great value to extend the assay system described in this thesis, to include both a range of target cells, and a number of different strains of GBS. Such variation has been deliberately avoided up to this point, to allow at least one mechanism to be analysed in detail. Now that such a mechanism has been described, it should be applied to different experimental assay systems, to see whether or not it accounts for attachment of all strains of GBS to a variety of epithelial surfaces. In particular, the relevance of this mechanism in attachment of GBS to vaginal epithelial cells should be studied, because if this interaction could be prevented, it may provide a powerful new approach to eradicating maternal colonisation with the organism. Prophylatic antibiotic treatment is not effective in this situation, and there is a continuing problem of neonatal group B streptococcal disease, following vertical transmission from the mother. An effective method for preventing adherence of the organism to the vaginal surface would represent a major advance in the management of this serious problem.

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REFERENCES

ABER, R.C., ALLEN, N., HOWELL, J.T., WILKINSON, H.W. and FACKLAM, R.R. (1976) Nosocomial transmission of group B streptococci. Pediatrics, 58, 346-353.

ABLOW, R.C., DRISCOLL, S.G., EFFMANN, E.L., GROSS, I., JOLLES, C.J., UAUY, R. and WARSHAW, J.B. (1976) A comparison of early-onset group B streptococcal neonatal infection and the respiratory-distress syndrome of the newborn. New Engl. J. Med., 294, 63-70.

ADLAM, C., BROUGHTON, E.S. and SCOTT, M.T. (1972) Enhanced resistance of mice to infection with bacteria following pretreatment with Corynebacterium parvum. Nature New Biol., 235, 219-220.

AGRAWAL, B.B.L. and GOLDSTEIN, I.J. (1968) Protein-carbohydrate interaction. VII Physical and chemical studies on concanavalin A, the hemagglutinin of the jack bean. Arch. Biochem. Biophys., 124, 218-229.

ALKAN, M.L. and BEACHEY, E.H. (1978) Excretion of lipoteichoic acid by group A streptococci: influence of penicillin on excretion and loss of ability to adhere to human oral mucosal cells. J. clin. Invest., 61, 671-677.

ALLEN, A.K., NEUBERGER, A. and SHARON, N. (1973) The purification, composition and specificity of wheat-germ agglutinin. Biochem. J., 131, 155-162.

ALLEN, J.L. and SPRUNT, M.D. (1978) Discrepancy between minimum inhibitory and minimum bactericidal concentrations of penicillin for group A and group B β-haemolytic streptococci. J. Pediat., 93, 69-71.

ANDERSON,/...

ANDERSON, M.J., WHITEHEAD, J.S. and KIM, Y.S. (1980) Interaction of Escherichia coli K88 antigen with porcine intestinal brush border membranes. Infect. Immun., 29, 897-901.

ANDERSSON, B., ERIKSSON, B., FALSEN, E., FOGH, A., HANSON, L.Å., NYLÉN, O., PETERSON, H. and SVANBORG-EDÉN, C. (1981) Adhesion of Streptococcus pneumoniae to human pharyngeal epithelial cells in vitro: differences in adhesive capacity among strains isolated from subjects with otitis media, septicaemia or meningitis or from healthy carriers. Infect. Immun., 32, 311-317.

ANTHONY, B.F. and CONCEPCION, N.F. (1975) Group B streptococcus in a general hospital. J. infect. Dis., 132, 561-567.

ANTHONY, B.F. and OKADA, D.M. (1977) The emergence of group B streptococci in infections of the newborn infant. Annu. Rev. Med., 28, 355-369.

ANTHONY, B.F., OKADA, D.M. and HOBEL, C.J. (1978) Epidemiology of group B streptococcus: longitudinal observations during pregnancy. J. infect. Dis., 137, 524-530.

ARONSON, M., MEDALIA, O., SCHORI, L., MIRELMAN, D., SHARON, N. and OFEK, I (1979) Prevention of colonisation of the urinary tract of mice with Escherichia coli by blocking of bacterial adherence with methyl α-D-mannopyranoside. J. infect. Dis., 139, 329-332.

ASH, S.G. (1979) Adhesion of microorganisms in fermentation processes. In: Ellwood, D.C., Melling, J. and Rutter, P. (eds.), Adhesion of Microorganisms to Surfaces, pp. 57-86. London: Academic Press.

ASHERSON,/...

ASHERSON, G.L. and ALLWOOD, G.G. (1971) Depression of delayed hypersensitivity by pretreatment with freund-type adjuvants. 1. Description of the phenomenon. Clin. exp. Immunol., 9, 249-258.

ATKINSON, H.M. and TRUST, T.J. (1980) Haemagglutination properties and adherence ability of Aeromonas hydrophila. Infect. Immun., 27, 938-946.

AXELSEN, N.H. (1973) Intermediate gel in crossed and in fused rocket immunoelectrophoresis. In: Axelsen, N.H., Krøll, J. and Weeke, B. (eds.), A Manual of Quantitative Immunoelectrophoresis, Methods and Application. Scand. J. Immunol., 2, No. 1, pp. 71-77.

BAGG, J., POXTON, I.R. and WEIR, D.M. (1981) The isolation of a lectin-like molecule from Corynebacterium parvum (NCTC 10390). J. Clin. Lab. Immunol., 6, 165-168.

BAIER, R.E., SHAFRIN, E.G. and ZISMAN, W.A. (1968) Adhesion: mechanisms that assist or impede it. Science, 162, 1360-1368.

BAKER, C.J. (1977) Summary of the workshop on perinatal infections due to group B streptococcus. J. infect. Dis., 136, 137-152.

BAKER, C.J. (1980) Group B streptococcal infections. Adv. int. Med., 25, 475-501.

BAKER, C.J., BARRETT, F.F., GORDON, R.C. and YOW, M.D. (1973) Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. J. Pediat., 82, 724-729.

BAKER, C.J., GOROFF, D.K., ALPERT, S., CROCKETT, V.A., ZINNER, S.H., EVRARD, J.R., ROSNER, B. and McCORMACK, W.M. (1977) Vaginal/...

Vaginal colonisation with group B streptococcus: a study in college women. J. infect. Dis., 135, 392-397.

BAKER, C.J. and KASPER, D.L. (1976) Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. New Engl. J. Med., 294, 753-756.

BAKER, C.J., KASPER, D.L., TAGER, I.B., PAREDES, A., ALPERT, S., McCORMACK, W.M. and GOROFF, D. (1977) Quantitative determination of antibody to capsular polysaccharide in infection with type III strains of group B streptococcus. J. clin. Invest., 59, 810-818.

BAKER, C.J., WEBB, B.J. and BARRETT, F.F. (1976) Antimicrobial susceptibility of group B streptococci isolated from a variety of clinical sources. Antimicrob. Agents Chemother., 10, 128-131.

BAR-SHAVIT, Z., GOLDMAN, R., OFEK, I., SHARON, N. and MIRELMAN, D. (1980) Mannose-binding activity of Escherichia coli: a determinant of attachment and ingestion of the bacteria by macrophages. Infect. Immun., 29, 417-424.

BAR-SHAVIT, Z., OFEK, I., GOLDMAN, R., MIRELMAN, D. and SHARON, N. (1977) Mannose residues on phagocytes as receptors for the attachment of Escherichia coli and Salmonella typhi. Biochem. biophys. Res. Commun., 78, 455-460.

BARTON, L.L., FEIGIN, R.D. and LINS, R. (1973) Group B β hemolytic streptococcal meningitis in infants. J. Pediat., 82, 719-723.

BAYER, A.S., CHOW, A.W., ANTHONY, B.F. and GUZE, L.B. (1976) Serious infections in adults due to group B streptococci. Clinical and serotypic characterisation. Am. J. Med., 61, 498-503.

BEACHEY,/...

BEACHEY, E.H. (1975) Binding of group A streptococci to human oral mucosal cells by lipoteichoic acid. Trans. Ass. Am. Physns., 88, 285-292.

BEACHEY, E.H. (1981) Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. infect. Dis., 143, 325-345.

BEACHEY, E.H., CHIANG, T.M., OFEK, I. and KANG, A.H. (1977) Interaction of lipoteichoic acid of group A streptococci with human platelets. Infect. Immun., 16, 649-654.

BEACHEY, E.H., DALE, J., GREBE, S., AHMED, A., SIMPSON, W.A. and OFEK, I. (1979a) Lymphocyte binding and T-cell mitogenic properties of group A streptococcal lipoteichoic acid. J. Immun., 122, 189-195.

BEACHEY, E.H., DALE, J.B., SIMPSON, W.A., EVANS, J.D., KNOX, K.S., OFEK, I. and WICKEN, A.J. (1979b) Erythrocyte binding properties of streptococcal lipoteichoic acids. Infect. Immun., 23, 618-625.

BEACHEY, E.H., EISENSTEIN, B.I. and OFEK, I. (1981) Sublethal concentrations of antibiotics and bacterial adhesion. In: Elliott, K., O'Connor, M. and Whelan, J. (eds.), Adhesion and Microorganism Pathogenicity, pp. 288-305. London: Pitman Medical Ltd.

BEACHEY, E.H. and OFEK, I. (1976) Epithelial cell binding of group A streptococci by lipoteichoic acid on fimbriae denuded of M protein. J. exp. Med., 143, 759-771.

BERNARD,/...

BERNARD, J.-P., FRANCIOLI, P. and GLAUSER, M.P. (1981) Vancomycin prophylaxis of experimental Streptococcus sanguis. Inhibition of bacterial adherence rather than bacterial killing. J. clin. Invest., 68, 1113-1116.

BHATTACHARJEE, J.W. and SRIVASTAVA, B.S. (1978) Mannose-sensitive haemagglutinins in adherence of Vibrio cholerae eltor to intestine. J. gen. Microbiol., 107, 407-410.

BLUMENSTOCK, E. and JANN, K. (1982) Adhesion of piliated Escherichia coli strains to phagocytes: differences between bacteria with mannose-sensitive pili and those with mannose-resistant pili. Infect. Immun., 35, 264-269.

BOMFORD, R. and CHRISTIE, G.H. (1975) Mechanisms of macrophage activation by Corynebacterium parvum. II. In vivo experiments. Cellular Immunol., 17, 150-155.

BOTTA, G.A. (1979) Hormonal and type-dependent adhesion of group B streptococci to human vaginal cells. Infect. Immun., 25, 1084-1086.

BOTTA, G.A. (1981) Surface components in adhesion of group A streptococci to pharyngeal epithelial cells. Current Microbiol., 6, 101-104.

BRECHER, S.M., VAN HOUTE, J. and HAMMOND, B.F. (1978) Role of colonisation in the virulence of Actinomyces viscosus strains T 14-Vi and T 14-Av. Infect. Immun., 22, 603-614.

BRINTON, C.C. (1965) The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in Gram-negative bacteria. Trans. N.Y. Acad. Sci., 27, 1003-1054.

BRINTON,/...

BRINTON, C.C., BRYAN, J., DILLON, J.-A., GUERINA, N., JACOBSON, L.J., LABIK, A., LEE, S., LEVINE, A., LIM, S., McMICHAEL, J., POLEN, S., ROGERS, K., TO, A.C.-C. and TO, S.C.-M. (1978) Uses of pili in gonorrhea control: role of bacterial pili in disease, purification and properties of gonococcal pili, and progress in the development of a gonococcal pilus vaccine for gonorrhea. In: Brooks, G.F., Gotschlich, E.C., Holmes, K.K., Sawyer, W.D. and Young, F.E. (eds.), Immunobiology of Neisseria gonorrhoeae, pp. 155-178. Washington: American Society for Microbiology.

BROOKS, D.E., MILLAR, J.S., SEAMAN, G.V.F. and VASSAR, P.S. (1967) Some physico-chemical factors relevant to cellular interaction. J. cellular Physiol., 69, 155-168.

BROUGHTON, D.D., MITCHELL, W.G., GROSSMAN, M., HADLEY, W.K. and COHEN, M.S. (1976) Recurrence of group B streptococcal infection. J. Pediat., 89, 183-185.

BROWN, J.H. (1920) The cultural differentiation of beta haemolytic streptococci of human and bovine origin. J. exp. Med., 31, 35-47.

BUCHANAN, R.E. and GIBBONS, N.E. (eds.) (1974) In: Bergey's Manual of Determinative Bacteriology, p. 340. Baltimore: Williams and Wilkins.

BUCHANAN, T.M., PEARCE, W.A. and CHEN, K.C.S. (1978) Attachment of Neisseria gonorrhoeae pili to human cells, and investigations of the chemical nature of the receptor for gonococcal pili. In: Brooks, G.F., Gotschlich, E.C., Holmes, K.K., Sawyer, W.D. and Young, F.E. (eds.), Immunobiology of Neisseria gonorrhoeae, pp. 242-249. Washington: American Society for Microbiology.

BURGER,/...

BURGER, M.M. and GOLDBERG, A.R. (1967) Identification of a tumour-specific determinant on neoplastic cell surfaces. Proc. natn. Acad. Sci. U.S.A., 57, 359-366.

BURROWS, M.R., SELLWOOD, R. and GIBBONS, R.A. (1976) Haemagglutinating and adhesive properties associated with the K99 antigen of bovine strains of Escherichia coli. J. gen. Microbiol., 96, 269-275.

CHARACKLIS, W.G. (1981) Microbial fouling: a process analysis. In: Knudsen, J.G. and Somerscales, E.F.C. (eds.), Proceedings of the International Conference on the Fouling of Heat Transfer Equipment, pp. 251-293. Washington: Hemisphere Publishing Corporation.

CHEN, P.S., TORIBARA, T.Y. and WARNER, H. (1956) Micro-determination of phosphorus. Analyt. Chem., 28, 1756-1758.

CHIANG, T.M., ALKAN, M.L. and BEACHEY, E.H. (1979) Binding of lipoteichoic acid of group A streptococci to isolated human erythrocyte membranes. Infect. Immun., 26, 316-321.

CHITNIS, D.S., SHARMA, K.D. and KAMAT, R.S. (1982a) Role of bacterial adhesion in the pathogenesis of cholera. J. med. Microbiol., 15, 43-51.

CHITNIS, D.S., SHARMA, K.D. and KAMAT, R.S. (1982b) Role of somatic antigen of Vibrio cholerae in adhesion to intestinal mucosa. J. med. Microbiol., 15, 53-61.

CHRISTIE, G.H. and BOMFORD, R. (1975) Mechanisms of macrophage activation by Corynebacterium parvum. 1. In vitro experiments. Cellular Immunol., 17, 141-149.

CHRISTIE,/...

CHRISTIE, R., ATKINS, N.E. and MUNCH-PETERSEN, E. (1944) A note on a lytic phenomenon shown by group B streptococci. Aust. J. exp. Biol. med. Sci., 22, 197-200.

CISAR, J.O., BARSUMIAN, E.L., CURL, S.H., VATTER, A.E., SANDBERG, A.L. and SIRAGANIAN, R.P. (1980) The use of monoclonal antibodies in the study of lactose-sensitive adherence of Actinomyces viscosus T 14V.. J. Reticuloendothelial Soc., 28, Supplement, 73s-79s.

CISAR, J.O. and VATTER, A.E. (1979) Surface fibrils (fimbriae) of Actinomyces viscosus T 14V.. Infect. Immun., 24, 523-531.

CISAR, J.O., VATTER, A.E. and McINTIRE, F.C. (1978) Identification of the virulence-associated antigen on the surface fibrils of Actinomyces viscosus T 14. Infect. Immun., 19, 312-319.

CLARK, W.B., BAMMAN, L.L. and GIBBONS, R.J. (1978a) Comparative estimates of bacterial affinities and adsorption sites on hydroxyapatite surfaces. Infect. Immun., 19, 846-853.

CLARK, W.B., BAMMANN, L.L. and GIBBONS, R.J. (1978b) Ability of Streptococcus mutans and a glucosyltransferase-defective mutant to colonize rodents and attach to hydroxyapatite surfaces. Infect. Immun., 21, 681-684.

CLARK, W.B. and GIBBONS, R.J. (1977) Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of Streptococcus mutans 6715 to hydroxyapatite surfaces. Infect. Immun., 18, 514-523.

CLARK, W.B., WEBB, E.L., WHEELER, T.T., FISCHLSCHWEIGER, W., BIRDSELL, D.C. and MANSHEIM, B.J. (1981) Role of surface fimbriae (fibrils)/...

(fibrils) in the adsorption of Actinomyces species to saliva-treated hydroxyapatite surfaces. Infect. Immun., 33, 908-917.

COLEY, J., DUCKWORTH, M. and BADDILEY, J. (1975) Extraction and purification of lipoteichoic acids from Gram-positive bacteria. Carbohydr. Res., 40, 41-52.

COLLIER, W.A. and DE MIRANDA, J.C. (1955) Bacterien-hemagglutination. I. Versuche mit einem hemagglutinerenden Stamm von E. coli. Antonie van Leeuwenhoek J. Microbiol. Serol., 21, 133-140.

COLLINS, J.C. and KNIGHT, C.A. (1978) Purification of the influenza hemagglutinin glycoprotein and characterisation of its carbohydrate components. J. Virol., 26, 457-467.

COSTELLO, A.H., CISAR, J.O., KOLENBRANDER, P.E. and GABRIEL, O. (1979) Neuraminidase-dependent haemagglutination of human erythrocytes by strains of Actinomyces. J. Dent. Res., (Special Issue A). Abstract No. 1000, p. 342.

DAZZO, F.B. (1980) Microbial adhesion to plant surfaces. In: Berkeley, R.C.W., Lynch, J.M., Melling, J., Rutter, P.R. and Vincent, B. (eds.), Microbial Adhesion to Surfaces, pp. 311-328. Chichester: Ellis Horwood Ltd.

DE CUENINCK, B.J. (1979) Surface protein of a Streptococcus agalactiae isolate. Infect. Immun., 24, 637-641.

DERJAGUIN, B.V. and LANDAU, L. (1941) Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. Acta physicochim., U.R.S.S. 14, 633-662.

DIXON,/...

DIXON, H.B.F. (1981) Defining a lectin. Nature, 292, 192.

DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A. and SMITH, F. (1956) Colorimetric method for the determination of sugars and related substances. Analyt. Chem., 28, 350-356.

DUGUID, J.P. (1946) The sensitivity of bacteria to the action of penicillin. Edinburgh Medical Journal, 53, 401-412.

DUGUID, J.P. (1959) Fimbriae and adhesive properties in Klebsiella strains. J. gen. Microbiol., 21, 271-286.

DUGUID, J.P., ANDERSON, E.S. and CAMPBELL, I. (1966) Fimbriae and adhesive properties in salmonellae. J. Path. Bact., 92, 107-138.

DUGUID, J.P., CLEGG, S. and WILSON, M.I. (1979) The fimbrial and non-fimbrial haemagglutinins of Escherichia coli. J. med. Microbiol., 12, 213-227.

DUGUID, J.P. and GILLIES, R.R. (1957) Fimbriae and adhesive properties in dysentery bacilli. J. Path. Bact., 74, 397-411.

DUGUID, J.P. and GILLIES, R.R. (1958) Fimbriae and haemagglutinating activity in Salmonella, Klebsiella, Proteus and Chromobacterium. J. Path. Bact., 75, 519-520.

DUGUID, J.P., SMITH, I.W., DEMPSTER, G. and EDMUNDS, P.N. (1955) Non-flagellar filamentous appendages ('fimbriae') and haemagglutinating activity in Bacterium coli. J. Path. Bact., 70, 335-348.

DUMA, R.J., WEINBERG, A.N., MEDREK, T.F. and KUNZ, L.J. (1969) Streptococcal infections: a bacteriologic and clinical study of streptococcal bacteraemia. Medicine, 48, 87-127.

DUPONT,/...

DUPONT, H.L., FORMAL, S.B., HORNICK, R.B., SNYDER, M.J., LIBONATI, J.P., SHEAHAN, D.G., LABREC, E.H. and KALAS, J.P. (1971) Pathogenesis of Escherichia coli diarrhoea. New Engl. J. Med., 285, 1-9.

EBISU, S., YAMAZAKI, Y. and OKADA, H. (1979) Abstract no. 10. J. Jpn. Assoc. Periodontol.

EICKHOFF, T.C., KLEIN, J.O., DALY, A.K., INGAL, D. and FINLAND, M. (1964) Neonatal sepsis and other infections due to group B beta-hemolytic streptococci. New Engl. J. Med., 271, 1221-1228.

EISENSTEIN, B.I., BEACHEY, E.H. and OFEK, I. (1980) Influence of sublethal concentrations of antibiotics on the expression of the mannose-specific ligand of Escherichia coli. Infect. Immun., 28, 154-159.

EISENSTEIN, B.I., OFEK, I. and BEACHEY, E.H. (1979) Interference with the mannose binding and epithelial cell adherence of Escherichia coli by sublethal concentrations of streptomycin. J. clin. Invest., 63, 1219-1228.

EISENSTEIN, B.I., OFEK, I. and BEACHEY, E.H. (1981) Loss of lectin-like activity in aberrant type I fimbriae of Escherichia coli. Infect. Immun., 31, 792-797.

ELBEIN, A.D., SANFORD, B.A., RAMSAY, M.A. and PAN, Y.T. (1981) Effect of inhibitors on glycoprotein biosynthesis and bacterial adhesion. In: Elliot, K., O'Connor, M. and Whelan, J. (eds.), Adhesion and Microorganism Pathogenicity, pp. 270-287. London: Pitman Medical Ltd.

ELLEN,/...

ELLEN, R.P., FILLERY, E.D., CHAN, K.H. and GROVE, D.A. (1980) Sialidase-enhanced lectin-like mechanism for Actinomyces viscosus and Actinomyces naeslundii haemagglutination. Infect. Immun., 27, 335-343.

ELLEN, R.P. and GIBBONS, R.J. (1972) M protein-associated adherence of Streptococcus pyogenes to epithelial surfaces: pre-requisite for virulence. Infect. Immun., 5, 826-830.

ELLEN, R.P., WALKER, D.L. and CHAN, K.H. (1978) Association of long surface appendages with adherence-related functions of the Gram-positive species Actinomyces naeslundii. J. Bact., 134, 1171-1175.

ENTLICHER, G., KOŠTÍŘ, J.V. and KOCOUREK, J. (1970) Isolation and characterisation of hemagglutinins from the pea (Pisum sativum L). Biochim. biophys. Acta, 221, 272-281.

ESHDAT, Y., OFEK, I., YASHOUV-GAN, Y., SHARON, N. and MIRELMAN, D. (1978) Isolation of a mannose-specific lectin from Escherichia coli, and its role in the adherence of the bacteria to epithelial cells. Biochem. biophys. Res. Commun., 85, 1551-1559.

EVANS, D.G. and EVANS, Jr., D.J. (1978) New surface-associated heat-labile colonisation factor antigen (CFA/II) produced by enterotoxigenic Escherichia coli of serogroups O6 and O8. Infect. Immun., 21, 638-647.

EVANS, D.G., EVANS, Jr., D.J. and TJOA, W. (1977) Haemagglutination of human group A erythrocytes by enterotoxigenic Escherichia coli isolated from adults with diarrhoea: correlation with colonisation factor. Infect. Immun., 18, 330-337.

EVANS,/...

EVANS, D.G., EVANS, Jr., D.J., TJOA, W.S. and DUPONT, H.L. (1978) Detection and characterisation of colonisation factor of enterotoxigenic Escherichia coli isolated from adults with diarrhoea. Infect. Immun., 19, 727-736.

EVANS, D.G., SILVER, R.P., EVANS, Jr., D.J., CHASE, D.G. and GORBACH, S.L. (1975) Plasmid-controlled colonisation factor associated with virulence in Escherichia coli enterotoxigenic for humans. Infect. Immun., 12, 656-667.

FACKLAM, R.R., PADULA, J.F., THACKER, L.G., WORTHAM, E.C. and SCONYERS, B.J. (1974) Presumptive identification of groups A, B and D streptococci. Appl. Microbiol., 27, 107-113.

FALKLER, Jr., W.A. and BURGER, B.W. (1981) Microbial surface interactions: reduction of the haemagglutination activity of the oral bacterium Fusobacterium nucleatum by absorption with Streptococcus and Bacteroides. Arch. oral. Biol., 26, 1015-1025.

FALKLER, Jr., W.A. and HAWLEY, C.E. (1977) Haemagglutinating activity of Fusobacterium nucleatum. Infect. Immun., 15, 230-238.

FELDMAN, W.E. (1976) Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. J. Pediat., 88, 549-552.

FITZGERALD, R.J., KEYES, P.H., STOUDT, T.H. and SPINELL, D.M. (1968) The effects of a dextranase preparation on plaque and caries in hamsters, a preliminary report. J. Am. dent. Ass., 76, 301-304.

FLETCHER,/...

FLETCHER, M., LATHAM, M.J., LYNCH, J.M. and RUTTER, P.R. (1980) The characteristics of interfaces and their role in microbial attachment. In: Berkeley, R.C.W., Lynch, J.M., Melling, J., Rutter, P.R. and Vincent, B. (eds.), Microbial Adhesion to Surfaces, pp. 67-78. Chichester: Ellis Horwood Ltd.

FLETCHER, M. and LOEB, G.I. (1979) Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. Appl. environ. Microbiol., 37, 67-72.

FOWLER, J.E. and STAMEY, T.A. (1977) Studies of introital colonization in women with recurrent urinary infections. VII. The role of bacterial adherence. J. Urol., 117, 472-476.

FRANCIOSI, R.A., KNOSTMAN, J.D. and ZIMMERMAN, R.A. (1973) Group B streptococcal neonatal and infant infections. J. Pediat., 82, 707-718.

FRETER, R. (1981) Mechanisms of association of bacteria with mucosal surfaces. In: Elliott, K., O'Connor, M. and Whelan, J. (eds.), Adhesion and Microorganism Pathogenicity, pp. 36-55. London: Pitman Medical Ltd.

FRETER, R. and JONES, G.W. (1976) Adhesive properties of Vibrio cholerae: nature of the interaction with intact mucosal surfaces. Infect. Immun., 14, 246-256.

FRETER, R., O'BRIEN, P.C.M. and HALSTEAD, S.A. (1978) Adhesion and chemotaxis as determinants of bacterial association with mucosal surfaces. Adv. exp. Med., 107, 429-437.

FRETER,/...

FRETER, R., O'BRIEN, P.C.M. and MACSAI, M.S. (1979) Effect of chemotaxis in the interaction of cholera vibrios with intestinal mucosa. J. clin. Nutr., 32, 128-132.

FRY, R.M. (1938) Fatal infections by haemolytic streptococcus group B. Lancet, 1, 199-201.

GALBRAITH, W. and GOLDSTEIN, I.J. (1970) Phytohemagglutinins: a new class of metalloproteins. Isolation, purification and some properties of the lectin from Phaseolus lunatus. FEBS Lett., 9, 197-201.

GARDNER, S.E., YOW, M.D., LEEDS, T.J., THOMPSON, P.K., MASON, E.O. and CLARK, D.J. (1979) Failure of penicillin to eradicate group B streptococcal colonisation in the pregnant woman. A couple study. Am. J. Obstet. Gynecol., 135, 1062-1065.

GERMAINE, G.R. and SCHACHTELE, C.F. (1976) Streptococcus mutans dextransucrase: mode of interaction with high-molecular-weight dextran and role in cellular aggregation. Infect. Immun., 13, 365-372.

GIBBONS, R.J. and FITZGERALD, R.J. (1969) Dextran-induced agglutination of Streptococcus mutans, and its potential role in the formation of microbial dental plaques. J. Bact., 98, 341-346.

GIBBONS, R.J. and QURESHI, J.V. (1978) Selective binding of blood group-reactive mucins by Streptococcus mutans and other oral organisms. Infect. Immun., 22, 665-671.

GIBBONS, R.J. and QURESHI, J.V. (1979) Inhibition of adsorption of Streptococcus mutans strains to saliva-treated hydroxy-apatite by galactose and certain amines. Infect. Immun., 26, 1214-1217.

GIBBONS,/...

GIBBONS, R.J. and VAN HOUTE, J. (1971) Selective bacterial adherence to oral epithelial surfaces, and its role as an ecological determinant. Infect. Immun., 3, 567-573.

GIBBONS, R.J. and VAN HOUTE, J. (1973) On the formation of dental plaques. J. Periodontol., 44, 347-360.

GILBOA-GARBER, N. (1972) Inhibition of broad spectrum haemagglutinin from Pseudomonas aeruginosa by D-galactose and its derivatives. FEBS Lett., 20, 242-244.

GILBOA-GARBER, N., MIZRAHI, L. and GARBER, N. (1972) Purification of the galactose-binding haemagglutinin of Pseudomonas aeruginosa by affinity column chromatography, using Sepharose. FEBS Lett., 28, 93-95.

GILBOA-GARBER, N., MIZRAHI, L. and GARBER, N. (1977) Mannose-binding haemagglutinins in extracts of Pseudomonas aeruginosa. Can. J. Biochem., 55, 975-981.

GIRARD, A.E. and JACIUS, B.H. (1974) Ultrastructure of Actinomyces viscosus and Actinomyces naeslundii. Arch. oral. Biol., 19, 71-79.

GLAZER, A.N. and SMITH, E.L. (1961) Studies on the ultraviolet difference spectra of proteins and polypeptides. J. biol. Chem., 236, 2942-2947.

GOLDSTEIN, I.J. and HAYES, C.E. (1978) The lectins: carbohydrate binding proteins of plants and animals. Adv. Carbohydr. Chem. & Biochem., 35, 127-340.

GOLDSTEIN, I.J., HOLLERMAN, C.E. and SMITH, E.E. (1965) Protein-carbohydrate interaction. II. Inhibition studies on the interaction of concanavalin A with polysaccharides. Biochemistry, 4, 876-883.

GOLDSTEIN,/...

GOLDSTEIN, I.J., HUGHES, R.C., MONSIGNY, M., OSAWA, T. and SHARON, N. (1980) What should be called a lectin? Nature, 285, 66.

GORDON, J.S. and SBARRA, A.J. (1976) Incidence, technique of isolation, and treatment of group B streptococci in obstetric patients. Am. J. Obstet. Gynecol., 126, 1023-1026.

GOULD, J.C. (1975) Tests for sensitivity to antimicrobial agents. In: Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (eds.), Medical Microbiology, 12th Edn., Vol. 2, pp. 190-208. Edinburgh, London and New York: Churchill Livingstone.

GRABOVSKAYA, K.B., TOTOLJAN, A.A., RÝC, M., HAVLÍČEK, J., BUROVA, L.A. and BÍCOVÁ, R. (1980) Adherence of group A streptococci to epithelial cells in tissue culture. Zbl. Bakt. Hyg., I. Abt. Orig. A, 247, 303-314.

GROSS, R.J., CRAVISTO, A., SCOTLAND, S.M., CHEASTY, T. and ROWE, B. (1978) The occurrence of colonisation factor (CF) in enterotoxigenic Escherichia coli. FEMS Lett., 3, 231-233.

GUGGENHEIM, B. (1970) Extracellular polysaccharides and microbial plaque. Int. dent. J., 20, 657-678.

GUGGENHEIM, B., REGOLATI, B. and MÜHLEMANN, H.R. (1972) Caries and plaque inhibition by mutanase in rats. Caries Res., 6, 289-297.

GUYOT, G. (1908) Über die bakterielle Hämagglutination. Zbl. Bakt. I. Abt. Orig., 47, 640-653.

HACKNEY, C.R., KLEEMAN, E.G., RAY, B. and SPECK, M.L. (1980) Adherence as a method for differentiating virulent and avirulent strains of Vibrio parahaemolyticus. Appl. environ. Microbiol., 40, 652-658.

HALL,/...

HALL, R.T., BARNES, W., KRISHNAN, L., HARRIS, D.J., RHODES, P.G., FAYEZ, J. and MILLER, G.L. (1976) Antibiotic treatment of parturient women colonised with group B streptococci. Am. J. Obstet. Gynecol., 124, 630-634.

HALPERN, B.N., BIOZZI, G., STIFFEL, C. and MOUTON, D. (1966) Inhibition of tumour growth by administration of killed Corynebacterium parvum. Nature, 212, 853-854.

HALPERN, B.N., PRÉVOT, A.-R., BIOZZI, G., STIFFEL, C., MOUTON, D., MORARD, J.C., BOUTHILLIER, Y. and DECREUSEFOND, C. (1964) Stimulation de l'activité phagocytaire du système reticulo-endothélial provoquée par Corynebacterium parvum. J. Reticuloendothelial Soc., 1, 77-96.

HAMMARSTRÖM, S. and KABAT, E.A. (1969) Purification and characterisation of a blood-group A reactive hemagglutinin from the snail Helix pomatia and a study of its combining site. Biochemistry, 8, 2696-2705.

HART, D.A. (1980) Lectins in biological systems: applications to microbiology. Am. J. clin. Nutr., 33, 2416-2425.

HEALY, T.W., HOMOLA, A., JAMES, R.O. and HUNTER, R.T. (1978) Coaggulation of amphoteric latex colloids, reversibility and specific ion effects. Disc. Faraday Soc., 65, 156-163.

HOMOLA, A. and JAMES, R.O. (1977) Preparation and characterisation of amphoteric polystyrene latexes. J. Colloid and Interface Sci., 59, 123-134.

HONDA, E. and YANAGAWA, R. (1974) Agglutination of trypsinised sheep erythrocytes by the pili of Corynebacterium renale. Infect. Immun., 10, 1426-1432

HORN,/...

HORN, K.A., ZIMMERMAN, R.A., KNOSTMAN, J.D. and MEYER, W.T. (1974) Neurological sequelae of group B streptococcal neonatal infection. Pediatrics, 53, 501-504.

HULL, R.A., GILL, R.E., HSU, P., MINSHEW, B.H. and FALKOW, S. (1981) Construction and expression of recombinant plasmids encoding type I or D-mannose-resistant pili from a urinary tract infection Escherichia coli isolate. Infect. Immun., 33, 933-938.

ISAACSON, R.E., FUSCO, P.C., BRINTON, C.C. and MOON, H.W. (1978) In vitro adhesion of Escherichia coli to porcine small intestinal epithelial cells: pili as adhesive factors. Infect. Immun., 21, 392-397.

ISAACSON, R.E. and RICHTER, P. (1981) Escherichia coli 987P pilus: purification and partial characterisation. J. Bact., 146, 784-789.

JAMES, A.M. (1979) Molecular aspects of biological surfaces. Chem. Soc. Rev., 8, 389-418.

JAMES, A.N., KNOX, J.M. and WILLIAMS, R.P. (1976) Attachment of gonococci to sperm. Influence of physical and chemical factors. Br. J. vener. Dis., 52, 128-135.

JAMES-HOLMQUEST, A.N., SWANSON, J., BUCHANAN, T.M., WENDE, R.D. and WILLIAMS, R.P. (1974) Differential attachment by piliated and non-piliated Neisseria gonorrhoeae to human sperm. Infect. Immun., 9, 897-902.

JOHNSON, A.P., TAYLOR-ROBINSON, D. and McGEE, Z.A. (1977) Species specificity of attachment and damage to oviduct mucosa by Neisseria gonorrhoeae. Infect. Immun., 18, 833-839.

JOHNSON,/...

JOHNSON, J.L. and CUMMINS, C.S. (1972) Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical propionibacteria and strains of Arachnia propionica. J. Bact., 109, 1047-1066.

JONES, G.W., ABRAMS, G.D. and FRETER, R. (1976) Adhesive properties of Vibrio cholerae: adhesion to isolated rabbit brush border membranes and haemagglutinating activity. Infect. Immun., 14, 232-239.

JONES, G.W. and FRETER, R. (1976) Adhesive properties of Vibrio cholerae: nature of the interaction with isolated rabbit brush border membranes and human erythrocytes. Infect. Immun., 14, 240-245.

JONES, G.W., RICHARDSON, L.A. and VANDEN BOSCH, J.L. (1980) Phases in the interaction between bacteria and animal cells. In: Berkeley, R.C.W., Lynch, J.M., Melling, J., Rutter, P.R. and Vincent, B. (eds.), Microbial Adhesion to Surfaces, pp. 211-219. Chichester: Ellis Horwood Ltd.

KALB, A.J. and LUSTIG, A. (1968) The molecular weight of concanavalin A. Biochim. biophys. Acta, 168, 366-367.

KÄLLENIUS, G., SVENSON, S.B., MÖLLBY, R., CEDERGREN, B., HULTBERG, H. and WINBERG, J. (1981) Structure of carbohydrate part of receptor on human uroepithelial cells for pyelonephritogenic Escherichia coli. Lancet, 2, 604-606.

KASPER, D.L., BAKER, C.J., BALTIMORE, R.S., CRABB, J.H., SCHIFFMAN, G. and JENNINGS, H.J. (1979) Immunodeterminant specificity of human immunity to type III group B streptococci. J. exp. Med., 149, 327-339.

KELLOGG,/...

KELLOGG, Jr., D.S., COHEN, I.R., NORINS, L.C., SCHROETER, A.L. and REISING, G. (1968) Neisseria gonorrhoeae. II. Colonial variation and pathogenicity during 35 months in vitro. J. Bact., 96, 596-605.

KELLOGG, Jr., D.S., PEACOCK, Jr., W.L., DEACON, W.E., BROWN, L. and PIRKLE, C.I. (1963) Neisseria gonorrhoeae. I. Virulence genetically linked to clonal variation. J. Bact., 85, 1274-1279.

KELSTRUP, J. and FUNDER-NIELSEN, T.D. (1974) Adhesion of dextran to Streptococcus mutans. J. gen. Microbiol., 81, 485-489.

KINANE, D.F., BLACKWELL, C.C., WEIR, D.M. and WINSTANLEY, F.P. (1982) Phagocyte recognition of Neisseria gonorrhoeae. J. Clin. Lab. Immunol., (in press).

KNOTT, L.J., ÖGMUNDSDÓTTIR, H.M. and WEIR, D.M. (Unpublished)

KOCOUREK, J. and HOŘEJŠÍ, V. (1981) Defining a lectin. Nature, 290, 188.

KOLENBRANDER, P.E. and WILLIAMS, B.L. (1981) Lactose-reversible coaggregation between oral actinomycetes and Streptococcus sanguis. Infect. Immun., 33, 95-102.

KORHONEN, T.K. (1979) Yeast cell agglutination by purified enterobacterial pili. FEMS Lett., 6, 421-425.

KORHONEN, T.K., EDÉN, S. and SVANBORG-EDÉN, C. (1980a) Binding of purified Escherichia coli pili to human urinary tract epithelial cells. FEMS Lett., 7, 237-241.

KORHONEN, T.K., LOUNATMAA, K., RANTA, H. and KUUSI, N. (1980c) Characterization of type I pili of Salmonella typhimurium LT2. J. Bact., 144, 800-805.

KORHONEN,/...

KORHONEN, T.K., NURMIAHO, E.-L., RANTA, H. and SVANBORG-EDÉN, C. (1980b) New method for isolation of immunologically pure pili from Escherichia coli. Infect. Immun., 27, 569-575.

LAEMMLI, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

LAMBDEN, P.R., HECKELS, J.E., JAMES, L.T. and WATT, P.J. (1979) Variations in surface protein composition associated with virulence properties in opacity types of Neisseria gonorrhoeae. J. gen. Microbiol., 114, 305-312.

LAMBDEN, P.R., ROBERTSON, J.N. and WATT, P.J. (1980) Biological properties of two distinct pilus types produced by isogenic variants of Neisseria gonorrhoeae P9. J. Bact., 141, 393-396.

LANCEFIELD, R.C. (1934) A serological differentiation of specific types of bovine hemolytic streptococci (group B). J. exp. Med., 59, 441-458.

LANCEFIELD, R.C. (1938) Two serological types of group B hemolytic streptococci with related, but not identical, type-specific substances. J. exp. Med., 67, 25-40.

LANCEFIELD, R.C., McCARTY, M. and EVERLY, W.N. (1975) Multiple mouse-protective antibodies directed against group B streptococci. Special reference to antibodies effective against protein antigens. J. exp. Med., 142, 165-179.

LEFFLER, H. and SVANBORG-EDÉN, C. (1980) Chemical identification of a glycosphingolipid receptor for Escherichia coli attaching to/...

to human urinary tract epithelial cells and agglutinating human erythrocytes. FEMS Lett., 8, 127-134.

LERNER, P.I., GOPALAKRISHNA, K.V., WOLINSKY, E., McHENRY, M.C., TAN, J.S. and ROSENTHAL, M. (1977) Group B streptococcus (S. agalactiae) bacteraemia in adults: analysis of 32 cases and review of the literature. Medicine, 56, 457-473.

LEVINE, M.J., HERZBERG, M.C., LEVINE, M.S., ELLISON, S.A., STINSON, M.W., LI, H.C. and VAN DYKE, T. (1978) Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycoproteins with Streptococcus sanguis and Streptococcus mutans. Infect. Immun., 19, 107-115.

LEVINE, M.M. and RENNELS, M.B. (1978) E. coli colonisation factor antigen in diarrhoea. Lancet, 2, 534.

LEVINE, M.M., RENNELS, M.B., DAYA, V. and HUGHES, T.P. (1980) Haemagglutination and colonisation factors in enterotoxigenic and enteropathogenic Escherichia coli that cause diarrhoea. J. infect. Dis., 141, 733-737.

LEVY, N.J. (1979) Wheat germ agglutinin blockage of chlamydial attachment sites: antagonism by N-acetyl-D-glucosamine. Infect. Immun., 25, 946-953.

LIENER, I.E. and PALLANSCH, M.J. (1952) Purification of a toxic substance from defatted soy bean flour. J. biol. Chem., 197, 29-36.

LORIAN, V. (1975) Some effects of subinhibitory concentrations of antibiotics on bacteria. Bull. N.Y. Acad. Med., 51, 1046-1055.

LOWRY,/...

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951) Protein measurement with the Folin Phenol Reagent. J. biol. Chem., 193, 265-275.

MANGAN, D.F. and SNYDER, I.S. (1979) Mannose-sensitive interaction of Escherichia coli with human peripheral leukocytes in vitro. Infect. Immun., 26, 520-527.

MARCHALONIS, J.J. and EDELMAN, G.M. (1968) Isolation and characterisation of a hemagglutinin from Limulus polyphemus. J. molec. Biol., 32, 453-465.

MÅRDH, P.-A. and WESTRÖM, L. (1976) Adherence of bacteria to vaginal epithelial cells. Infect. Immun., 13, 661-666.

MARSHALL, K.C., STOUT, R. and MITCHELL, R. (1971) Mechanism of the initial events on the sorption of marine bacteria to surfaces. J. gen. Microbiol., 68, 337-348.

McBRIDE, W.H., JONES, J.T. and WEIR, D.M. (1974) Increased phagocytic cell activity and anaemia in Corynebacterium parvum treated mice. Brit. J. exp. Path., 55, 38-45.

McCABE, M.M., HAMELIK, R.M. and SMITH, E.E. (1977) Purification of dextran-binding protein from cariogenic Streptococcus mutans. Biochem. biophys. Res. Commun., 78, 273-278.

McCRACKEN, Jr., G.H. and FELDMAN, W.E. (1976) Editorial comment. J. Pediat., 89, 203-204.

McINTIRE, F.C., VATTER, A.E., BAROS, J. and ARNOLD, J. (1978) Mechanism of coaggregation between Actinomyces viscosus T14V and Streptococcus sanguis 34. Infect. Immun., 21, 978-988.

MILLER, /...

MILLER, C.H., PALENIK, C.J. and STAMPER, K.E. (1978) Factors affecting the aggregation of Actinomyces naeslundii during growth and in washed cell suspensions. Infect. Immun., 21, 1003-1009.

MILLIGAN, T.W., BAKER, C.J., STRAUS, D.C. and MATTINGLY, S.J. (1978) Association of elevated levels of extracellular neuraminidase with clinical isolates of type III group B streptococci. Infect. Immun., 21, 738-746.

MIRELMAN, D., ALTMANN, G. and ESHDAT, Y. (1980) Screening of bacterial isolates for mannose-specific lectin activity by agglutination of yeasts. J. clin. Microbiol., 11, 328-331.

MONGIELLO, J.R. and FALKLER, Jr., W.A. (1979) Sugar inhibition of oral Fusobacterium nucleatum haemagglutination and cell binding. Arch. oral Biol., 24, 539-545.

NAGY, B., MOON, H.W. and ISAACSON, R.E. (1977) Colonisation of porcine intestine by enterotoxigenic Escherichia coli. Selection of piliated forms in vivo, adhesion of piliated forms to epithelial cells in vitro, and incidence of a pilus antigen among porcine enteropathogenic E. coli. Infect. Immun., 16, 344-352.

NEVEU, T., BRANELLEC, A. and BIOZZI, G. (1964) Propriétés adjuvantés de Corynebacterium parvum sur la production d'anticorps et sur l'induction de l'hypersensibilité retardée envers les protéines conjuguées. Annales de L'Institut Pasteur, 106, 771-777.

NICKLAS, J.M. (1978) Serious group B beta-hemolytic streptococcal infections in adults: report of two cases and review of the literature. John Hopkins med. J., 142, 39-45.

OFEK,/...

OFEK, I. and BEACHEY, E.H. (1978) Mannose binding and epithelial cell adherence of E. coli. Infect. Immun., 22, 247-254.

OFEK, I., BEACHEY, E.H., EISENSTEIN, B.I., ALKAN, M.L. and SHARON, N. (1979) Suppression of bacterial adherence by subminimal inhibitory concentrations of β-lactam and aminoglycoside antibiotics. Rev. infect. Dis., 1, 832-837.

OFEK, I., BEACHEY, E.H., JEFFERSON, W. and CAMPBELL, G.L. (1975) Cell membrane-binding properties of group A streptococcal lipoteichoic acid. J. exp. Med., 141, 990-1003.

OFEK, I., MIRELMAN, D. and SHARON, N. (1977) Adherence of Escherichia coli to human mucosal cells mediated by mannose receptors. Nature, 265, 623-625.

OFEK, I., SIMPSON, W.A., JOHNSON, R., PINALS, E. and BEACHEY, E.H. (1980) Interaction between M proteins and lipoteichoic acid of group A streptococci. Israel J. med. Sci., 16, 479-480.

ÖGMUNDSDÓTTIR, H.M. and WEIR, D.M. (1976) The characteristics of binding of Corynebacterium parvum to glass-adherent mouse peritoneal exudate cells. Clin. exp. Immunol., 26, 334-339.

OJO, E., HALLER, O. and WIGZELL, H. (1978) Corynebacterium parvum-induced peritoneal exudate cells with rapid cytolytic activity against tumour cells are non-phagocytic cells with characteristics of natural killer cells. Scand. J. Immunol., 8, 215-222.

OKUDA, K., SLOTS, J. and GENCO, R.J. (1981) Bacteroides gingivalis, Bacteroides asaccharolyticus and Bacteroides melaninogenicus subspecies: cell surface morphology and adherence to erythrocytes and human buccal epithelial cells. Current Microbiol., 6, 7-12.

OKUDA/...

OKUDA, K. and TAKAZOE, I. (1974) Haemagglutinating activity of Bacteroides melaninogenicus. Arch. oral Biol., 19, 415-416.

OLD, D.C. (1972) Inhibition of the interaction between fimbrial haemagglutinins and erythrocytes by D-mannose and other carbohydrates. J. gen. Microbiol., 71, 149-157.

ØRSKOV, I. and ØRSKOV, F. (1966) Episome-carried surface antigen K88 of Escherichia coli. I. Transmission of the determinant of the K88 antigen and influence on the transfer of chromosomal markers. J. Bact., 91, 69-75.

ØRSKOV, I. and ØRSKOV, F. (1977) Special O:K:H serotypes among enterotoxigenic E. coli strains from diarrhoea in adults and children. Occurrence of the CF (colonisation factor) antigen and of haemagglutinating abilities. Med. Microbiol. Immunol., 163, 99-110.

ØRSKOV, I., ØRSKOV, F. and BIRCH-ANDERSEN, A. (1980) Comparison of Escherichia coli fimbrial antigen F7 with type I fimbriae. Infect. Immun., 27, 657-666.

ØRSKOV, I., ØRSKOV, F., SMITH, H.W. and SOJKA, W.J. (1975) The establishment of K99, a thermolabile, transmissible Escherichia coli K antigen, previously called "Kco", possessed by calf and lamb enteropathogenic strains. Acta Pathol. Microbiol. Scand. Sect. Microbiol. Immunol., 83, 31-36.

OTTOW, J.C.G. (1975) Ecology, physiology and genetics of fimbriae and pili. Annu. Rev. Microbiol., 29, 79-108.

PAREDES,/...

PAREDES, A., WONG, P. and YOW, M.D. (1976) Failure of penicillin to eradicate the carrier state of group B streptococci in infants. J. Pediat., 89, 191-193.

PARSONS, C.L., ANWAR, H., STAUFFER, C. and SCHMIDT, J.D. (1979) In vitro adherence of radioactively labelled E. coli in normal and cystitis-prone females. Infect. Immun., 26, 453-457.

PEACOCK, A.C. and DINGMAN, C.W. (1967) Resolution of multiple ribonucleic acid species by polyacrylamide gel electrophoresis. Biochemistry, 6, 1818-1827.

PEROS, W.J. and GIBBONS, R.J. (1982) Influence of sublethal antibiotic concentrations on bacterial adherence to saliva-treated hydroxyapatite. Infect. Immun., 35, 326-334.

PETHICA, B.A. (1961) The physical chemistry of cell adhesion. Expl. Cell Res., Suppl. 8, 123-140.

POXTON, I.R. (1979) Serological identification of Bacteroides species by an enzyme-linked immunosorbent assay. J. clin. Path., 32, 294-298.

POXTON, I.R. and BROWN, R. (1979) Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of cell-surface proteins as an aid to the identification of the Bacteroides fragilis group. J. gen. Microbiol., 112, 211-217.

POXTON, I.R. and SUTHERLAND, I.W. (1976) The butanol-soluble proteins of Klebsiella aerogenes. Microbios, 15, 93-103.

PRÉVOT,/...

PRÉVOT, A.R. (1975) Bacteriological aspect of anaerobic corynebacteria in relation to RES stimulation. In: Halpern, B. (ed.), Corynebacterium parvum: Applications in Experimental and Clinical Oncology, pp. 3-10. New York: Plenum Press.

PUNSALANG, Jr., A.P. and SAWYER, W.D. (1973) Role of pili in the virulence of Neisseria gonorrhoeae. Infect. Immun., 8, 255-263.

QUIRANTE, J., CEBALLOS, R. and CASSADY, G. (1974) Group B β-hemolytic streptococcal infection in the newborn. Am. J. Dis. Child., 128, 659-664.

QURESHI, J.V. and GIBBONS, R.J. (1979) Differences in the adsorptive behaviour of A. viscosus and A. naeslundii to saliva-treated hydroxyapatite. J. Dent. Res., 58, Special Issue A, # 996 p. 341.

ROE, M.H., TODD, J.K. and FAVARA, B.E. (1976) Nonhemolytic group B streptococcal infections. J. Pediat., 89, 75-77.

ROSAN, B. and HAMMOND, B.F. (1974) Extracellular polysaccharides of Actinomyces viscosus. Infect. Immun., 10, 304-308.

ROTTINI, G., CIAN, F., SORANZO, M.R., ALBRIGO, R. and PATRIARCA, P. (1979) Evidence for the involvement of human polymorphonuclear leucocyte mannose-like receptors in the phagocytosis of Escherichia coli. FEBS Lett., 105, 307-312.

RUSSELL, R.R.B. (1979) Glucan-binding proteins of Streptococcus mutans serotype c. J. gen. Microbiol.,112, 197-201.

RUTTER, J.M., BURROWS, M.R., SELLWOOD, R. and GIBBONS, R.A. (1975) A genetic basis for resistance to enteric disease caused by E. coli. Nature, 257, 135-136.

SALIT,/...

SALIT, I.E. and GOTSCHLICH, E.C. (1977a) Hemagglutination by purified type I Escherichia coli pili. J. exp. Med., 146, 1169-1181.

SALIT, I.E. and GOTSCHLICH, E.C. (1977b) Type I Escherichia coli pili: characterisation of binding to monkey kidney cells. J. exp. Med., 146, 1182-1194.

SALIT, I.E. and MORTON, G. (1981) Adherence of Neisseria meningitidis to human epithelial cells. Infect. Immun., 31, 430-435.

SANDBERG, T., STENQVIST, K. and SVANBORG-EDÉN, C. (1979) Effects of subminimal inhibitory concentrations of ampicillin, chloramphenicol and nitrofurantoin on the attachment of Escherichia coli to human uroepithelial cells in vitro. Rev. infect. Dis., 1, 838-844.

SAUNDERS, J.M. and MILLER, C.H. (1980) Attachment of Actinomyces naeslundii to human buccal epithelial cells. Infect. Immun., 29, 981-989.

SCHAUF, V., DEVEIKIS, A., RIFF, L. and SEROTA, A. (1976) Antibiotic-killing kinetics of group B streptococci. J. Pediat., 89, 194-198.

SCHELD, W.M., ZAK, O., VOSBECK, K. and SANDE, M.A. (1981) Bacterial adhesion in the pathogenesis of infective endocarditis. Effect of subinhibitory antibiotic concentrations on streptococcal adhesion in vitro and the development of endocarditis in rabbits. J. clin. Invest., 68, 1381-1384.

SCOTT, M.T. (1972a) Biological effects of the adjuvant Corynebacterium parvum. I. Inhibition of PHA, mixed lymphocyte and GVH reactivity. Cellular Immunol., 5, 459-468.

SCOTT,/...

SCOTT, M.T. (1972b) Biological effects of the adjuvant Corynebacterium parvum. II. Evidence for macrophage-T-cell interaction. Cellular Immunol., 5, 469-479.

SEGREST, J.P. and JACKSON, R.L. (1972) Molecular weight determination of glycoprotein by polyacrylamide gel electrophoresis in sodium dodecyl sulphate. Methods Enzymol., 28, 54-63.

SHARON, N. and LIS, H. (1972) Lectins: cell-agglutinating and sugar-specific proteins. Science, 177, 949-959.

SIMPSON, W.A., OFEK, I. and BEACHEY, E.H. (1980a) Binding of streptococcal LTA to the fatty acid binding sites on serum albumin. J. biol. Chem., 255, 6092-6097.

SIMPSON, W.A., OFEK, I. and BEACHEY, E.H. (1980b) Fatty acid binding sites of serum albumin as membrane receptor analogs for streptococcal lipoteichoic acid. Infect. Immun., 29, 119-122.

SIMPSON, W.A., OFEK, I., SARASOHN, C., MORRISON, J.C. and BEACHEY, E.H. (1980) Characteristics of the binding of streptococcal lipoteichoic acid to human oral epithelial cells. J. infect. Dis., 141, 457-462.

SINGER, S.J. (1971) The molecular organisation of biological membranes. In: Rothfield, L. (ed.), Structure and Function of Biological Membranes, pp. 145-222. New York: Academic Press.

SLOTS, J. and GIBBONS, R.J. (1978) Attachment of Bacteroides melaninogenicus subsp. asaccharolyticus to oral surfaces and its possible role in colonisation of the mouth and of periodontal pockets. Infect. Immun., 19, 254-264.

SMITH,/...

SMITH, H.W. and LINGGOOD, M.A. (1971) Observations on the pathogenic properties of the K88, Hly and Ent plasmids of Escherichia coli, with particular reference to porcine diarrhoea. J. med. Microbiol., 4, 467-485.

SOBEL, J.D., SCHNEIDER, J., KAYE, D. and LEVISON, M.E. (1981) Adherence of bacteria to vaginal epithelial cells at various times in the menstrual cycle. Infect. Immun., 32, 194-197.

SOCRANSKY, S.S. (1977) Microbiology of periodontal disease - present status and future considerations. J. Periodontol., 48, 497-504.

SPINELL, D.M. and GIBBONS, R.J. (1974) Influence of culture medium on the glucosyl transferase and dextran-binding capacity of Streptococcus mutans 6715 cells. Infect. Immun., 10, 1448-1451.

SRIVASTAVA, R., SINHA, V.B. and SRIVASTAVA, B.S. (1980) Events in the pathogenesis of experimental cholera: role of bacterial adherence and multiplication. J. med. Microbiol., 13, 1-9.

STAAT, R.H., LANGLEY, S.D. and DOYLE, R.J. (1980) Streptococcus mutans adherence: presumptive evidence for protein mediated attachment followed by glucan-dependent cellular accumulation. Infect. Immun., 27, 675-681.

STANLEY, P. and CARVER, J.P. (1978) Binding of [^{125}I] wheat germ agglutinin to Chinese hamster ovary cells under conditions which affect the mobility of membrane components. J. Cell Biol., 79, 617-622.

STECK,/...

STECK, T. and DAWSON, G. (1974) Topographical distribution of complex carbohydrate in the erythrocyte membrane. J. biol. Chem., 249, 2135-2142.

STIRM, S., ØRSKOV, F., ØRSKOV, I. and BIRCH-ANDERSEN, A. (1967) Episome-carried surface antigen K88 of Escherichia coli. III. Morphology. J. Bact., 93, 740-748.

'STUDENT' (1908) The probable error of a mean. Biometrika, 6, 1-25.

SUMNER, J.B., GRALÉN, N. and ERIKSSON-QUENSEL, I.-B. (1938) The molecular weights of canavalin, concanavalin A, and concanavalin B. J. biol. Chem., 125, 45-48.

SUMNER, J.B. and HOWELL, S.F. (1936) The identification of the hemagglutinin of the jack bean with concanavalin A. J. Bact., 32, 227-237.

SVANBORG-EDÉN, C., ERIKSSON, B. and HANSON, L.Å. (1977) Adhesion of Escherichia coli to human uroepithelial cells in vitro. Infect. Immun., 18, 767-774.

SVANBORG-EDÉN, C., HAGBERG, L., HANSON, L.Å., KORHONEN, T., LEFFLER, H. and OLLING, S. (1981) Adhesion of Escherichia coli in urinary tract infection. In: Elliott, K., O'Connor, M. and Whelan, J. (eds.), Adhesion and Microorganism Pathogenicity, pp. 161-187. London: Pitman Medical Ltd.

SVANBORG-EDÉN, C. and HANSSON, H.A. (1978) Escherichia coli pili as possible mediators of attachment to human urinary tract epithelial cells. Infect. Immun., 21, 229-237.

SVANBORG-EDÉN,/...

SVANBORG-EDÉN, C., HANSON, L.Å., JODAL, U., LINDBERG, U. and ÅKERLUND, A.S. (1976) Variable adherence to normal human urinary-tract epithelial cells of Escherichia coli strains associated with various forms of urinary tract infection. Lancet, 2, 490-492.

SWANSON, J. (1973) Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. J. exp. Med., 137, 571-589.

SWANSON, J., HSU, K.C. and GOTSCHLICH, E.C. (1969) Electron microscopic studies on streptococci. I. M antigen. J. exp. Med., 130, 1063-1091.

SWANSON, J., KRAUS, S.J. and GOTSCHLICH, E.C. (1971) Studies on gonococcus infection. I. Pili and zones of adhesion: their relation to gonococcal growth patterns. J. exp. Med., 134, 886-906.

SWANSON, J., SPARKS, E., YOUNG, D. and KING, G. (1975) Studies on gonococcus infection. X. Pili and leukocyte association factor as mediators of interactions between gonococci and eukaryotic cells in vitro. Infect. Immun., 11, 1352-1361.

TADROS, T.F. (1980) Particle-surface adhesion. In: Berkeley, R.C.W., Lynch, J.M., Melling, J., Rutter, P.R. and Vincent, B. (eds.), Microbial Adhesion to Surfaces, pp. 93-116. Chichester: Ellis Horwood Ltd.

TANZER, J.M., FREEDMAN, M.L., FITZGERALD, R.J. and LARSON, R.H. (1974) Diminished virulence of glucan synthesis-defective mutants of Streptococcus mutans. Infect. Immun., 10, 197-203.

THALER,/...

THALER, M.M., HIRSCHBERGER, M. and MIRELMAN, D. (1977) Adherence of E. coli to immature intestine is mediated by mucosal receptors of mannose-specific bacterial lectins. Clin. Res., 25, 469A.

TINANOFF, N., TANZER, J.M. and FREEDMAN, M.L. (1978) In vitro colonization of Streptococcus mutans on enamel. Infect. Immun., 21, 1010-1019.

TORREY, J.C. (1916) Bacteria associated with certain types of abnormal lymph glands. J. med. Res., 34, 65-80.

TOWNSEND, R. and STAHL, P. (1981) Isolation and characterisation of a mannose/N-acetyl-glucosamine/fucose binding protein from rat liver. Biochem. J., 194, 209-214.

TRAMONT, E.C. (1977) Inhibition of adherence of Neisseria gonorrhoeae by human genital secretions. J. clin. Invest., 59, 117-124.

TRAMONT, E.C. (1981) Adhesion of Neisseria gonorrhoeae and disease. In: Elliott, K., O'Connor, M. and Whelan, J. (eds.), Adhesion and Microorganism Pathogenicity, pp. 188-201. London: Pitman Medical Ltd.

TRAMONT, E.C., CIAK, J., BOSLEGO, J., McCHESNEY, D.G., BRINTON, C.C. and ZOLLINGER, W. (1980) Antigenic specificity of antibodies in vaginal secretions during infection with Neisseria gonorrhoeae. J. infect. Dis., 142, 23-31.

TRAMONT, E.C., SADOFF, J.C., BOSLEGO, J.W., CIAK, J., McCHESNEY, D., BRINTON, C.C., WOOD, S. and TAKAFUJI, E. (1981) Gonococcal pilus vaccine: studies of antigenicity and inhibition of attachment. J. clin. Invest., 68, 881-888.

TRUOG,/...

TRUOG, W.E., DAVIS, R.F. and RAY, C.G. (1976) Recurrence of group B streptococcal infection. J. Pediat., 89, 185-186.

TUSZYNSKI, G.P., DAMSKY, C.H., FUHRER, J.P. and WARREN, L. (1977) Recovery of concentrated protein samples from sodium dodecyl sulfate-polyacrylamide gels. Analyt. Biochem., 83, 119-129.

TUSZYNSKI, G.P. and WARREN, L. (1975) Removal of sodium dodecyl sulfate from proteins. Analyt. Biochem., 67, 55-65.

TWEEDY, J.M., PARK, R.W.A. and HODGKISS, W. (1968) Evidence for the presence of fimbriae (pili) on vibrio species. J. gen. Microbiol., 51, 235-244,

TYLEWSKA, S., HJERTÉN, S. and WADSTRÖM, T. (1981) Effect of sub-inhibitory concentrations of antibiotics on the adhesion of Streptococcus pyogenes to pharyngeal epithelial cells. Antimicrob. Agents Chemother., 20, 563-566.

TYLEWSKA, S. and WADSTRÖM, T. (1981) Glycoconjugate receptors on human pharyngeal and buccal epithelial cells for Streptococcus pyogenes. In: Holm, S. and Christensen, P. (eds.), Basic Concepts of Streptococci and Streptococcal Diseases. Surrey: Reedbooks (in press).

TYLEWSKA, S.K., WADSTRÖM, T. and HJERTÉN, S. (1980) The effect of subinhibitory concentrations of penicillin and rifampicin on bacterial cell surface hydrophobicity and on binding to pharyngeal epithelial cells. J. antimicrob. Chemother., 6, 292-294.

UY, R. and WOLD, F. (1977) 1,4-butanediol diglycidyl ether coupling of carbohydrates to Sepharose: affinity adsorbents for lectins and glycosidases. Analyt. Biochem., 81, 98-107.

VAN HOUTE,/...

VAN HOUTE, J., BURGESS, R.C. and ONOSE, H. (1976) Oral implantation of human strains of Streptococcus mutans in rats fed sucrose or glucose diets. Arch. oral Biol., 21, 561-564.

VARIAN, S.A. and COOKE, E.M. (1980) Adhesive properties of Escherichia coli from urinary-tract infections. J. med. Microbiol., 13, 111-119.

VERWEY, E.J.W. and OVERBEEK, J. TH. G. (1948) Theory of the Stability of Lyophobic Colloids. Amsterdam: Elsevier.

VOSBECK, K., HANDSCHIN, H., MENGE, E.-B. and ZAK, O. (1979) Effects of subminimal inhibitory concentrations of antibiotics on adhesiveness of Escherichia coli in vitro. Rev. infect. Dis., 1, 845-851.

WALKER, S.H., SANTOS, A.Q. and QUINTERO, B.A. (1976) Recurrence of group B III streptococcal meningitis. J. Pediat., 89, 187-188.

WALLIN, J. and FORSGREN, A. (1975) Group B streptococci in venereal disease clinic patients. Brit. J. vener. Dis., 51, 401-404.

WANG, J.L., CUNNINGHAM, B.A. and EDELMAN, G.M. (1971) Unusual fragments in the subunit structure of concanavalin A. Proc. natn. Acad. Sci. U.S.A., 68, 1130-1134.

WASHINGTON, J.A. (1979) The effects and significance of subminimal inhibitory concentrations of antibiotics. Rev. infect. Dis., 1, 781-786.

WATT, P.J., WARD, M.E., HECKELS, J.E. and TRUST, T.J. (1978) Surface properties of Neisseria gonorrhoeae: attachment to and invasion of mucosal surfaces. In: Brooks, G.F., Gotschlich, E.C., Holmes, K.K., Sawyer, W.D. and Young, F.E. (eds.), Immunobiology/...

Immunobiology of Neisseria gonorrhoeae, pp. 253-257. Washington: American Society for Microbiology.

WEEKE, B. (1973) General remarks on principles, equipment, reagents and procedures. In: Axelsen, N.H., Krøll, J. and Weeke, B. (eds.), A Manual of Quantitative Immunoelectrophoresis, Methods and Application. Scand. J. Immunol., 2, No. 1, pp. 15-35.

WEIR, D.M. and ÖGMUNDSDÓTTIR, H.M. (1980) Cellular recognition by phagocytes: role of lectin-like receptors. In: van Furth, R. (ed.), Mononuclear Phagocytes, pp. 865-881. The Hague: Martinus Nijhoff.

WEISS, L. (1968) Studies on cellular adhesion. An experimental and theoretical approach to interaction forces between cells and glass. Expl. Cell Res., 53, 603-614.

WEISS, L. and HARLOS, J.P. (1977) Cell contact phenomena and their implications in cell communication. In: de Mello, W.C. (ed.), Intercellular Communications, pp. 33-59. New York: Plenum Publishing Corporation.

WICKEN, A.J., BROADY, K.W., EVANS, J.D. and KNOX, K.W. (1978) New cellular and extracellular amphipathic antigen from Actinomyces viscosus NY1. Infect. Immun., 22, 615-616.

WICKEN, A.J. and KNOX, K.W. (1975) Lipoteichoic acids: a new class of bacterial antigen. Science, 187, 1161-1167.

WIENER, E. (1975) Analysis of the Corynebacterium parvum adjuvant effects at the cellular and subcellular levels. In: Halpern, B. (ed.), Corynebacterium parvum: Applications in Experimental and Clinical Oncology, pp. 59-65. New York: Plenum Press.

WILKINSON,/...

WILKINSON, H.W. (1977) CAMP-disk test for presumptive identification of group B streptococci. J. clin. Microbiol., 6, 42-45.

WILKINSON, H.W. (1978) Analysis of group B streptococcal types associated with disease in human infants and adults. J. clin. Microbiol., 7, 176-179.

WILKINSON, H.W. and EAGON, R.G. (1971) Type-specific antigens of group B type Ic streptococci. Infect. Immun., 4, 596-604.

WOODRUFF, M., DUNBAR, N. and GHAFFAR, A. (1973) The growth of tumours in T-cell deprived mice and their response to treatment with Corynebacterium parvum. Proc. Roy. Soc. Lond. B, 184, 97-102.

YAMAZAKI, Y., EBISU, S. and OKADA, H. (1981) Eikenella corrodens adherence to human buccal epithelial cells. Infect. Immun., 31, 21-27.

YANAGAWA, R. and HONDA, E. (1976) Presence of pili in species of human and animal parasites and pathogens of the genus Corynebacterium. Infect. Immun., 13, 1293-1295.

YARIV, J., KALB, A.J. and LEVITZKI, A. (1968) The interaction of concanavalin A with methyl-α-D-glucopyranoside. Biochim. biophys. Acta., 165, 303-305.

ZAWANEH, S.M., AYOUB, E.M., BAER, H., CRUZ, A.C. and SPELLACY, W.N. (1979) Factors influencing adherence of group B streptococci to human vaginal epithelial cells. Infect. Immun., 26, 441-447.

APPENDIX

Some of the material presented in this thesis has been previously submitted for publication. The relevant references are as follows:

BAGG, J., POXTON, I.R., DOYLE, J., ROSS, P.W. and WEIR, D.M. (1980) The isolation and purification of a mannose-binding agglutinin from E. coli strains and Corynebacterium parvum NCTC 10390. In: Berkeley, R.C.W., Lynch, J.M., Melling, J., Rutter, P.R. and Vincent, B. (eds.), Microbial Adhesion to Surfaces, pp. 528-530. Chichester: Ellis Horwood Ltd.

BAGG, J., POXTON, I.R. and WEIR, D.M. (1981) The isolation of a lectin-like molecule from Corynebacterium parvum (NCTC 10390). J. Clin. Lab. Immunol., 6, 165-168.

BAGG, J., POXTON, I.R., WEIR, D.M. and ROSS, P.W. (1982) Binding of type III group-B streptococci to buccal epithelial cells. J. med. Microbiol., 15, (in press).