ABO BLOOD GROUP, SECRETOR STATUS AND SUSCEPTIBILITY TO INFECTION OF THE GENITOURINARY TRACT

by

DENIS F. KINANE, B.D.S.

Thesis presented for the Degree of Doctor of Philosophy

at the University of Edinburgh in the Faculty of Medicine

1983

One can summarise the phenomenon (of inflammation) shortly, and to the point: a defense reaction of mesodermal cells against a foreign invader. These cells must destroy invading microbes that are ingested and broken down - and consequently healing is brought about (Metchnikoff, 1905).

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

CONTENTS

Contents (contd.) Page

Contents (contd.) Page

Contents (contd.) Page

Contents (contd.) Page

Contents (contd.) Page

ABSTRACT

Epidemiological studies confirming the correlation between particular ABO blood groups and susceptibility to both recurrent urinary tract infection and infection with Neisseria gonorrhoeae have been undertaken and reported here. Host-parasite interactions that may be involved in the increased incidence of blood group B individuals with gonorrhoea have been investigated by examining gonococcal interaction with phagocytes of the four ABO blood groups under various conditions.

The survey of recurrent urinary tract infection revealed that women of blood groups B and AB who were non-secretors of blood group substances showed a significant relative risk of recurrent urinary tract infection of 3.12 (95% confidence limits, 1.49 and 6.52) in comparison with other types. This appears to be a genuine example of synergy in which absence of anti-B isohaemagglutinin combined with non-secretion of blood group substances confers an increased risk of recurrent urinary tract infection. Determination of blood group and secretor status may provide clinicians with additional information for the identification of women who are at increased risk of recurrent urinary tract infection.

ABO blood group and secretor status were determined for patients attending a sexually transmitted diseases clinic. This survey revealed that among patients with gonorrhoea, 20.1% were of blood group B, whereas among those without gonorrhoea only 12% were of blood group B. A higher percentage (20.9%)

of patients with no anti-B isohaemagglutinin had gonorrhoea compared with those without gonorrhoea (12.1%) and this difference was statistically significant ($X^2 = 4.947$, $p < 0.05$). Using the relative risk method of Woolf (1954/55) the relative risk of gonococcal infection was calculated to be 1.93 for individuals without anti-B isohaemagglutinin. This means that with all other considerations equal, they are 93% more susceptible to gonorrhoea than persons with anti-B isohaemagglutinin. There were no significant differences in secretor status for patients with or without gonorrhoea nor between the total patients and controls. In contrast to the recurrent urinary tract infection survey, no synergy between the non-secretion of blood group substances and the absence of anti-B isohaemagglutinin was found.

Further research was carried out to determine the underlying host-parasite interactions responsible for the increased susceptibility to gonorrhoea for individuals with no anti-B isohaemagglutinins. Initial experiments indicated very different patterns of association for pilate and non-pilate gonococci to polymorphonuclear leukocytes (PMN) and monocytes. PMN bound appreciably more gonococci than monocytes and, as others have found, PMN did not differ in their binding of pilate and non-pilate variants of the same strain. Human monocytes, like epithelial cells, showed an increased ability to bind pilate rather than non-pilate gonococci.

The presence of pili was shown to affect the stimulation of the intercellular oxygen-dependent bactericidal activity of PMN. Non-pilate gonococci stimulated nitroblue tetrazolium

reduction significantly more than pilate gonococci. This lowered stimulation of the phagocytes by pilate gonococci suggests a role for pili in virulence in addition to their ability to increase gonococcal binding to epithelial cells.

The association of gonococci to phagocytes of the four different ABO blood groups was examined under a variety of conditions. In the absence of serum no differences were noted for the association of gonococci to PMN or monocytes of the four blood groups at 4°C or 37°C. In these experiments, strains of gonococci with different LPS structures showed differences in their ability to bind to PMN; those gonococci with a "complex" LPS bound to a greater proportion of PMN than those with a "simple" LPS. Thus the expression of ABO antigens on PMN is not important in binding of gonococci, whereas the strain of gonococci appeared to be the factor that had the greater influence.

Binding of gonococci to monocytes of the four ABO groups in the presence of heat inactivated autologous serum (HIS) gave a statistically significant pattern in which group B monocytes bound more gonococci than A, O or AB monocytes. The importance of isohaemagglutinins in opsonisation of gonococci for monocytes was demonstrated by a reduction in association after the absorption of isohaemagglutinins. This increase was not dependent on the gonococcal strain used and contrasted with the equivalent experiment using PMN. This series of experiments showed no significant pattern in binding of gonococci for PMN of the four ABO groups in the presence of autologous HIS.

The association of gonococci to phagocytes was examined for the presence of 'lectin-like' receptors capable of binding these bacteria. The data suggests such 'lectin-like' receptors are present on PMN and monocytes but that there are quantitative and qualitative differences in expression of these receptors. These findings may have importance in investigation of the interaction of gonococci with phagocytes.

The epidemiological studies have confirmed previous findings linking blood groups with susceptibility to recurrent urinary tract infection and gonorrhoea. The studies on host-parasite interactions have extended the current information on the pathogenesis of gonorrhoea by defining the role of certain components of the gonococcal outer surface and isohaemagglutinins in the interaction of the bacteria with the phagocytic cells of the immune system.

DECLARATION

I confirm that this study has been designed and carried out by myself.

Denis Kinane

INTRODUCTION

GENERAL INTRODUCTION

During the last century the western developed countries have come to live in relative freedom from the fatal infections of the past. This has come about through improvements in nutrition and living conditions as well as the successful recognition of etiological agents of disease and development of specific therapy such as prevention by vaccines. Various diseases including smallpox and typhoid fever, have been controlled by measures such as correct diagnosis and treatment of infections, full implementation of immunisation programmes, alert epidemiological surveillance and rigorous environmental sanitation. There are however still a number of infections for which the agent has been isolated and disease effectively treated but for which there is no effective control. Both urinary tract infection (UTI) and gonorrhoea are infections against which the host does not appear to develop a long term protective immune response. Repeated attacks of gonorrhoea are common (Watt and Heckels, 1982); one of Edinburgh's most famous citizens, James Boswell, recorded approximately 30 separate infections in his famous diary (Boswell, 1980). UTI infections commonly recur (Bergstrom et al., 1968) and it has been difficult to identify the protective immune response (Holmgren and Smith, 1975), or indeed to determine to what extent the immune response is helpful (Angell et al., 1978). For the

successful induction of an immune response, microbial antigens must first become established in the host tissues and these antigens must survive the effects of phagocytic degredation and must be presented to the cells of the immune system in an appropriate form. This study examines some of the susceptibility factors that have a bearing on the outcome of bacterial infection in the non-immune host, with particular emphasis on the interactions of bacteria and phagocytes.

Recent work in this department has identified lectin-sugar mediated interactions between bacteria and membranes of epithelial and phagocytic cells, the evidence for which will be described in a following section. In view of this work, the role of the ubiquitous ABO blood group sugar-containing antigens in these lectin-sugar interactions presents a fertile area for investigation. The ABO blood groups have been linked with susceptibility to infectious diseases, but the nature of their relationship to the host parasite interactions that occur in gonorrhoea and UTI have not been extensively characterized. The ABO substances are expressed on the membranes of many cells, including phagocytes and mucosal epithelial cells. They are also found in water soluble forms in the body fluids of many individuals.

The purposes of this introduction are twofold:
1) to draw together evidence from various sources that might explain the association between ABO blood groups, secretion of water soluble blood group antigens and susceptibility to infectious disease; 2) to review relevant aspects of the

genetics and biochemistry of the ABO system, and the biology of N. gonorrhoeae as it relates to the pathogenicity of the infection and interaction with the immune system.

BLOOD GROUPS AND GENITOURINARY DISEASE CORRELATIONS

The ABO blood groups, secretor genes and Lewis system

1. The ABO blood groups

The history of blood groups begins in 1900 with Landsteiner's discovery of the ABO blood group system (Landsteiner, 1900). A fuller account of his work appeared in 1901, in which he described how humans could be divided into 3 distinct groups which he named A, B and C (Landsteiner, 1901). These groups were to be subsequently called A, B and O. The fourth, and rarest, blood group AB, was discovered in the following year by two of Landsteiner's collaborators (Decastello and Sturli, 1902). Landsteiner appreciated that only two antigens, A and B, were required to explain the four groups, and further that a person's serum will not contain antibodies against the antigens present on his own red blood cells. The present nomenclature ABO, originated in 1910 by Von Dungren and Hirschfeld, was accepted as the standard international nomenclature by the Hygiene Commission of the League of Nations in 1928 (Von Dungern and Hirschfeld, 1911).

Von Dungern and Hirschfeld (1910) discovered that blood groups were inherited as Mendelian characters. This discovery increased the biological interests in blood groups, and was one of the earliest achievements in human genetics (Vogel and Motulsky, 1982). Until then it had not been possible to show that the fundamental genetical work of Mendel fully applied to man. In fact, Bateson in his book on 'Mendel's Principles of Hereditary' stated that apart from eye colour there was little evidence as yet for Mendelian inheritance of normal characteristics

in man (Bateson, 1909).

The exact manner of ABO inheritance was elucidated by Bernstein (1924), who proposed the 'Three Genes Theory'. Von Dungern and Hirschfeld (1911) however, had discovered the A_1 and A_2 subgroups of A, and therefore, Thomsen et al. (1930) extended Bernstein's theory by proposing the four allele theory of inheritance. Table I shows how this four allele theory gives ten possible genotypes and six phenotypes.

GENOTYPES	PHENOTYPES
$A_1\ A_1$	A_1
$A_1\ A_2$	
$A_1\ 0$	
$A_2\ A_2$	A_2
$A_2\ 0$	
B B	B
B 0	
A_1 B	A_1B
A_2 B	A_2B
0 0	0

Table I. The A_1A_2BO groups as defined by anti-A, anti-A_1 and anti-B.

2. Secretor genes

After the discovery of the ABO blood groups in 1900 intense interest was generated in this field. Despite many workers applying their talents to this new research, it was not until 1924 that Schiff was able to demonstrate the presence of ABO antigens in serum (Schiff, 1924). Two years later Yamakami demonstrated A and B antigens in saliva (Yamakami, 1926). Schiff and Sasaki (1932) showed that the ability to secrete the A, B or O antigens in saliva was controlled by a pair of allelic genes, now generally called Se and se (se is the amorph or non-secretor allele). Friedenreich and Hartmann concluded that two forms of the ABO antigens were present (Friedenreich and Hartmann, 1938). These forms were: (1) an alcohol soluble form, present in all tissues (except the brain) and; (2) a water soluble form not present in red cells or serum but present in most of the body fluids and organs of secretors. The presence of this water soluble form is determined by the secretor gene. Although the secretor genes are not linked to the ABO genes, the water soluble form is influenced by the secretor gene in that secretors will have in their body fluids and saliva the A, B or H(O) substances corresponding to their particular blood group.

3. The Lewis system

Lewis has been called a system rather than a blood group as the antigens involved are primarily located in the secretions and plasma (Grubb, 1950) and are only secondarily absorbed on

to red cells (Sneath and Sneath, 1955). Grubb in 1948 noticed that individuals whose red cells were Le (a+) were non-secretors of ABH substances, those with Le (a-b+) were secretors, and those whose red cells were Le (a-b-) could be either secretors or non-secretors (Table II).

Table II. Lewis phenotypes and ABH secretion

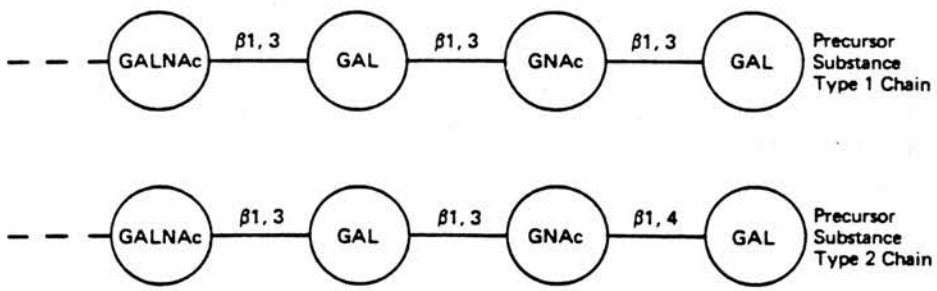
Lewis phenotype	ABH secretor status	Lewis secretor status
Le (a+b-)	All ABH nonsecretors	All Le^a secretors
Le (a-b+)	All ABH secretors	All Le^a and Le^b secretors
Le (a-b-)	80% ABH secretors 20% ABH nonsecretors	Secrete precursor substance

Two further Lewis antigens have been discovered Le^c (Iseki et al., 1957) and Le^d (Potapov, 1970). Despite the independent inheritance of these genes subsequent work has revealed a remarkable and complex set of interactions between the Lewis, secretor, Hh and ABO genes. An excellent review of the blood group substances and the genetic pathways involved in their production is given by Watkins (1972).

4. Biochemistry of the A, B, H and Lewis substances

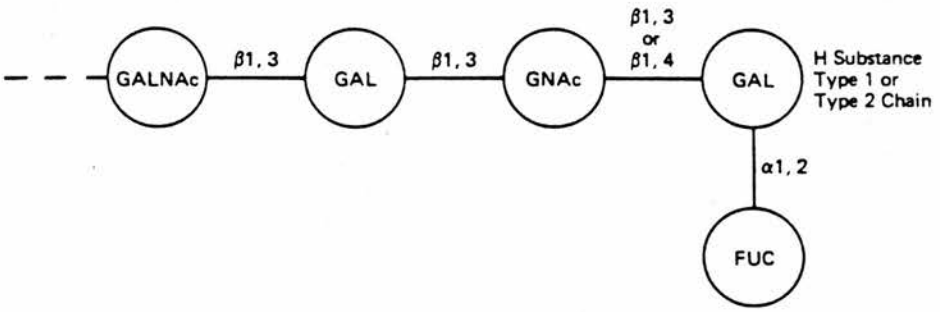
The biochemistry of ABH and Lewis substances is presented together because of the intimate relationship of the genes that produce them. ABH antigens expressed on cells are glycolipids and those in secretions, glycoproteins. On the

basis of molar ratios of component sugars (Hakomori and Strycharz, 1968), it is strongly suggested that the serologically active components are the same in both the glycolipid and glycoprotein substances (Watkins, 1972). The ABH and Lewis substances from secretions have their specificity in the carbohydrate portion of a glycoprotein molecule with molecular weight of about 300,000 (Issitt and Issitt, 1979). The ABH and Lewis substances are composed of a common basic structure, specificity being due to the sugar residues at the terminal nonreducing end of the molecule. The common basic glycoprotein structures are termed precursor substances and two types have been identified. These chains differ in the linkage of the terminal galactose molecule to the subterminal N-acetyl glucosamine molecule:

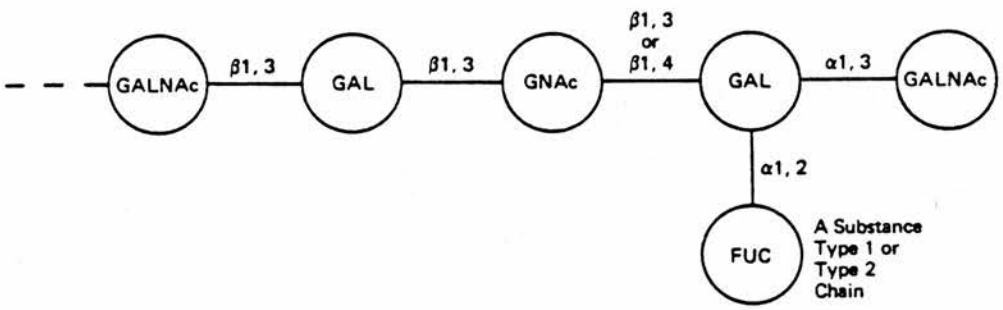


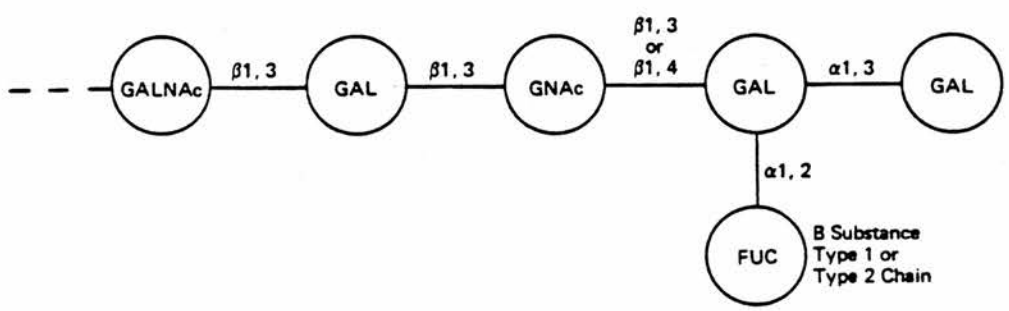
GALNAc – N-acetyl-D-galactosamine
GNAc – N-acetyl-D-glucosamine
GAL – D-galactose
FUC – L-fucose

H specificity results from the addition of a fucose molecule to either type 1 or type 2 chains:

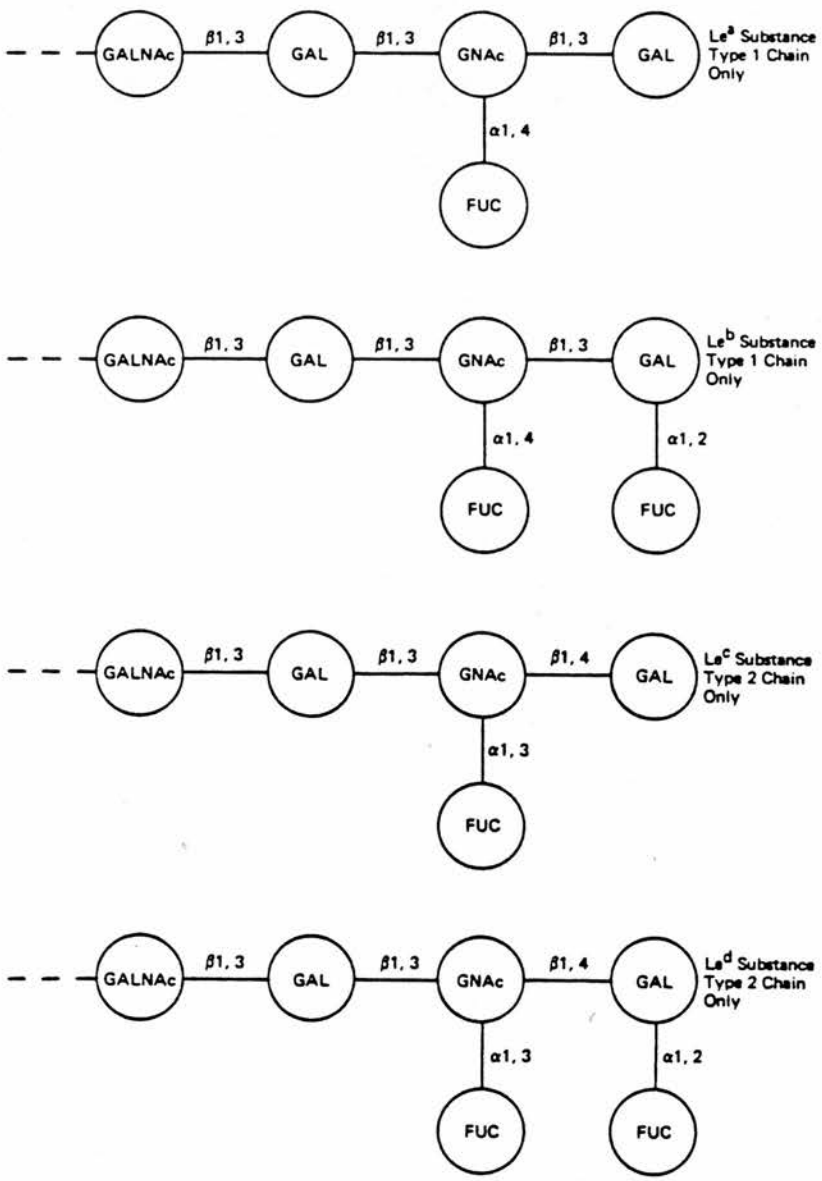


The presence of this fucose unit is necessary for the production of A and B antigens. A antigen specificity results from the addition of N-acetylgalactosamine to the terminal galactose residue of H active type 1 or type 2 chains; B specificity results from the addition of galactose rather than N-acetylgalactosamine as for A:





Lewis substances Le^a and Le^b are derived from Type 1 precursor chain and Le^c and Le^d from Type 2 precursor chains. Le^a substance results from the addition of fucose to carbon 4 of the subterminal N-acetylglucosamine in Type 1 precursor chains. Le^c substance results from the addition of fucose to carbon 3 of the subterminal N-acetylglucosamine unit of the Type 2 precursor chain. Le^b specificity results when both fucose residues are present on the Type 1 precursor chain and Le^d when both fucose residues are present on the Type 2 precursor chain:



From the preceding figures it can be seen that the basic differences between the ABH and Lewis substances are small. The presence of the fucose unit that confers specificity to the precursor chains is thought to be necessary for the production of antigens A and B. It is not known whether the fucose residue is part of the A or B antigen structure or whether the presence of fucose causes changes in conformational shape necessary in forming the A or B antigen structure. The structure of the sugars that confer A and B specificity are shown in Figure 1.

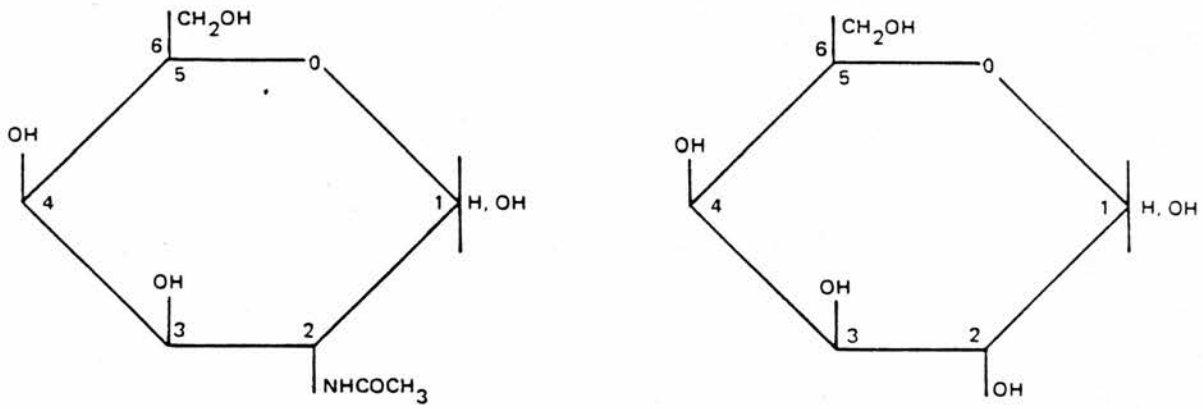


Figure 1. The immunodominant monosaccharides of A (N-acetyl-D-galactosamine) and B (D-galactose) substances.

The fact that these sugars differ only at the substituents occupying carbon 2 is an excellent example of the exquisite specificity of antigen antibody recognition.

ABO, secretor status and susceptibility to disease

1. Blood groups and disease associations

1.1 Introduction

Hirschfeld and Hirschfeld (1919) first showed that different peoples had variations in ABO blood-group frequencies. Mourant et al. (1976) in their book 'The Distribution of the Human Blood Groups and Other Polymorphisms', provided convincing data on the tremendous variation in blood group frequencies around the world. In Western Europe there are considerable numbers of populations differing significantly in ABO frequencies. Although slight trends among Rh and MN blood groups are seen on a continental scale, these are very much less than for ABO blood groups. 'There can be no doubt, however, as to the uneveness of distribution of the ABO blood groups as compared with MN and Rh groups, and it is probable that this is due to a greater susceptibility of the ABO phenotypes than the MN and Rh ones to be selected with regard to varying environmental conditions' (Mourant et al., 1976).

This hypothesis linking disease susceptibility and ABO phenotype is not a new one. Within three decades of the discovery of the ABO blood groups comprehensive lists of correlations between blood groups and disease were being compiled. Buchanan and Higley (1921) carried out extensive studies, blood grouping a total of 2446 patients suffering from a variety of diseases. They concluded that 'Nationality should be taken into consideration in the presentation of statistical studies of blood-grouping', and that 'There is no relationship between blood groups and any disease in which sufficient data

are available to justify a conclusion'. Their comment regarding consideration of nationality followed Hirschfeld and Hirschfeld's (1919) work on variations in blood groups among peoples, and they used this to criticise Alexander's work on blood-groups and malignancy (Alexander, 1921). They could find no correlation between blood groups and malignancy and commented that Alexander failed to consider nationality in his series. In fairness to Alexander, he did state that he had too few numbers of cases (50) to draw definite conclusions. This point was taken up again much later by Mourant et al. (1978) commenting on Buchanan and Higley's (1921) conclusions that there was no relationship between blood groups and disease, 'With hindsight it is possible to realise that the numbers tested were much too small to yield statistically significant results, even for disease where we now know that associations exist'.

1.2 Criticisms of blood group and disease correlations

Almost as soon as correlations between blood groups and various characters appeared, criticism also appeared. It is interesting to note the examples reviewed by Prokop and Uhlenbruck (1969) in 'Human Blood and Serum Groups'. They cited, among others, a paper published in 1927 which stated 'hangover' is more pronounced in group A persons and that group B persons defaecate the most. It became fashionable to correlate psychological conditions and blood group. One of the best examples of this

work is that of Furakawa (1930) who concluded, 'We have shown that there seems to be a correlation between blood-type and temperament. It should therefore be possible to determine temperament scientifically without the subjective judgement of different observers'. Unfortunately this problem still exists; an article entitled 'Blood Groups and Personality Traits' was published as recently as December 1964 (Cattell et al., 1964) and received strong criticism. Weiner (1970) criticised much of the work on correlations between blood groups and disease; when diseases are selected at random and without clear rationale, in order to determine correlation with blood group, the a priori probabilities of an association are extremely remote. In Furakawa's paper of 1930 the a priori probabilities of the claimed associations between blood groups and temperament were never mentioned, much less taken into account.

A recent article on ABO blood groups and socio-economic classes is a good example of the difficulties that arise in interpretation (Beardmore and Karimi-Booshehri, 1983). This study reveals a highly significant excess of group A individuals in socio-economic classes 1 and 2 and group O individuals in classes III, IV and V. The authors suggest a genetic rationale for these findings. They state that either pleiotropic effects of the ABO genes themselves or linked genes, for which the ABO genes may be markers, might be responsible for this socio-economic association. These conclusions are speculative and a relatively long-standing differentiation within the population may be more important as a cause of this

association. A further criticism of this study is the absence of sound a priori reasons for an association of socio-economic classes and ABO blood groups. Although these studies give a highly significant result it is statistically erroneous to survey data in search of correlations with no prior consideration of a rationale for an association (R.A. Elton, personal communication; Weiner, 1970).

Weiner (1970), excluded the association between blood groups and particular infectious diseases from criticism on lack of a priori probabilities, as many micro-organisms have been shown to have A, B and H-like antigens present (Springer, 1966). For a comprehensive account of blood group and disease relationships the reader is referred to the book 'Blood Groups and Disease' (Mourant et al., 1978).

2. ABO blood groups and susceptibility to infectious diseases

2.1 Early work

The original studies in the field of blood groups and infection were those of Pettenkofer and Bickerich (1960), who injected killed cultures of Pasteurella pestis into rabbits. They detected anti-H antibodies and postulated that the bacillus contained H antigen. They stated because persons of group A, B and AB possess little H antigen, it is possible to assume they are more able to produce anti-H than O persons; and thus, group O might be disadvantageous as regards natural selection. In 1960 Vogel, Pettenkofer and Helmbold put forward a comprehensive

hypothesis concerning the relationship between the great epidemics and the diversity of the world distribution of the ABO blood groups. This hypothesis was based on: historical records of the distribution and severity of recorded epidemics of plague, smallpox and cholera; previous discoveries of blood-group-like antigens in bacteria; and the finding of H-like activity in Pasteurella pestis, and an A-like one in vaccinia virus (Pettenkofer and Bickerich, 1960). From their observations they deduced that group O individuals would have a raised susceptibility to plague, and group A persons to smallpox. In 1962 Springer and Weiner published a severe criticism of their work. The most important objections raised were: Pettenkofer et al.'s lack of knowledge on the antigenic structure of the strains involved in these epidemics; the finding by Springer et al. (1961) of no significant blood group activity in 3 Pasteurella pestis strains; and the erroneous statement in Pettenkofer's hypothesis regarding the ability of non-O individuals to form anti-H antibodies and thus have selective advantage against P. pestis. Each of these, and other criticisms of Springer and Weiner (1962) were replied to by Pettenkofer et al. (1962). On the criticism of anti-H production by non-O individuals, they stated that certain non-O persons had no H-antigen and were thus able to produce anti-H; and these individuals were involved in the selection mechanism. This reply was unconvincing as the non-O individuals capable of producing anti-H are very rare (Levine et al., 1955; Levine et al., 1961) and thus unlikely to have been important in

selection processes.

The idea that the world distribution of blood groups is due to selection associated with disease is still open to investigation although it is difficult to substantiate due to the lack of verifiable scientific information on historic epidemics. Muschel (1966) has reviewed this topic in detail.

3. ABO blood groups and infection correlations

There is much evidence to suggest that there are correlations between an individual's ABO blood group and susceptibility to certain infectious diseases (Table III).

3.1 Enteric bacteria

In studies of a Chilean population it was found that persons of blood group B had a 50% greater probability than non-B persons of *Escherichia coli* urinary tract infections (Cruz-Coke *et al.*, 1965). Robinson *et al.* (1971) investigating enteric infections found that patients of groups B and AB were more susceptible to enteric infection, especially with *E. coli* and *Salmonella* species. These findings agreed with Socha *et al.* (1969) who found children of blood group B had a 55% greater risk of having infantile diarrhoea than non-B children.

3.2 Neisseria gonorrhoeae

In 1976 Foster and Labrum investigated the ABO blood groups of 584 asymptomatic female carriers and found a high incidence

of blood group B individuals among them. Miler et al. (1977) found that within a Caucasian population, group B persons were more susceptible than non-B persons, but they could not show the same susceptibility within a black population. Johnson et al. (1983) found no correlation with group B and increased susceptibility to gonorrhoea in male Caucasians. These findings suggest that the increased susceptibility to gonorrhoea for group B individuals might only occur with females and in well defined ethnic groups. Miler et al. (1977) studied male and female gonorrhoea patients in their survey but made no analysis of sex in their results.

3.3 Cholera

Barua and Paguio (1977) determined the ABO blood groups of cholera patients and found an increased percentage of group O persons over controls. Chaudhuri and Das Adhikary (1978) also found group O persons were significantly more susceptible to cholera and further that non-secretors of blood group substances were also more susceptible.

3.4 Viral infections

A higher incidence of influenza A_2 virus infections among group O individuals and a corresponding deficiency in group A individuals has been observed (McDonald and Zuckerman, 1962). In the same study the opposite trend was seen for adenovirus infections, group A being more susceptible and group O being less susceptible. Potter (1969) also found that influenza A

was more common in O persons, although more recent work by Mackenzie and Fimmel (1978) found that group B individuals were more susceptible. Possible reasons for the discrepancy in these results could be that different strains of influenza A were looked at. Mackenzie and Fimmel (1978) were working with influenza A (H3,N2) infections whereas the previous studies were with influenza A (H2,N2). Mackenzie and Fimmel cited other reports that expose the wide variation between blood groups and susceptibility to influenza A infections. Their own results suggested that genetic factors linked to ABO blood groups are involved in this susceptibility, but that any association must be indirect.

3.5 Miscellaneous infections

Group O and AB individuals have been found to have a higher incidence of periodontal disease, although in the same study no differences were found between susceptibility of secretors and non-secretors (Pradhan et al., 1971). Susceptibility to malaria has been correlated with blood group A (Gupta and Chowdhuri, 1980) and pulmonary tuberculosis with group O (Viskum, 1975).

4. Secretor status and infection correlations

The inability to secrete the water soluble blood group substances has been linked with the susceptibility to certain infectious diseases as shown in Table IV. Chaudhuri and Das Adhikary (1978) found that non-secretors were more susceptible

Table III Correlations between ABO blood groups and infectious agents/diseases

Infectious agent/disease	Susceptible blood group	Authors
E. coli (urinary)	B	Cruz-Coke *et al.*, 1965
E. coli (enteric)	B	Socha *et al.*, 1969
E. coli, Salmonella (enteric)	B+AB	Robinson *et al.*, 1971
N. gonorrhoeae	B	Foster and Labrum, 1976
N. gonorrhoeae	B	Miler *et al.*, 1977
N. gonorrhoeae	No correlation found	Johnson *et al.*, 1983
Cholera	O	Barua and Paguio, 1977
Cholera	O	Chaudhuri and Das Adhikary, 1978
Influenza A_2	O	McDonald and Zuckerman, 1962
Adenovirus	A	McDonald and Zuckerman, 1962
Influenza A	O	Potter, 1969
Influenza A	B	Mackenzie and Fimmel, 1978
Periodontal Disease	O+AB	Pradhan *et al.*, 1971
Malaria	A	Gupta and Chowdhuri, 1980
M. tuberculosis (pulmonary)	O	Viskum, 1975
Rheumatic fever and rheumatic heart disease	non-O	Haverkorn and Goslings, 1969 (16 pooled studies)

Table IV Correlations between secretor status and infectious agents/diseases

Infectious agent/disease	Susceptible group	Authors
Cholera	Non-secretors	Chaudhuri and Das Adhikary, 1978
Periodontal Disease	No correlation found	Pradhan *et al.*, 1971
N. gonorrhoeae	No correlation found	Johnson *et al.*, 1983
Rheumatic fever	Non-secretors	Glynn *et al.*, 1959
Rheumatic fever and rheumatic heart disease	Non-secretors	Haverkorn and Goslings, 1969 (9 pooled studies)
Carriage of Group A streptococci	Non-secretors	Haverkorn and Goslings, 1969

to cholera. Pradhan et al. (1971) found no correlation with periodontal disease and secretor state. The relationship between rheumatic fever and secretor status, first suggested by Glynn et al. (1956), is probably the best documented example of secretor status and disease correlation (Haverkorn and Goslings, 1969).

Haverkorn and Goslings (1969) pooled a total of 16 studies on relationships between ABO blood group and rheumatic fever and concluded that persons of non-O blood group were more susceptible to rheumatic fever. In 8 of 9 studies of rheumatic fever and secretor status they found that non-secretors were more susceptible. Haverkorn and Gosling cultured the throats of secretors and non-secretors and found an increased carriage of group A streptococci among non-secretors. Their hypothesis was that increase in carriage of group A streptococci among non-secretors may account for the increased frequency of non-secretors in rheumatic fever patients.

5. Common antigenic determinants between blood groups and bacteria

As with the H antigen detected in Pasteurella pestis and the A antigen in vaccinia virus (Pettenkofer and Bickerich, 1960), many other micro-organisms have been shown to have blood group like activity. Springer et al. (1961), employing a haemagglutination inhibition technique, demonstrated blood group antigenic activity among many strains of Gram-negative bacteria.

They selected 282 Gram-negative bacteria from 12 different genera and found blood group activity in half of them (Table V).

High activity for group B and H substance was noted with no bacteria having high A activity. Similarly, Drach et al. (1971) found 47% of 34 urinary tract pathogens with blood group A and B activity, many with both group A and B activity on the same strain of bacteria. Miler et al. (1977) found that gonococci were capable of absorbing anti-A and, to a reduced degree anti-B, isohaemagglutinins. They suggested that gonococci have group A and to a lesser extent group B antigens on their surfaces.

Table V. Distribution of blood-group activity among Gram-negative bacteria (from Springer et al., 1961).

Genus	Strains tested	A_1	B	H(O)	$A_1BH(O)$	A_1B	$A_1H(O)$	BH(O)	Inactive
Escherichia	135	8	18	22	6	3	3	4	71
Salmonella	19	1	2	9	0	0	1	0	6
Arizona	3	0	1	1	0	0	0	1	0
Klebsiella	42	2	6	4	3	1	1	5	20
Citrobacter	24	2	2	2	0	2	2	3	11
Pasteurella	8	0	1	0	0	0	0	2	5
Proteus	20	0	6	2	0	0	0	1	11
Pseudomonas	15	1	1	2	0	0	1	0	10
Serratia	2	0	0	2	0	0	0	0	0
Alcaligenes	8	0	0	0	0	0	1	1	6
Shigella	5	0	0	0	0	0	0	0	5
Herrellea	1	0	1	0	0	0	0	0	0
	282	14	38	44	9	6	9	17	145

6. Aspects of ABO groups that might affect susceptibility to infections

6.1 Direct action of isohaemagglutinin

The presence of blood group substances on bacteria provides evidence for possible correlations between ABO blood group and

bacterial infection. It might be predicted, that if the bacteria possessed an antigen resembling that of blood group A, then group A persons, being unable to make an anti-A antibody would be less protected than group O and B persons who already have the anti-A antibody. The possible protective effects of the isohaemagglutinins have been investigated. Muschel and Osawa (1959) have shown anti-B to be bactericidal against E. coli 086.

6.2 Isohaemagglutinin binding to bacteria

Check et al. (1972) found that anti-B was capable of opsonising E. coli 086. Other effects of isohaemagglutinins, such as interaction with blood group-like antigens on bacterial cell walls, thus inhibiting attachment to mucosal cells, are theoretically possible.

6.3 Effect of ABO antigens in attachment

Boat et al. (1978) found that water soluble ABO antigens inhibited haemagglutination of influenza B virus and suggested that some of these substances may interfere with access of influenza virus to binding sites. One of the protective roles of these blood group substances may be their ability to occupy or in some way interfere with binding sites either on the bacterium or on the epithelial cell, preventing attachment of the bacteria. The ABO antigens on epithelial cells, as in the case of P antigens in pyelonephritic urinary tract infection (Källenius et al., 1981), may be involved in binding of bacterial adhesins.

Epidemiological studies of genitourinary tract infections

1. Clinical and epidemiological aspects of urinary tract infections

1.1 General

Urinary tract infections (UTI) represent a common group of diseases characterised by significant bacteriuria. Aerobic Gram-negative bacilli cause the majority of UTI, but occasionally other micro-organisms such as staphylococci or enterococci are involved. *E. coli* is the predominant pathogen accounting for 80-90% of primary non-obstructive UTI (Holmgren and Smith, 1975). The symptoms and severity of the acute disease vary markedly and UTI may be divided into pyelonephritis patients, and patients with uncomplicated UTI. Uncomplicated UTI includes asymptomatic bacteriuria patients and those patients with dysuria, frequency and significant bacteriuria (cystitis). Pyelonephritis is an ascending infection of the urinary tract which affects the kidneys and is characterised by pyrexia, rigors, loin pain, frequency, dysuria and significant bacteriuria.

1.2 Complications

The risk of renal damage from UTI is considerable. Winberg *et al.* (1974) in a study of UTI in children, monitored patients from their first known infection and noted a reduction in parenchyma in 13% of boys and 4.5% of girls. This reduction in renal parenchyma may result in renal insufficiency and hypertension.

1.3 Epidemiology

Urinary tract infection is probably one of the most common nonepidemic bacterial infections. It is estimated that 10 to 20%

of women, with otherwise normal urinary tracts, will have at least one urinary tract infection during their lifetime and many will have repeated infections (Santoro and Kaye, 1978). Recurrences of UTI are common. Bergstrom et al. (1968) noted a recurrence rate of 30% for children, and for those with recurrences, 60% go on to have further infections.

In school screening programmes the prevalence of significant bacteriuria is around 1% in girls, and about 3% of all girls will have had at least one symptomatic infection before the age of 10 years (Winberg et al., 1974). In males UTI is less common (women outnumber men in the ratio of 30:1) but a high frequency is found during the first years of life (Winberg et al., 1974). UTI is generally a disease of young women with the probability of UTI increasing as they become sexually active and begin to bear children. Several factors may give an increased risk of UTI and these include vesicoureteric reflux and incomplete bladder emptying.

The prevalence of UTI, as noted above, with the significant risk of renal damage makes it an important cause of morbidity whilst the majority of UTI are undramatic, a large population nevertheless suffers from recurrent UTI and this makes considerable demands on medical resources. Recurrences are high and complications involving renal parenchymal reduction (particularly in children) can be severe. Thus, studies examining underlying mechanisms of resistance or susceptibility may be of value in recognising 'at risk' populations.

2. Clinical and epidemiological aspects of gonorrhoea

2.1 General

Gonorrhoea, an infection generally of the mucosal surfaces of the genito-urinary tract with the bacterium Neisseria gonorrhoeae, is transmitted mainly by sexual intercourse. Infection, in 90% of males, is characterised by a purulent urethral discharge 4-10 days after contact and by dysuria and frequency of micturition; also in males the infection may spread to the epididymis and prostate. In women the urethra and cervix are commonly infected, and in 25-50% of cases, the rectal mucosa may also be involved. In contrast to males, gonorrhoea in females is largely asymptomatic, and lack of treatment predisposes to complications. Pregnant women with gonorrhoea can transmit eye infections to their infants during birth. Although eye infections are rare in adults, pharyngeal colonisation occurs occasionally (3-11%) in gonorrhoea patients of both sexes (Noble et al., 1979). Homosexual men may also contract rectal infections.

2.2 Complications

In most cases gonorrhoea is uncomplicated and is a relatively innocent disease of the accesible membranes. However, in about 10% of women the infection may spread from the cervix to the endometrium and fallopian tubes (salpingitis). Further, in some 2 to 15% of women, genital gonorrhoea may give rise to pelvic inflammation (Falk, 1965) which may result in infertility (Weström, 1980). Systemic spread of gonorrhoea, disseminated

gonococcal infection (DGI), appears to occur in up to 3% of infected patients (Knapp and Holmes, 1975). DGI may give rise to such manifestations as arthritis, endocarditis and skin lesions.

2.3 Epidemiology

Gonorrhoea is a disease with world-wide distribution. In the United States each year, approximately 2.6 million infections occur. (Today's VD Control Problem, 1975). The incidence of the disease in England and Wales fell after the Second World War when there was a peak of 47,343 cases in 1946, to a trough of 17,536 cases reported in 1954. The incidence began to rise in the late 1950's, and the figures for 1980 show 54,433 cases of gonorrhoea reported in that year (Extract from the Annual Report of the Chief Medical Officer of the Department of Health and Social Security 1981, 1983). Gonorrhoea is mainly a disease of young adults in the 15-24 year age group (WHO, 1978). Previously the incidence for males was greater than that for females but now this ratio is tending towards unity.

3. Aims of the epidemiological studies

The foregoing sections indicate the likelihood of associations of blood group with UTI and gonorrhoea. Secretor status, although implicated in various infections, has not been considered in susceptibility to UTI or gonorrhoea - thus these studies set out to confirm associations of blood group with UTI and

gonorrhoea and to determine any associations with secretor status. The aims of the epidemiological studies were:

1. To determine if the distribution of ABO blood groups among women with recurrent UTI differed from that of the general population.
2. To determine if secretor status influenced susceptibility to recurrent UTI in women.
3. To determine if ABO blood group influenced susceptibility to gonococcal infection.
4. To determine if secretor status influenced susceptibility to gonorrhoea.
5. If there were correlations of incidence of UTI and gonorrhoea with blood group and/or secretor status, to propose and test hypotheses that would explain why these factors influence susceptibility.

HOST-PARASITE INTERACTIONS

Attachment in host-parasite interactions

1. General introduction

Although bacteria can enter the host directly at sites of trauma, attachment of the bacteria to host surfaces is generally a prerequisite for infection to occur (Smith, 1977).

Attachment to host cells can be viewed in two ways: as a virulence attribute for micro-organisms assisting their colonisation and invasion; or as an important initial step in phagocytosis and killing of microbes. In some circumstances, however, micro-organisms can resist phagocytic digestion and this step is to the advantage of the micro-organism (Veale et al., 1977; Smith, 1976; Novotny et al., 1977). Thus attachment can be both beneficial and harmful for micro-organisms.

2. Contact and adherence in colonisation and invasion

The attachment process may be a passive adsorption of the microbe to the cell surface or an active process in which the motile organism actively moves towards its target site. Both host and bacterial cell surfaces have anionic charge densities. Van der Waal's forces, salt bridging and surface tension may help to overcome these mutual charge repulsion effects. In addition both the host and parasite can exhibit cell surface properties that help to reduce the effect of these electrostatic forces.

2.1 Microbial factors

Bacterial characteristics that help to overcome the repulsion of like charges include specialised structures such as pili, capsules and surface arrays. For example, the carbohydrate-rich coat of lactobacilli may alter the surface charge attributes and so aid the adherence to chicken crop epithelium (Brooker and Fuller, 1975). Surface structures of bacteria such as the pili of gonococci, or fimbriae of E. coli are cell extensions that may be of great importance in attachment. Cell extensions overcome the repulsive forces between bodies. This repulsion is directly related to the radius; as the radius becomes smaller so the energy barrier between the objects is reduced. Pili or fimbriae are ideal for this purpose. Their tips have extremely small radii that allow the bacterium to approach the host surface closely enough for hydrogen or ionic bonds to form. Once contact is made with the host cell, various consequences may ensue: the initial weak contact may be lost due to shearing; further appendages may attach; or the probability of surface to surface contact may be increased due to the proximity of the two cell membranes, and in this way strong adhesion may occur. A further consideration is that in surface to surface interactions of physical bodies, electrostatic forces are relatively long range, and as the surfaces come closer, the shorter range attractive forces between bodies come into play (Watt and Heckels, 1982). Such interactions between micro-organism and host surface are essential

for strong adherence to occur. Neisseria gonorrhoeae would appear to be capable of firm attachment as they can withstand the fluid flows of micturition (Ward and Watt, 1972), and commensal Neisseria remain attached despite pharyngeal washing action (Weistreich and Baker, 1971).

2.2 Host factors

Particular host cells may have receptors or extracellular products for binding specific bacteria (Gibbons and van Houte, 1971) that are also capable of aiding the adhesion phenomena. Fibronectin isolated from fibroblasts has been shown to actively attach to Staphylococcus aureus (Kuusela, 1978). Many of these interactions are very specific. The interaction between the glycosphingolipid of the P blood group and P-fimbriae of pyelonephritic strains of E. coli is a particularly good example (Leffler and Svanborg-Edén, 1980; Källenius et al., 1981).

This glycosphingolipid, with a specific galactosyl-galactose structure, is part of the P blood group antigens. Among E. coli strains isolated from the urinary tract of acute pyelonephritis patients 91% had P blood group-specific adhesions (P-fimbriae) (Källenius et al., 1981). The P blood group antigens have been proposed as receptors for the P-fimbriae of the pyelonephritis-associated E. coli. Only individuals of the rare blood group $\overline{p}$ (1 in 10^5) have cell membrane glycolipids devoid of this receptor. These receptors are present on many cell types including red blood cells and urinary tract epithelium.

P-fimbriae can be considered to be a virulence marker and

their detection on E. coli strains may be a useful indicator of pyelonephritis in patients with bacteriuria. It has also been suggested that persistence of E. coli with P-fimbriae in the faecal and periurethral flora might be an indication for long-term prophylaxis (Källenius et al., 1981).

Individuals with the P-blood group antigens form the majority of the population and screening patients for their P-blood group, to determine risk groups, would be of little clinical importance. This contrasts with the associations of ABO blood group with infections whereby individuals are considered to be more susceptible to an infection if they belong to a particular blood group. Examination of ABO antigens for similar roles in bacterial adhesion is needed.

Contact and adherence of micro-organisms to host cell surfaces is a complex event with many different physical and chemical forces involved. Specific surface properties of both micro-organism and host cell must be considered in the analysis of these interactions, and one of the recurring criticisms of a number of studies on attachment of N. gonorrhoeae to epithelial surfaces is that the buccal or HeLa cells used are not target cells for the gonococcus.

3. Contact and adherence with phagocytes

Once the microbe has breached the mucosal barrier, the phagocytic cells provide a second line of defence, enhancing the elimination of the invading pathogen by phagocytosis and intracellular killing.

Macrophages, in addition, have an important role in presentation of antigen to lymphocytes. For this to occur the bacterium or its antigens have to adhere to the macrophage. Reduction or prevention of such binding may interfere with the induction of the immune response against a micro-organism. Macrophages and neutrophils are both capable of chemotaxis, directed migration towards sites of infection and are part of the inflammatory response. Many chemotactic factors for PMN and macrophages have been described (Gallin, 1980), most being chemotactic for both cells, but some have a preferential effect on one cell type or another. Staphylococci and streptococci attract more neutrophils than monocytes, the opposite being true for typhoid and tubercle bacilli. Bacterial products themselves can be chemotactic and some, such as the LPS of Gram-negative bacteria, activate complement and so generate other chemotactic factors. Gonococci are also capable of budding off LPS (Stead et al., 1975) which may further enhance chemotaxis.

Once the phagocyte has migrated towards the bacterium adherence may occur with subsequent phagocytosis. Both monocytes and neutrophils have receptors for the Fc fragment of immunoglobulin and for complement components (Allison, 1982). These receptors can function as recognition sites on phagocytic cells and mediate the binding of bacteria coated with antibody and/or complement. Certain bacteria can also activate the alternative complement pathway and so can adhere via complement receptors, in the absence of antibody (Jasin, 1972).

In the non-immune host, in the absence of antibody and without activation of complement, binding of bacteria to phagocytes can still occur. The mechanisms whereby such interactions take place are largely unclear and physicochemical interactions such as charge effects and hydrophobic binding have been proposed (Wilkinson, 1976).

In the last few years lectin-like receptors on phagocytic cells have been described. These recognise bacterial cell wall sugars and are responsible for adherence of bacteria to the phagocyte membrane (Ögmundsdóttir and Weir, 1976; Freimer et al., 1978; Ögmundsdóttir et al., 1978; Glass et al., 1981; Weir, 1980). These receptors may be important in the pathogenesis of bacterial infections and discussion of gonococcal interaction with phagocytes will be covered in the next section.

Neisseria gonorrhoeae

1. Introduction

Gonorrhoea is the model chosen to investigate the recognition of genito-urinary pathogens by phagocytic cells and the possible role of ABO blood groups in binding interactions. Neisseria gonorrhoeae is a small kidney-shaped Gram-negative diplococcus, with the long axes parallel and the opposed surfaces slightly concave. In addition Neisseria species are recognised by their ability to oxidise dimethyl-, or tetramethyl-paraphenylene diamine (oxidase reagent). The gonococcus cannot be distinguished from other Neisseria by the oxidase test or microscopy; sugar utilisation tests are required to distinguish one species from another (Table VI).

Table VI Sugar utilisation of Neisseria species

Species		Glucose	Maltose	Fructose	Sucrose	Lactose
N. gonorrhoeae		+	-	-	-	-
N. meningitidis		+	+	-	-	-
N. lactamica		+	+	-	-	+
N. subflava	N. pharyngis	+	+	-	-	-
N. flava	N. pharyngis	+	+	+	-	-
N. perflava	N. pharyngis	+	+	+	+	-
N. sicca	N. pharyngis	+	+	+	+	-

1.1 Colonial characteristics

Gonococci grown on solid medium give rise to a variety of different colony forms. Kellogg et al. (1963) described four

frequently observed colony types, naming them T1 to T4. They observed that only certain colony types were virulent when inoculated into the urethra of male volunteers and that T1 colonies predominate in primary cultures from clinical material. Additional colony types have been recognised, T5 (Jephcott and Reyn, 1971) and T_1^1 (Chan and Wiseman, 1975). Chan and Wiseman (1975), investigating the virulence of these colony types in the chick embryo found T_1, T_2 and T_1^1 to be virulent whereas T_3, T_4 and T_5 are essentially the avirulent laboratory associated types. Daily selective subculture of T_1, T_2 and T_1^1 colonies is required if these colonies are to be maintained <u>in vitro</u> as non-selective subculture leads to the emergence of T_3, T_4 and T_5 colonies.

Swanson (1978a) noted that each of these colony types may represent a combination of several phenotypic characteristics, including pilation, degree of pilation, colour and opacity. All of these phenotypic characteristics may have relevance in virulence, particularly colony opacity and pilation (Swanson, 1980). Although the majority of gonococci isolated from males or females give T_1 or T_2 colonies (Sparling and Yobs, 1967), variations in degree of pilation and colonial opacity occur particularly among women at different stages of their menstrual cycle (Swanson, 1980).

2. The gonococcal cell envelope

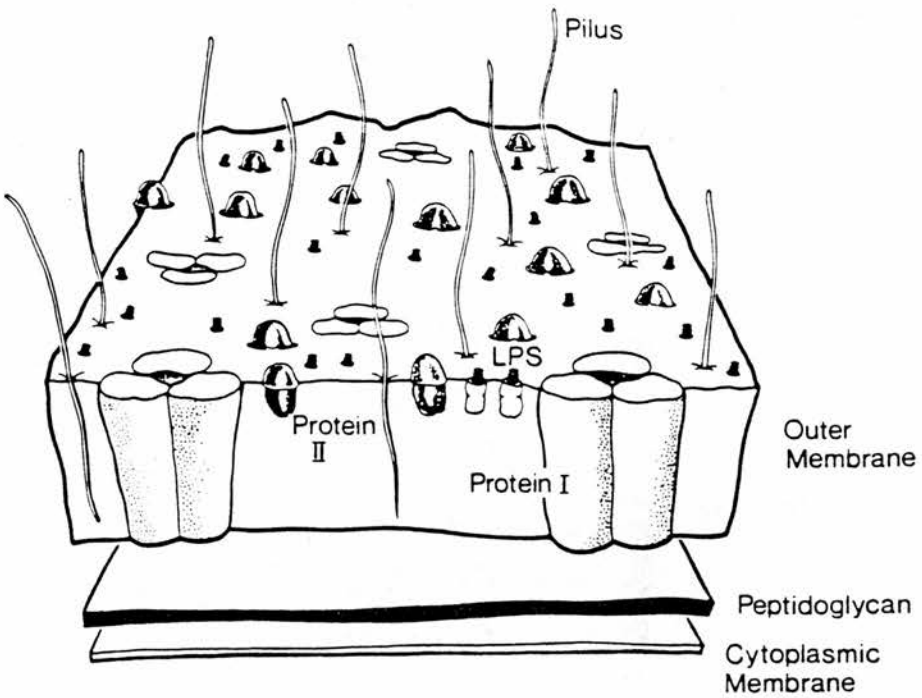


Figure 2 Schematic diagram of the gonococcal outer surface (from Watt and Heckels, 1982)

2.1 General comments

The gonococcal cell envelope (Figure 2) conforms to the general characteristics of a Gram-negative cell: outer membrane structure, peptidoglycan layer and an inner cytoplasmic membrane (Swanson et al., 1971). Components of the gonococcal cell envelope will be discussed with regard to their importance in host parasite interactions.

2.2 Pili - structure

Non-flagellar protein appendages were termed 'fimbriae' by Duguid *et al.* (1955) but Brinton (1965) suggested that 'pili' should replace this term. Although fimbriae was the first designation given to these appendages, workers studying gonococci use the term pili, and to avoid confusion this term will be used here.

Pili are filamentous protein appendages which extend several microns from the surface of gonococci. Swanson *et al.* (1971) observed that virulent colonial types of gonococci were pilate whereas non-virulent colonial types were not. Following this observation attention was focused on pili as a virulence factor. Earlier, Kellogg *et al.* (1963) had shown that type 1 colonies were virulent, producing infection in volunteers. Type 4, the non-pilate gonococci, were unable to produce infection. Similar results were obtained with chimpanzees (Brown *et al.*, 1972) and in the chick embryo model (Buchanan and Gotschlich, 1973).

The association of pilation with adhesion and increased virulence has promoted considerable interest in the structure and immunochemistry of pili. Gonococcal pili have been analysed by several workers. They consist of a repeating polymeric array of a single pilin protein subunit of molecular weight between 17,000 and 21,000 daltons depending on the individual strain (Buchanan and Pearce, 1976).

Pili from different strains are similar in overall amino acid composition and have a high proportion of hydrophobic amino

acids (Robertson et al., 1977; Hermodsen et al., 1978). Robertson et al. (1977) detected additional pilus components including phosphate groups, galactose and trace amounts of glucose. Despite some structural homogeneity, pili can show considerable antigenic heterogeneity between strains (Buchanan, 1978) and within strains (Lambden et al., 1980 and Lambden et al., 1981). Antigenic intra-strain variation was found among the pili from opaque and transparent colonies of a number of strains (Salit et al., 1980). Further variation has been noted during the course of natural gonorrhoea infections (Watt and Heckels, 1982) and these observations suggest a role for pilus variation in the pathogenesis of gonorrhoea. The apparent ability of gonococci to modify their immunodominant regions and create unique antigenic types is important in considering the development of anti-pilus vaccine in prevention of gonorrhoea.

Lambden et al. (1980) have shown two distinct types of pili from isogenic variants of strain P9. These pili, termed alpha and beta, are from transparent and intermediate/opaque colony types respectively. They differ not only in the molecular weight of their subunits, alpha having subunits of 19,500 and beta of 20,500 Daltons, but also in their attachment to buccal epithelial cells.

2.3 The attachment role of pili in virulence

The importance of attachment in colonisation and infection was described earlier, as was the mutual repulsion of electrostatic forces between negatively charged bacteria and host cells. An

explanation of the association of pili with virulence and adherence is offered by Heckels et al. (1976). Experiments using gonococci which have been chemically modified to alter their surface charge suggest that one role of pili in adhesion is to overcome the repulsive electrostatic barrier existing between gonococci and the host cell surface. Initial attachment by pili would increase the probability of a close approach and favour attachment since attractive forces act over shorter range than the repulsive forces. Stable adhesion would then occur by direct interactions of the two surfaces. These interactions would presumably occur at a lower frequency in non-pilate gonococci.

Pilate gonococci have been found to adhere more efficiently to certain human tissues than non-pilate organisms; epithelial cells (Punsalang and Sawyer, 1973), sperm cells (James-Holmquest et al., 1974), erythrocytes (Punsalang and Sawyer, 1973), vaginal epithelial cells (Mardh and Weström, 1976), fallopian tube epithelium (Ward et al., 1974), and tissue culture cells - human amnion (Swanson, 1973) and HeLa (Swanson et al., 1975).

Punsalang and Sawyer (1973) preincubated pili with the following simple sugars; D-glucose, maltose, D-mannitol, dulcitol, D-sorbitol, raffinose, saccharose, lactose, D-fructose, D-galactose, D-mannose and inulin. They were unable to demonstrate any inhibition of pili binding to rabbit erythrocytes. The specificity of the pilus-cell interaction has also been investigated by comparing the binding of pilate and non-pilate variants to buccal epithelial cells. Attachment was unaffected by the presence of the simple sugars, lactose, melibiose, mannose, α-methyl mannoside,

galactose, α-methyl galactoside, β-methyl galactoside, fucose, N-acetylglucosamine and N-acetylgalactosamine (Trust et al., 1980). Attachment of pilate gonococci, however, was reduced to the lower level of non-pilate gonococci by the action of the following mixture of glycosidases on the host cell membrane: α- and β-N-acetylhexosaminidase, α- and β-mannosidase, α- and β-glucosidase, α- and β-galactosidase, α-L-fucosidase and β-xylosidase. They suggested that an oligosaccharide on the surface of the host cell may function as a pilus receptor.

Buchanan et al. (1978) came to a similar conclusion from binding studies of ^{125}I-labelled purified pili to a range of human cell types. Attachment to buccal cells was inhibited by gangliosides suggesting that an oligosaccharide structurally related to the ganglioside was present in a surface glycolipid or glycoprotein, acting as a pilus receptor. This may be similar to the glycosphingolipid associated with the adhesion of pyelonephritic E. coli to erythrocytes and uroepithelial cells described previously (Leffler and Svanborg-Edén, 1980).

Host and bacterial factors involved in the binding of gonococci vary during the course of a natural infection. The gonococci are found in different anatomical locations in contact with different epithelial cell types and there are variations in their environment such as pH and fluid flows. James and Swanson (1978) found that pilate gonococci varied in their ability to attach to vaginal epithelial cells depending on the course of the menstrual cycle.

As described previously, there is also great variation in the pili themselves; intra-strain variation in pili can result in differences in binding to buccal cells (Lambden et al., 1980; Lambden et al., 1981). Alpha pili bound optimally at pH 6.5 and beta pili at the more acidic pH of 4.5. In general alpha bound better than beta. Alpha also differed from beta pili in that the removal of sialic acid residues from buccal cell surface carbohydrates by neuraminidase markedly reduced alpha pili binding but had no effect on beta-pili binding. Further treatment of the neuraminidase - modified cells by a mixture of exoglycosidases, removing sugar residues, reduced the level of alpha binding to that of the beta pili. It was suggested that alpha pili bind to buccal cells through a specific receptor involving sialic acid and other unspecified sugar residues whereas beta pili are unable to recognise such receptors (Lambden et al., 1980). Heckels (1982) has shown, in adhesion studies, that 4 different pilus types from strain P9, have specificities that vary with cell type. He has also shown that pilus type may differ within the same strain depending on the environment, for example, cervix and urethra of females and the urethra of a male partner. These facts suggest that during their growth in vivo, gonococci are capable of varying their structural make-up, with effects on their affinity for various target cells.

Properties of pili that are not associated with adhesion, but may be involved in virulence have been described, for example in their interaction with phagocytes. Whilst no difference

has been observed between binding of pilate and non-pilate gonococci to polymorphonuclear leukocytes (PMN) (Swanson et al., 1974; Swanson et al., 1975), pili do appear to slow down ingestion of gonococci (Thomas et al., 1973) and pilate gonococci may be more resistant to phagocytosis than non-pilate variants (Dilworth et al., 1975; Blake and Swanson, 1975). Densen and Mandell (1978) suggested that pilate gonococci adhering to PMN have pili radiating across the PMN surface which may disrupt membrane motility and thus interfere with phagocytosis.

Pilate gonococci may be more capable of binding iron than non-pilate gonococci (Payne and Finkelston, 1975). This may enhance the virulence of pilate gonococci in the in vivo situation as iron in vitro enhances growth (Kellogg et al., 1963) and competition with the host for iron is held to be an important factor in establishment of many bacterial infections (Weinberg, 1974).

Lastly, pili may be involved in exchange of genetic material between gonococci. It has been found that pilate gonococci are highly efficient in the uptake of transforming DNA (Sparling et al., 1980). During the course of the natural infection, transformation may play an important role in the rapid exchange of genetic information between strains.

2.4 Outer membrane proteins

Analysis of the gonococcal outer membrane has shown that, like other Gram-negative bacteria, it contains lipopolysaccharide

and relatively few proteins. The outer membrane protein profile of gonococci varies considerably even within a single strain. The major proteins present, however, can be classified into three major types: I, II and III.

Protein I, formerly called the major or principal outer membrane protein (Swanson and Heckels, 1980), comprises 50% of the total protein in the outer membrane (Sandström et al., 1982b), is present in all gonococcal phenotypes and has a subunit molecular weight between 32,000 and 40,000 depending on the strain. Protein I probably exists as a trimer (Newhall et al., 1980) spanning the outer membrane, linked to the underlying peptidoglycan (Heckels, 1979) and extending onto the surface. The structure of the Protein I trimer suggests its function is structural in maintaining a hydrophilic diffusion pore analagous to the transmembrane proteins of E. coli allowing access into the cell of essential nutrients (Heckels and James, 1980). Douglas et al. (1981) have, to a large extent, confirmed this hypothesis.

One or more types of Protein II can be found in gonococci from opaque colonies and have molecular weights varying from 24,000 to 30,000 (Swanson, 1978b). Proteins I and II are both readily labelled by ^{125}I and lacto-peroxidase, indicating their presence on the gonococcal surface (Heckels, 1978 and Swanson, 1978b). This contrasts with protein III, which is poorly labelled (Heckels, 1978) suggesting it is largely absent from the cell surface. Protein III has a molecular weight of 34,000 and is probably found in close association with protein I, perhaps replacing one unit in a protein I trimer (Swanson, 1981).

In colonial variants of the same strain, protein II can vary to such an extent that less than 5% shared antigenicity can be detected when antisera raised against one type of protein II is reacted with other protein II species (Diaz and Heckels, 1982). Protein III cross reactivity between strains is considerable, suggesting it would not be of value in serological typing (Swanson, 1981).

Serological classification systems based primarily on protein antigens have been developed (Johnston et al., 1976; Wang et al., 1977; Buchanan and Hildebrandt, 1981; Sandström and Danielsson, 1980). Protein I, stable within a strain, is the principal protein involved in these typing systems.

These protein I serotypes would appear to have clinical significance. Sandström and Danielsson (1980) have developed a W-antigen serological classification which divides gonococci into two major (WI and WII) and one minor (WIII) serogroup by coagglutination (COA). These serogroups correlate with different clinical syndromes. WI serogroup has been correlated with disseminated gonococcal infection (DGI) and resistance to the bactericidal action of normal human serum; WII and WIII serogroups have been correlated with resistance to antimicrobial agents; and WIII serogroup is uniformly sensitive to the bactericidal action of normal human serum (Sandström et al., 1982a). Buchanan and Hildebrandt (1981), using purified protein I have defined nine different serotypes, and it would appear that different clinical syndromes such as salpingitis, may be associated

with particular protein I serotypes (Buchanan et al., 1980). These various protein I based typing schemes overlap. The COA reagents, however, detect the more common antigenic determinants and other serotyping schemes can detect subsets within the COA serogroups (Sandström et al., 1982a).

2.5 The role of outer membrane proteins in adhesion to epithelial cells

Lambden et al. (1979) have shown that opaque variants with various protein II profiles show enhanced binding to buccal cells over the transparent variants without protein II. Heckels and James (1980) confirmed this finding and further found that trypsin treatment reduced protein II binding to that of the non-protein II or transparent variants. No decrease in binding occurred within trypsin treated transparent variants that had protein I but no protein II. This suggested that protein I does not play a significant role in adhesion to buccal cells. Buccal cells are not the natural target cell for gonococci and although these data demonstrate the importance of protein II species in attachment of opaque colony forms to buccal cells, this adhesion model is obviously not ideal. James et al. (1980) have shown that with different tissue culture lines - two HeLa and one human embryonic lung fibroblast line - transparent variants can attach more than opaque variants.

An explanation for the isolation of transparent colonies from salpingitis cases is offered by Draper et al. (1980) who

found an increased adhesion of transparent variants over opaque to human fallopian tubes. Lambden et al. (1979) have shown that specificity in attachment occurs not only between opaque and transparent forms, but also between gonococci with different protein II species in the same strain. Further differences in attachment to buccal and tissue culture cells for gonococci with different protein II species have been demonstrated (Heckels, 1982). This differential adhesion to different cell types suggests that variations in outer membrane protein structure may play an important part in gonococcal colonisation of different surfaces in the natural infection.

2.6 The role of outer membrane proteins in attachment to phagocytes

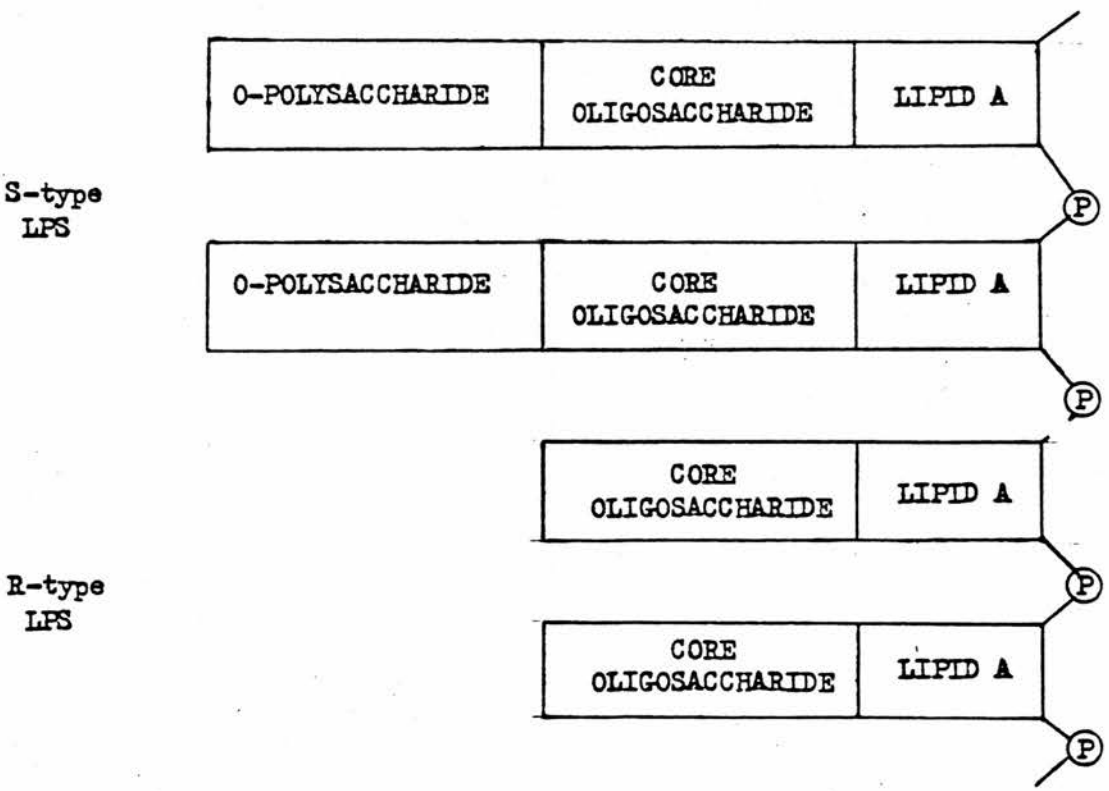
King and Swanson (1978) have isolated a protein of 28,000 or 29,000 Daltons, (depending on the strain) which mediates the attachment of gonococci to PMN. Antisera raised against this protein inhibits binding of the bacteria to PMN. King and Swanson have termed this protein leukocyte association protein (LAP) and find abundant quantities on some gonococci (LA+) and much smaller quantities on other gonococci (LA-). LA+ and LA- designations are independent of colonial opacity or degree of pilation. LAP binding may be mediated by a sugar moiety as periodate treatment of gonococci markedly reduced association. King and Swanson (1978) were unable to block gonococcal binding to PMN with the simple sugars mannose, galactose, fucose and xylose but paradoxically found that pretreatment of LA+ GC with

periodate markedly reduced the association with PMN. They concluded that the contribution of sugars in LAP and other components of the bacterial outer surface to interaction between PMN and gonococci is unclear. Heckels (1982) found variations in attachment to leukocytes that depend on differences in protein II species of the gonococci. This suggests that variations in protein II can influence attachment to phagocytic cells as well as epithelial cells.

2.7 Lipopolysaccharide

Lipopolysaccharide (LPS) is located on the outermost aspect of the Gram-negative cell envelope (Shands, 1965) (Figure 2).

The detailed structure of gonococcal lipopolysaccharide (LPS) has not yet been elucidated, but two types of LPS, rough (R) and smooth (S) are possible. These are diagramatically represented below:

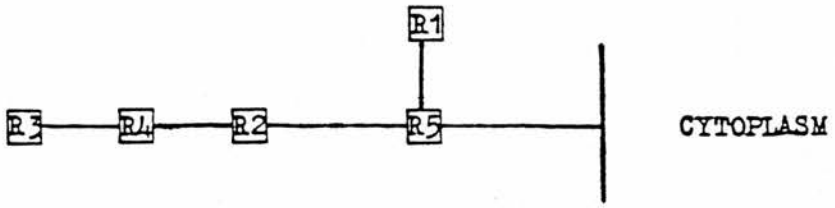


Contradictory reports have appeared on the ability of gonococci to produce rough or smooth LPS. Stead et al. (1975) suggested an R-type LPS structure for gonococci with lipid A attached through 2-keto-3-deoxyoctulosonic acid (KDO) to an oligosaccharide containing glucose, galactose, glucosamine and heptose. Perry et al. (1975) confirmed this, although they found pilate gonococci had additional O-polysaccharides containing sugars such as galactosamine, rhamnose, xylose or fucose depending on the strain examined. Wiseman and Caird (1977) reported analyses that suggested smooth type LPS for all colony types of gonococci, but that rhamnose was only present in non-pilate variants. However, Perry (1978) reported that S-type LPS could only be detected in minor amounts. Since it has been shown that LPS can be affected by factors such as environmental conditions (McDonald and Adams, 1971), it is possible that these differences were due to variations in media and cultural conditions. Watt and Heckels (1982) state that from their own and other unpublished observations, gonococci grown under usual conditions (unspecified) have largely, if not entirely, LPS of the R-type with a short oligosaccharide linked to lipid A.

Immunochemical evidence suggests that LPS from different strains have a large degree of structural homology (Wallace et al., 1978).

Differences in LPS structure, presumably due to differences in sugar linkages in the oligosaccharide, have been detected by ELISA and immunodiffusion techniques, and used as a serotyping scheme (Apicella and Gagliardi, 1979). The receptor site for

the rod type (R-type) pyocins of Pseudomonas aeruginosa has been shown to be in the LPS of Pseudomonas (Govan, 1974; Koval and Meadows, 1977). A similar location has been found for these R-type pyocins in gonococci (Sadoff et al., 1978). Kageyama (1975) proposed pyocin receptor sites for his groups R1-R5 in the LPS of Pseudomonas, and these are depicted diagramatically below:



The 'R' numbers correspond to sites thought to be essential for attachment of each group of pyocins; mutants lacking the receptor for R3 were resistant to these pyocins but still had R4, R2, R1 and R5 receptor sites, and were sensitive to those pyocin groups. Gonococci were found to be sensitive to pyocins of groups R1 and R5, suggesting that there may be similarities in the 'deeper' portions of the LPS of Pseudomonas and Neisseria. Gonococcal strains have been classified into two broad groups: group I strains, that were sensitive to Kageyama group R5 pyocins and group II strains, that were sensitive to groups R5 and R1. The LPS of group I strains was termed 'simple' and the LPS of group II strains 'complex'. The major application of the pyocin typing system has been to define these two groups that interact very differently with the immune system (Blackwell et al., 1979; Winstanley et al., 1983).

2.8 Biological role of lipopolysaccharide

Gonococcal LPS possesses endotoxic activity and gonococci can bud off outer membrane containing LPS (Stead et al., 1975). LPS released from gonococci can be lethal to ciliated cells of the human fallopian tube (Melly et al., 1981). Thus LPS may contribute to virulence and anti-LPS antibodies have been shown to be protective in embryonated eggs, from hens which were pre-immunised with LPS (Diena et al., 1978).

Antibodies directed against LPS are bactericidal for gonococci and are more effective in killing than antibodies raised against purified outer membrane proteins or pili (Ward et al., 1978). The bactericidal effect of normal human serum is largely due to the presence of antibodies cross-reactive with LPS (Rice and Kasper, 1977). Gonococci that are not sensitive to the bactericidal effect of normal human serum lack the LPS antigen to which the lytic antibody is directed (Schneider et al., 1981). Further *in vitro* evidence for this has been reported by Winstanley et al. (1983), who examined gonococcal strains that differed in their LPS components. Gonococci were classified, as described previously, into two broad groups: group I and group II. Group II strains, those with a complex LPS, were found to be serum sensitive and group I strains, those with a simple LPS, were resistant. This suggests that the bactericidal effect of normal human serum (NHS) for strains of *N. gonorrhoeae* is mediated by antibodies directed against an LPS component only present in gonococci of group II. Group I strains are resistant to NHS which may be due to the absence of the more distal LPS component(s) associated with group II strains.

Frasch (1980) suggested that LPS is the specific outer membrane component involved in agglutination of gonococci by wheat germ agglutinin (specific for N-acetyl-glucosamine). Watt et al. (1978) suggested that the terminal sugar groups on gonococcal LPS might be involved in attachment to receptors present on host cell surfaces. They tested their hypothesis by preparing liposomes with the immunodeterminants of the LPS expressed at the surface and carried out binding assays with HEp-2 cells. Non-specific binding of liposomes occurred at high concentrations. At very low concentrations of liposomes, however, they found enhanced binding, compatible with specific binding to receptors that are present in only small numbers. Thus these terminal LPS sugar groups would seem to be capable of binding to specific receptors on the surface of the epithelial cells. This adhesive role for sugar groups on the gonococcal surface was further supported by the abolition of binding to HEp-2 cells and 50% reduction in binding to buccal cells after chemical disruption of the gonococcal surface sugars. This data suggests that sugar residues on the gonococcus may be involved in attachment and that these residues could be the terminal LPS sugar groups. Sugar residues may however be associated with other gonococcal surface components but the detailed structure of the major proteins has yet to be resolved with respect to their carbohydrate content.

2.9 Capsule

Gonococcal capsular-like material has been described for

organisms grown in a variety of special conditions (Hendley et al., 1977; James and Swanson, 1977; Richardson and Sadoff, 1977; Demarco de Hormaeche et al., 1978). More recent observations suggest that this capsule may be an artefact (Melly et al., 1979). Demarco de Hormaeche et al. (1983) compared "encapsulated" and "non-encapsulated" variants and found only the encapsulated variant could produce infection in chambers subcutaneously implanted in mice. These encapsulated and non-encapsulated variants also differed in their serum sensitivities and antigenic composition. It appears that there is some controversy about the existence of a gonococcal capsule, and until capsular material is isolated and characterised, its significance in gonorrhoea cannot be properly assessed.

3. The pathogenesis of gonorrhoea

Having described the major components of the gonococcal outer membrane and their possible roles in virulence, it is now necessary to consider the pathogenesis of gonorrhoea.

3.1 Attachment of gonococci to mucosal surfaces

Transmission of gonococcal infection requires close physical contact allowing gonococci to pass from one infected mucosal surface to another. This is probably because gonococci are susceptible to drying and do not readily survive outside the human body. Gonorrhoea is usually transmitted during intimate sexual contact. Exceptions to this are gonococcal conjunctivitis and oro-gastric infection, either or both of which can be passed

on to babies born to mothers with genital gonorrhoea (Handsfield et al., 1973). The most common sites of infection are the genital mucosa, but the rectal and pharyngeal mucosa can also be involved following ano-genital and oro-genital contact respectively.

Once deposited on a mucosal surface, gonococci establish themselves rapidly. This is suggested by the observation that micturition after exposure is not protective (Holmes et al., 1970). The mechanisms by which gonococci attach to and invade epithelial cells is unknown, but it is likely to involve both gonococcal and epithelial surface structures. As described previously, gonococcal binding may be affected by pili, surface proteins and the terminal sugar residues of LPS attaching to receptors on the mucosal epithelial cell. Whether any one of these gonococcal components is solely involved in binding is uncertain. There are likely to be differences in the relative importance of each, in infection of different host sites. It is interesting to note that non-pilate gonococci are common in specimens of pus from patients (Novotny et al., 1975; Evans, 1977). Furthermore, non-pilate gonococci can attach to and invade epithelial cells (Taylor-Robinson et al., 1974) suggesting that pili are not essential for attachment and invasion. It is more likely that the particular host environment may determine the predominant binding mechanisms involved, and it is possible that two or more specific mechanisms may be involved in sequence.

Gonococci attach to organ cultures of human genital mucosa

but not to non-human cells, suggesting that gonococcal receptors are only found on human cells (Taylor-Robinson et al., 1974; Johnson et al., 1977). This finding may explain why man is the only known natural host for the gonococcus.

3.2 Invasion

Once established on a mucosal surface, gonococci penetrate the epithelial layer and multiply in the subepithelial tissue (Harkness, 1948). Electron microscopy of urethral scrapings has shown gonococci attaching and entering urethral epithelial cells, apparently by endocytosis (Ward and Watt, 1972). This endocytosis by epithelial cells differs from invasion by other bacteria such as salmonellae, which enter the intestinal cell by erosion of the brush border. It is not clear whether gonococci can also invade by a similar mechanism as gonococcal LPS can cause tissue damage (Mardh et al., 1979; Gregg et al., 1981).

4. Interaction of gonococci with host defence mechanisms

4.1 Non-specific defence mechanisms - phagocytic cells

Once gonococci have penetrated into the sub-mucosa, the initial response consists of an inflammatory reaction with large numbers of PMN infiltrating the site of infection. What initiates the inflammatory response is as yet unclear, but PMN may be attracted by gonococcal cytotoxins (James and Williams, 1978)

or by activation of complement (Ward et al., 1978). As described previously, the surface protein 'leucocyte association factor' (LAP) (King and Swanson, 1978) has been reported as the major factor involved in association of gonococci to PMN. However, its influence on the severity of the disease is unknown. Other surface components that may exert an antiphagocytic role include pili (Dilworth et al., 1975; Ofek et al., 1974; Thomas et al., 1973) and a material whose expression is affected by EDTA that may be LPS (Rosenthal et al., 1977). Polymorphs degranulate normally into vacuoles containing gonococci and damaged gonococci can be visualised in older vacuoles (WHO, 1978). Some polymorphs containing large numbers of gonococci are themselves damaged by the infection, releasing viable gonococci. Veale and Smith (1975) suggested that gonococci can multiply in healthy polymorphs. Also, a recent study suggested that viable gonococci produce a cytotoxin that lyses PMN (Casey et al., 1983).

Electron microscopic studies of gonorrhoeal exudate apparently show that gonococci can survive and grow within phagocytic leukocytes (Novotny et al., 1975). The leukocytes die and the resulting clusters of gonococci surrounded by the remnants of dead leukocytes have been termed 'infectious units' (Novotny et al., 1977). Novotny et al. (1977) have suggested that gonococci in 'infectious units', that is surrounded by leukocyte remnants, may be protected from humoral and cellular immunity. These infectious units have an affinity for epithelial cells and the gonococci multiply utilising the host cell remnants,

then damage the epithelial cells and infiltrate the submucosa. Free gonococci are phagocytosed. There is evidence based on electron microscopic histology that suggests the host cell remnants are macrophage derived. Novotny's hypothesis suggests that if gonococci are phagocytosed by PMN they are killed but, if phagocytosed by macrophages, they interfere in some way with the internal regulation of the macrophage and multiply. These multiplying gonococci become surrounded by remnants of the killed macrophage forming an 'infectious unit' capable of infecting more epithelial cells. When gonorrhoea is transmitted, presumably infectious units and free gonococci are passed on and begin the 'infectious unit' cycle in the new host. This hypothesis implies that it is advantageous to the gonococcus to be phagocytosed by macrophages and detrimental if phagocytosed by PMN.

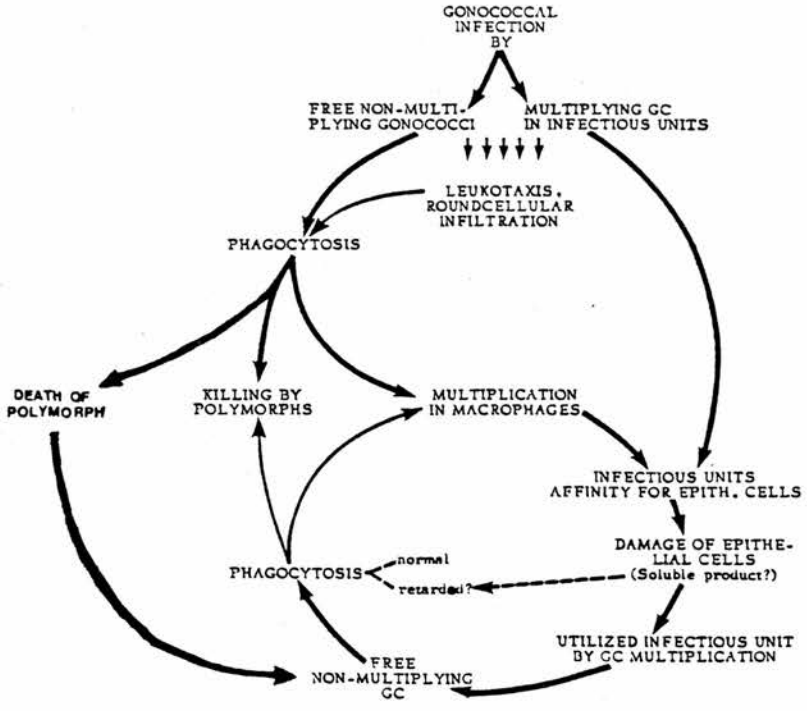


Figure 3 Summary of gonococcal infectious process (modified from Novotny et al., 1977 and Casey et al., 1983)

4.2 Disseminated gonococcal infection

In 1 to 3 per cent of patients, gonococci may spread haematogenously from the primary infection site giving disseminated gonococcal infection (DGI) (Barr and Danielsson, 1971) which may result in endocarditis, arthritis and skin lesions. Patients deficient in the terminal components of complement are particularly at risk of DGI (Peterson et al., 1976; Lee et al., 1978), suggesting that complement's bactericidal effect is a major factor in preventing haematogenous spread of gonococci. The bactericidal activity of serum is dependent on activation of the classical complement pathway, typically initiated by antigen-antibody reactions (Ingwer et al., 1978). In general most gonococcal strains isolated from DGI are resistant to the complement-dependent bactericidal activity of normal human serum, whereas gonococci from uncomplicated gonorrhoea are very sensitive (Schoolnik et al., 1976). Also the majority of gonococci isolated from DGI are very sensitive to penicillin, belong to the auxotype Arg^-, Hyx^-, Ura^-, and are resistant to the bactericidal activity of normal human serum (Knapp and Holmes, 1975; Eisenstein et al., 1977).

This 'natural' bactericidal activity of normal human serum is associated with the IgM class of immunoglobulins (Schoolnik et al., 1979; Michael and Rosen, 1963) and appears to be directed towards LPS (Glynn and Ward, 1970; Rice and Kasper, 1977) and possibly outer membrane proteins (Tramont et al., 1974). Schneider et al. (1982) investigated the immunological basis

of serum resistance in certain strains of Neisseria gonorrhoeae. They found that serum resistance was not due to intrinsic resistance to activated complement, inaccessibility of the cell membrane to C5b, or IgA or IgG 'blocking' antibody. They concluded that serum resistance resulted from the absence of specific LPS determinants to which the lytic antibody of normal human sera is directed. As previously described, Winstanley et al. (1983) grouped gonococcal strains into two broad categories (group I and group II) according to their sensitivities to R-type pyocins. They found that group I were serum resistant and group II were serum sensitive. This would imply that group II should be restricted to localised infection whereas group I would predominate in DGI. Analysis of data from a previous study (Blackwell et al., 1979) revealed that 13.6% of isolates from localised infections were of group I and 86.4% group II. In contrast 41.6% of isolates from DGI were group I and 58.3% group II. Thus there was an increased percentage of group I strains in localised infections than in DGI. Group II organisms have been isolated from DGI indicating that LPS type does not seem to be the sole factor in the serum resistance of a disseminated strain.

As previously described, the coagglutination serogroup WI has been correlated with DGI and resistance to the bactericidal effect of normal human serum. This suggestion that DGI and serum resistance are associated with particular protein I types was first put forward by Hildebrandt et al. (1978). They postulated that this protein may in some way mask the LPS surface

antigens and prevent their recognition by bactericidal antibodies in human serum. Clearly correlation of protein I type (by coagglutination for example) and the LPS (by pyocin sensitivity), is required for strains exhibiting serum resistance and involved in DGI.

James and Swanson (1978) reported that gonococci from opaque colonies are serum sensitive whereas those from transparent colonies are relatively resistant. Transparent colonies are isolated from the cervix around the time of menstruation (James and Swanson, 1978) which is when DGI in females is most likely to occur (Holmes et al., 1971).

4.3 Specific immune responses

Cohen (1967) has shown by indirect fluorescence tests that both normal sera and sera from patients with gonorrhoea contain IgG, IgM and IgA classes of anti-gonococcal immunoglobulins. McMillan et al. (1979a) examined urethral exudates from patients with gonorrhoea and found gonococcal antibodies of the IgA class to be present in 98%, IgG in 90% and IgM in 49%. In a similar study of anti-gonococcal antibodies in cervical secretions of infected female patients, a comparable distribution of immunoglobulin classes was found (McMillan et al., 1979b).

The development of a specific cell-mediated immune response to gonorrhoea has been demonstrated by the blastogenic response of lymphocytes in patients with gonorrhoea after exposure to gonococcal antigens (Kearns et al., 1973b).

Although specific immune responses are found following infection the organism appears to evade the host response in a number of ways and patients may suffer repeated attacks. One explanation may be the wide antigenic heterogeneity of the components of the gonococcal outer membrane surface noted previously. Thus the possibility exists that an individual once infected with a particular gonococcal strain may be resistant to reinfection with the homologous strain but not to gonococci with different antigenic profiles. Buchanan et al. (1980) found that an episode of gonococcal salpingitis may provide immunity to a further episode of salpingitis with the same protein I serotype but does not prevent reinfection at other sites. Thus it is feasible that immune responses may provide a site specific and serotype-specific protection against gonococcal salpingitis.

In males gonorrhoea is, with prompt treatment, generally of short duration as the incubation period is short and onset of symptoms fast. This limits the exposure of gonococcal antigens to the host's immune system. In females, many infections are asymptomatic, allowing more time for antigenic stimulation before treatment. Novotny's 'infectious unit' hypothesis suggests protection of the gonococci from host defence mechanisms by the physical covering of gonococci with host derived substances (Novotny et al., 1977).

Another factor to consider in terms of susceptibility to gonococcal reinfection is the ability of gonococci to produce a proteolytic enzyme capable of cleaving antibodies of the IgA 1

subclass at the hinge region (Plaut et al., 1975). This enzyme has no effect however against the IgG and IgM also found in genital secretions (McMillan et al., 1979a and McMillan et al., 1979b), and therefore its significance is unclear. It has been suggested that secretory IgA, which appears early in gonococcal infections, might act by coating or agglutinating gonococci, thus limiting their replication or preventing attachment and invasion of mucosal surfaces (Kearns et al., 1973a; O'Reilly et al., 1976).

In conclusion, it appears that because of the limitation of human studies, no clear understanding has yet emerged of the extent and significance in protection of the anti-gonococcal immune response.

ABO blood group and susceptibility to gonorrhoea - the hypotheses

Anti-gonococcal antibodies of classes IgG, IgM and IgA have been found both in individuals with no history of gonorrhoea (natural antibodies) and in patients with gonorrhoea (induced antibodies) (Cohen, 1967). As described previously, IgM is the natural antibody associated with normal serum bactericidal activity (Ingwer et al., 1978). Miler et al. (1977) found that anti-A and anti-B isohaemagglutinins induced by gonococcal infection are predominately of the IgM class, as are isohaemagglutinins of normal sera. They also found that gonococci are capable of absorbing both anti-A and anti-B isohaemagglutinins suggesting the presence of A- and B-like antigens on gonococci. Two hypotheses have been suggested to explain the increased susceptibility of group B individuals to gonorrhoea -

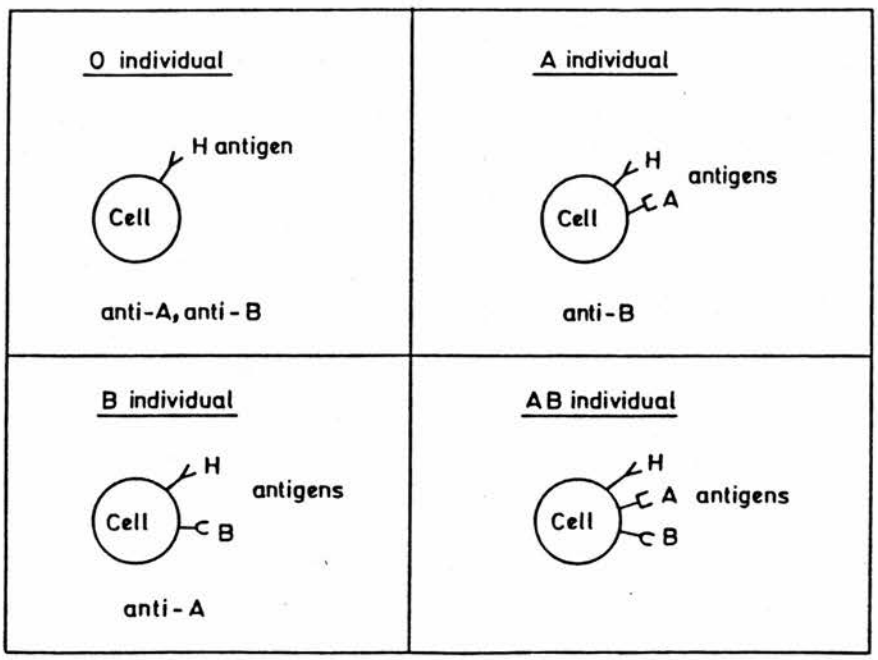
1) as with E. coli 086 (Muschel and Osawa, 1959), anti-B is bactericidal for particular strains of gonococci,
2) as with E. coli 086 (Check et al., 1972), anti-B is capable of opsonising particular strains of gonococci.

The first hypothesis, that differences in susceptibility to gonorrhoea might be associated with the bactericidal effect of anti-B isohaemagglutinins present in individuals of blood groups A and O, has been tested. Winstanley et al. (1983) examined normal human serum for bactericidal activity against gonococci from individuals of blood groups A and B. They concluded that the bactericidal activity of normal human serum was not associated with anti-A or anti-B isohaemagglutinins.

There is evidence for the second hypothesis from a number

of sources. Natural IgG has been ascribed the role of heat-stable opsonin involved in phagocytosis of gonococci by PMN (Schiller et al., 1979). They also found that IgG-mediated phagocytosis could be enhanced in the presence of complement, although complement itself was not capable of promoting phagocytosis. Haegart (1979) demonstrated the presence of IgM receptors on human peripheral monocytes, suggesting that IgM antibodies could be involved in opsonisation. IgM or IgG is required for the heat-labile opsonic activity of the alternative complement pathway (Jasin, 1972). Foster and Labrum (1976) suggested that the anti-B isohaemagglutinin might be a more efficient opsonin than the anti-A isohaemagglutinin.

In addition to differences in isohaemagglutinins, individuals of the four ABO blood groups have different antigens expressed on their cells. These differences are summarised diagramatically below:



These are stable host characters that are not significantly altered by environmental factors such as the hormones of the menstrual cycle. All tissues colonised by gonococci have the ABH antigens present (Miler et al., 1977), as have leukocytes. The structure and important biological roles of pili and LPS have been described previously. The pyocin sensitivities that reflect composition of LPS groups I and II have been shown to be stable over a long period (Blackwell et al., 1979). Pilation of gonococci is consistent with colony morphology which is readily checked by viewing colonies and verified by electron-microscopy. These characteristics of the gonococci were examined in their interaction with leukocytes of different ABO groups under various conditions.

Interaction between gonococci and PMN or monocytes may be mediated by lectins present either on the bacterium or the leukocyte. These lectins, or sugar-binding proteins, can be demonstrated by inhibition of binding reactions after preincubation of cells or bacteria with sugars. Various workers have attempted to demonstrate lectins of gonococci involved in binding to host cells, as described in the sections on the gonococcal outer surface. Punsalang and Sawyer (1973) using eleven simple sugars, were unable to inhibit the binding of pili to rabbit erythrocytes. Trust et al. (1980) were also unable to inhibit attachment of gonococci to buccal cells with ten simple sugars. They did however find evidence to suggest that an oligosaccharide on the surface of the epithelial cell may act as a pilus receptor. Buchanan et al. (1978) came to a similar conclusion as did Lambden

et al. (1980). King and Swanson (1978) were unable to block gonococcal binding to PMN with four simple sugars but found that pretreatment of specific gonococci with periodate which disrupts sugar moieties reduced the binding of gonococci to PMN. They concluded that in gonococcal binding to PMN the importance of sugars on proteins and other components of the gonococcal outer surface is unclear. Watt et al. (1978) investigated the possibility that gonococcal surface lectins may bind to sugar groups of human fallopian tube cells. They found no inhibition of binding using lectins specific for the following sugars; N-acetyl-galactosamine, galactose, fucose (incidentally the immunodominant sugars of blood group substances A, B and H) and N-acetyl-glucosamine. Watt et al. (1978) however, presented evidence suggesting that sugar groups of gonococcal LPS can bind to HEp-2 cells. This evidence has been described earlier in the section on LPS. Watt et al. concluded that sugars on the gonococcal surface are likely to be involved in attachment to mucosal cells but that appropriate inhibition experiments were required.

Aims of the studies on host-parasite interaction

These studies were carried out to examine the host-parasite interactions that might underly the reported increase in susceptibility of blood group B individuals to gonorrhoea. ABH antigens on leukocytes might be part of a mechanism similar to that reported for P blood group antigens and P-fimbriate E. coli. Because of the different roles suggested for PMN and monocytes/macrophages

in gonococcal infection, these host cells were investigated to determine if there were differences in their interaction with gonococci.

The aims of the host-parasite studies were:

1. To examine the non-immune, possibly lectin-mediated interactions, and binding characteristics of pilate and non-pilate gonococci of LPS groups I and II to PMN, monocytes and macrophages.
2. to determine the role, if any, of ABH antigens in this binding.
3. to determine if the isohaemagglutinins in normal human serum were capable of opsonising gonococci of group I and group II as assessed by attachment to PMN and monocytes; and if so, were there differences in attachment to phagocytes of the four ABO blood groups in the presence of autologous sera.
4. to determine if pilation or LPS group influenced the stimulation of intracellular bactericidal activity in phagocytes as determined by the nitroblue tetrazolium (NBT) reaction.

SUBJECTS, MATERIALS AND METHODS

Subjects

1. Subjects for recurrent urinary tract infection (UTI) survey

At the pyelonephritis clinic of the City Hospital, Edinburgh, regular attenders were asked to provide a specimen of blood and saliva for determination of blood group and secretor status. All patients in this survey were females aged 10-80 (mean 49) years. None had predisposing factors such as obstructions or stones.

The patients were divided prospectively into two categories:

a) Pyelonephritis patients

(i) Those with unequivocal radiological evidence of chronic pyelonephritis, that is, focal loss of parenchyma associated with distortion of calyces.

or,

(ii) those with a history and clinical and laboratory findings consistent with acute pyelonephritis.

b) Uncomplicated UTI patients

Those patients with normal intravenous pyelograms, but a history of recurrent UTI and the detection of significant bacteriuria (10^5 organisms/ml).

2. Subjects for sexually transmitted diseases (S.T.D.) survey

During a three-month period all new patients attending the department of genitourinary medicine at the Royal Infirmary, Edinburgh, were asked to provide a specimen of blood and saliva for determination of blood group and secretor status.

3. Control populations

ABO blood group and secretor status control data were produced by testing 334 plasma samples of female donors from the survey area, age-ranges 18-60, for the presence of secretor substances. Further control data for blood group frequency were available for 6662 donors from the same area as that from which our patients were drawn (Kopeć, 1970).

Materials

1. Buffers and reagents

All chemicals were analytical grade obtained from BDH Chemicals Ltd., Poole, Dorset unless otherwise stated.

1.1 Phosphate buffered saline (PBS)

PBS pH 7.2, 10mM phosphate buffer, containing 0.15 M Na Cl.

1.2 Dulbecco's phosphate buffered saline (modified) (D.PBS)

This was prepared by dissolving 8.0g NaCl, 0.2g KCl, 1.08g Na_2HPO_4, 0.2g KH_2PO_4, 0.089g $CaCl_2.2H_2O$ and 0.203g $MgCl_2.6H_2O$ up to 1 litre with distilled water (pH 7.3).

1.3 HEPES buffer

HEPES buffer was prepared by dissolving 23.83g of HEPES buffer (Flow Laboratories, Irvine, Scotland, UK) in approximately 90ml distilled water. Sufficient 5N NaOH (about 5 ml) was

added to adjust the pH to 7.6 - 7.7 and the final solution was adjusted to 100 ml with distilled water. This gave a one molar solution.

1.4 Eagle's minimum essential medium (MEM)

Eagle's MEM was prepared by mixing 10 ml to 10 times concentrated Eagle's MEM (Flow Laboratories, Irvine, Scotland, UK), 3 ml 1M HEPES buffer, 1 ml of 0.2M glutamine and 0.1 ml of penicillin and streptomycin (200,000 $U.ml^{-1}$) and adjusting the volume to 1 litre with distilled water (pH 7.3).

1.5 RPMI

RPMI 1640 (Flow Laboratories, Irvine, Scotland, UK) was supplemented with 0.2 ml of penicillin and streptomycin (200,000 $U.ml^{-1}$) and 10 mls of foetal calf serum, per 100 ml (pH 7.2).

1.6 Lysis buffer

This was prepared by dissolving 8.2g NH_4Cl, 1.0g $KHCO_3$ and 0.037g Na_2 EDTA in 1 litre of distilled water (pH 7.4).

1.7 PPO/POPOP scintillation fluid

This solution was prepared by dissolving 2.5g 2,5-diphenyloxazole (PPO) and 0.15g 1,4-bis-[2-(5-phenyloxazolyl)]-benzene in toluene made up to a volume of 1 litre.

1.8 Ficoll-Hypaque solution

Ficoll-Hypaque (Ficoll, Pharmacia, London) solution was

made by combining 9% (w/v) Ficoll solution with 33.9% (w/v) Hypaque solution. These solutions were mixed in a ratio of one part Hypaque solution to 2.4 parts of Ficoll solution. 0.1% (w/v) Na_2 EDTA was added. The specific gravity of the final solution was 1.078.

2. Stains

2.1 White cell diluting fluid

A stock solution was prepared by adding 0.1g gentian violet to 100 ml of 1% acetic acid. For use, the stock was diluted 1:10 with 1% acetic acid. 1 part cell suspension was added to 9 parts stain.

2.2 May Grunwald/Giemsa stain

May Grunwald stain was diluted 1:5 with PBS. Giemsa stain was diluted 1:30 with PBS.

3. Bacterial culture media

3.1 GC agar

GC medium (Difco) was autoclaved and the following addition per 960 ml were made: 0.5% (w/v) ferric nitrate (British Drug House) 10 ml, 20% (w/v) glucose 10 ml and 20 ml of GC supplement which was made up in the following way: 0.5g L-glutamine (Koch Light Chemicals), 0.001g cocarboxylase (British Drug House), water to 100 ml. Media were used within 5 days of preparation.

3.2 Modified New York City medium (MNYC)

MNYC medium was prepared using Difco GC medium base enriched with 10% (w/v) human blood lysed with 0.5% (w/v) saponin, 2.5% (w/v) yeast dialysate (Faur et al., 1973), 0.1% (w/v) glucose, lincomycin (1.0 µg/ml), colistin (6 µg/ml), amphotericin B (1.0 µg/ml) and trimethoprim lactate (6.5 µg/ml). Media were used within 4 days of preparation.

3.3 ANM liquid media

This medium was prepared by mixing Proteose Peptone (Difco, Detroit, Michigan, USA) 15g, corn or soluble starch 1.0g, NaCl 5.0g, K_2PO_4 1.0g, KH_2PO_4 1.0g $NaHCO_3$ 0.15g and distilled water to 1 litre.

3.4 GC broth

This broth was prepared as for GC medium but without the addition of agar.

4. Long term storage media for N. gonorrhoeae

4.1 Trypticase soy broth

Trypticase soy broth (Oxoid) was supplemented with 6% lactose and aliquoted into bijoux for use. Gonococcal suspensions were made in the broth and then frozen at -70°C.

4.2 Skimmed milk storage

Bijoux of skimmed milk were used to suspend gonococci

which were then snap frozen in a mixture of dry ice and acetone. The bijoux were stored frozen at -70°C and thawed when required.

Methods

1. ABO blood group and secretor status determination

Blood from each patient was collected at the clinic and stored in heparinized tubes. The saliva from each patient was boiled for 20 min, centrifuged at 5000 x g for 10 min and the supernate stored briefly at 4°C until tested for the presence of blood group antigen.

Blood group was determined by agglutination tests in plastic wells. Secretor status was determined as described by Mollinson (1979) with saliva or plasma samples. Saliva or plasma from secretors and non-secretors representative of the four blood groups were used as controls for each experiment. Agglutinins used were anti-A and anti-B sera and Ulex europaeus lectin. The red blood cells used were A_2, B and O. Tables VII and VIII depict how these tests were carried out and how the results were read. The Blood Transfusion Service, Royal Infirmary, Edinburgh, kindly supplied the reagents for blood grouping and determination of secretor status and also did random verification checks on selected saliva samples (50) and plasma samples (48).

Table VII Screening inhibition technique

Agglutinin	Doubling dilutions eg. 1/2	1/4	1/8	1/16	1/32
Anti-A					
Anti-B					
Anti-H (Ulex europaeus)					

(a) 100 µl of test saliva was added to each of the 15 wells containing the above dilutions of agglutinin.
(b) These were incubated at 20°C, 20 min. A_2, B or O cells were added to the appropriate wells.
(c) Agglutination was recorded.

Table VIII Interpretation of results

	Blood Group	Substances secreted	Agglutination A	B	H
Secretors	O	H only	+	+	-
	A	A + H	-	+	-
	B	B + H	+	-	-
	AB	A, B + H	-	-	-
Non-secretors	O, A, B, AB	None	+	+	+

2. Bacteria

2.1 Bacterial strains

Neisseria gonorrhoeae strains P152 and E757 recently isolated from patients attending the Department of Genito-urinary Medicine were used throughout these experiments. A further six strains were used in the nitroblue tetrazolium reduction assay and these are listed with P152 and E757 in Table IX. Table IX shows the pyocin sensitivities of the eight strains and divides them into two major groups according to their pyocin sensitivities (Blackwell *et al.*, 1979). Group I strains were sensitive to pyocins attaching to structures nearest the cytoplasmic membrane whereas group II were sensitive to the same pyocins, but in addition, to pyocins attaching to LPS components in positions more distal from the cytoplasmic membrane (Blackwell *et al.*, 1983). P152 and E757 were used throughout the attachment studies; P152 belonged to coaglutination group WI and E757 to group WII (K. Reid, personal communication; Sandström and Danielson, 1980).

Opaque colony types 2 (pilate) (T_2) and 3 (non-pilate) (T_3) were differentiated with a Zeiss stereoscope microscope with a double system of substage lighting and selectively sub-cultured and maintained on GC agar (Figure 4). T_2 and T_3 growths, with more than 95% of the colonies stable, were employed. Bacteria were harvested from GC agar plates at 14-16 hr, suspended in Dulbecco's phosphate buffered saline (D.PBS) by gentle pipetting, washed and finally resuspended gently in D.PBS.

Table IX Pyocin types of N. gonorrhoeae strains

N. gonorrhoeae strain	R1 strain					Unclassified strain		R2 strain			R3 strain	R4 strain	
	ISD	2285	IS4	IS6	IS8	R205	9579	ISB	R21	430	ISE	ISA	ISC
Group I													
E757	-	-	-	-	-	-	+	-	-	-	-	+	±
M9131	-	-	-	-	-	-	+	-	-	-	-	+	+
Group II													
F62	+	±	+	+	+	+	+	-	-	-	-	+	+
9	+	-	+	+	+	+	+	-	-	-	-	+	+
M8865	+	±	+	+	+	+	+	-	-	-	-	+	+
E760	+	-	+	+	+	+	+	-	-	-	-	+	+
P152	+	-	+	+	+	+	+	-	-	-	-	+	+
M6967	+	-	+	+	+	+	+	-	-	-	-	+	+

+ inhibition; ± inhibition with some growth; - no inhibition

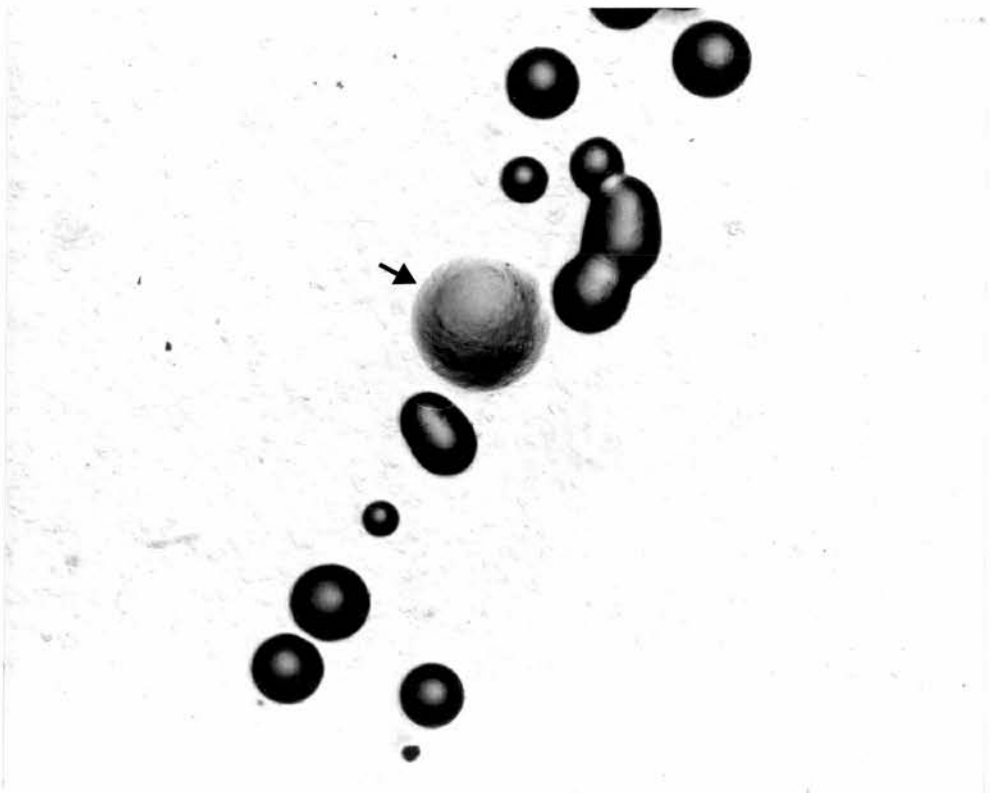


Figure 4 T_2 and T_3 (arrow) opaque colonies grown on GC agar. X 30.

Bacterial concentrations were determined at E650nm on a CE292 spectrophotometer (Cecil Instruments, Cambridge, England) by reference to standards enumerated by microscope counts with a counting chamber (Figure 5).

2.2 Removal of pili

The gonococcal pili were sheared by vortexing a suspension of gonococci in D.PBS with a Rotamixer Deluxe (Hook and Tucker Ltd., England) at maximum setting for 5 min. Electron microscopy confirmed the presence of pili on type 2 gonococci and their absence on type 3 and vortexed type 2 and 3 gonococci (Figures 6 and 7).

2.3 Viable counts

Viable counts were performed by making serial ten-fold dilutions of the appropriate suspensions, and plating 3 x 20 µl drops of each dilution onto the appropriate growth medium. Duplicate plates were used, and the viable count was made from the mean of the 6 x 20 µl drops of the dilution giving the highest number of colonies which could be reliably counted.

2.4 Incubation

Standard incubation conditions were overnight at 37°C in an aerobic CO_2-enriched (10 per cent) humidified atmosphere. Broth cultures were incubated at 37°C in an orbital incubator.

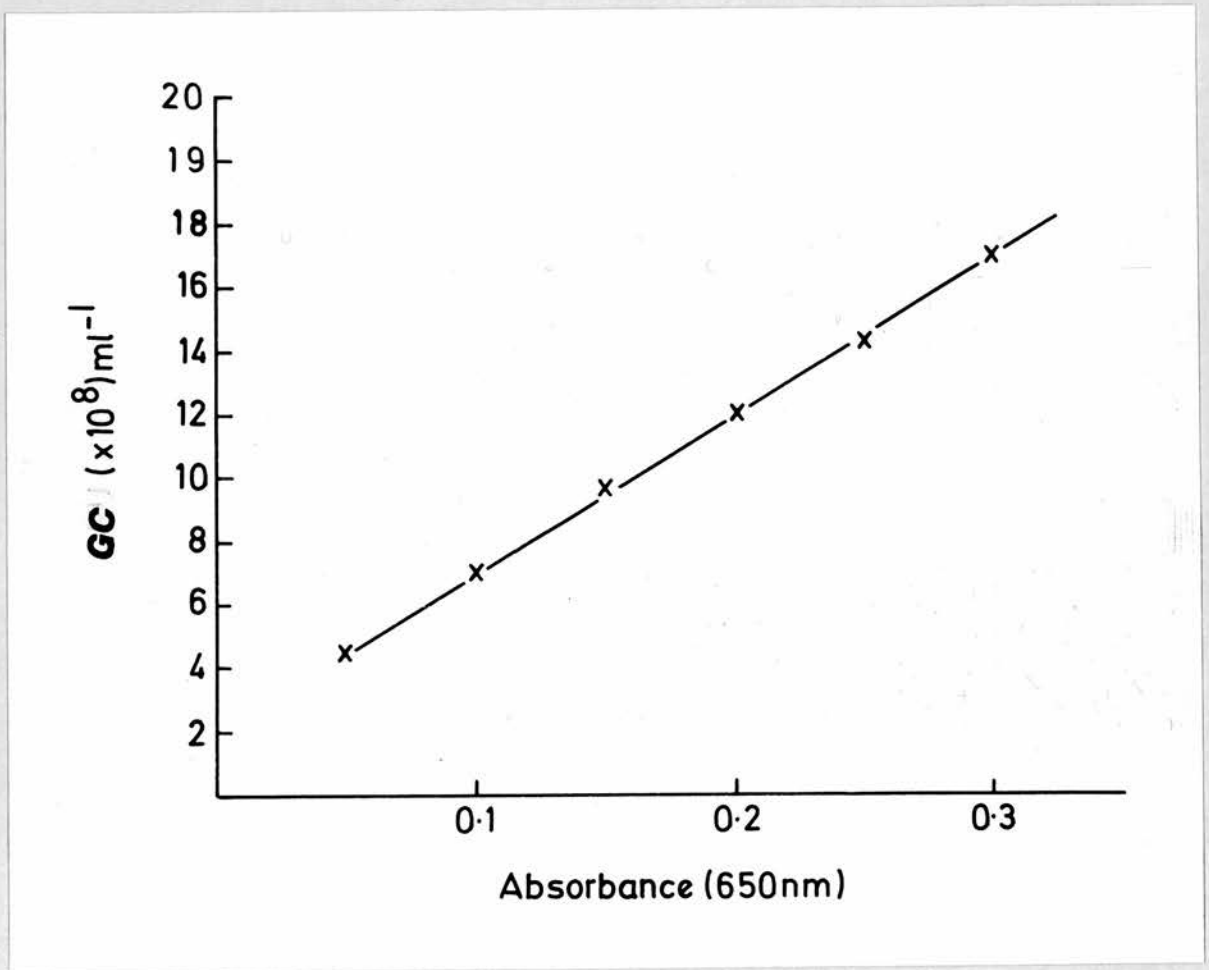


Figure 5 Calibration curve for determination of gonococcal (GC) concentration in PBS.

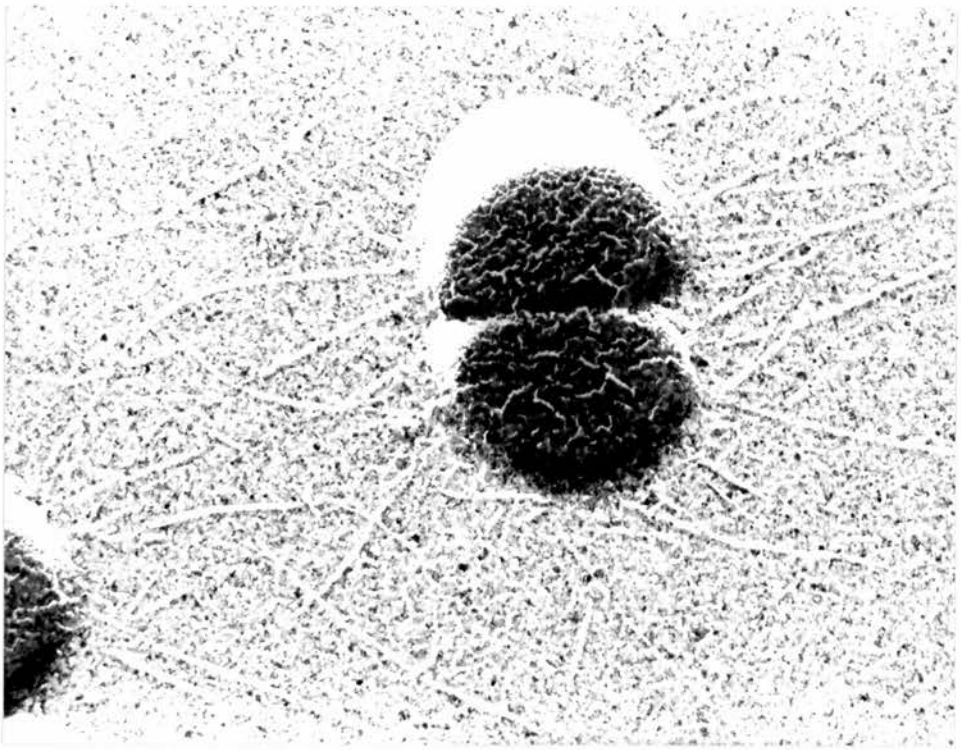


Figure 6 Shadowed electronmicrograph showing a pilate gonococcus. X 55,000

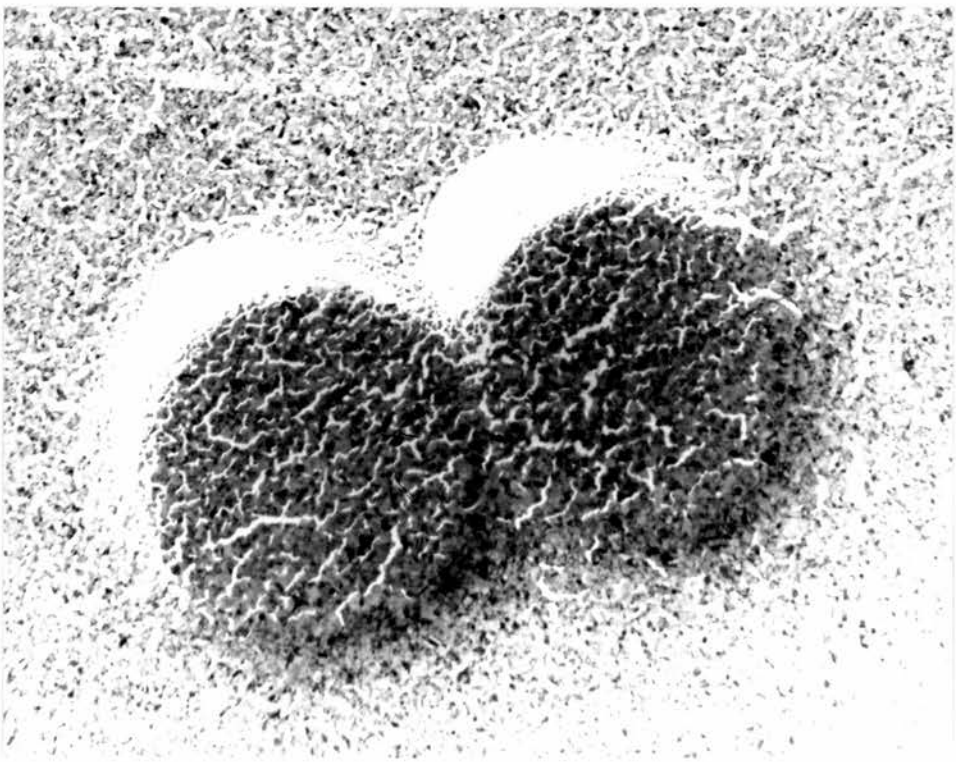


Figure 7 Shadowed electronmicrograph showing a non-pilate gonococcus. X 100,000

3. Assays with phagocytic cells

3.1 Phagocytes

Blood from healthy donors with no history of gonorrhoea was collected in plastic tubes containing 10 u. of heparin ml^{-1}. Monocytes and PMN were separated on Ficoll-Hypaque cushions, at 400g for 30 min according to the method of Böyum (1968). After the interface cells (monocytes and lymphocytes) were collected, the pellet containing granulocytes and red blood cells were re-suspended in 0.82% ammonium chloride to lyse the erythrocytes (Boyle, 1968). The separated cells were finally washed twice in D.PBS.

3.2 Preparation of monolayers

PMN and monocytes were re-suspended in Eagle's MEM to give a total cell count of 2×10^5/ml. One millilitre of the above cell preparations was layered onto 13mm diameter (No. 1) glass coverslips in 16mm well diameter tissue culture plates (Costar, 295 Broadway, Cambridge, Mass.) and incubated for 1 hr at 37°C. Non-adherent cells were removed by washing seven times with D.PBS.

3.3 Binding assay

Coverslips with monolayers of PMN or monocytes were overlaid with 1 ml of gonococci in D.PBS containing Ca^{++} and Mg^{++} ions (0.9 mM and 0.5 mM, respectively) and incubated for either 30 min at 37°C or 2 hr at 4°C. In those experiments involving

sera, 0.1 ml of the appropriate sera was added to the 0.9 ml of gonococci in D.PBS. Non-attached organisms were removed by repeated washing with D.PBS. Coverslips were air dried, fixed in methanol and stained with May Grunwald for 3 min, rinsed and stained with Giemsa for 3 min. Bacterial binding was estimated by counting monocytes with bacteria attached at two or more discrete points. Duplicate coverslips were used: for each coverslip, 200 leucocytes were counted and the results expressed as the percentage of cells binding organisms.

3.4 Sera

For those experiments which involved opsonisation autologous sera was obtained at the same time as the blood for the cells was taken. In some experiments heat inactivated sera (HIS) was used and this was obtained by inactivating sera in a water bath at 56°C for 30 min. Fresh human sera (FHS) and heat inactivated sera (HIS) were used at a final concentration of 10%. The isohaemagglutinin levels of all sera used were the same (titres equal to 64).

3.5 Absorption of ABO antibodies

The group O sera used for these experiments had both anti-A and anti-B titres equal to 256. The isohaemagglutinins anti-A and anti-B were absorbed by an equal mixture of A_1 and B washed RBC for 1 hour at 37°C and then overnight at 4°C. The absorption mixture of sera and RBC was approximately 50% (vol/vol). The antibody titres were rechecked after absorption

and where necessary absorption was repeated until both anti-A and anti-B titres were reduced to zero. Haemagglutination tests to check original titres and titres after absorption were carried out as described by Miler et al. (1977).

3.6 Nitroblue tetrazolium (NBT) reduction

The NBT reduction in PMN was assayed as described by Kowolik and Moody (1979) with pilate and non-pilate variants of gonococcal strains of group I and group II. Blood for these experiments was from healthy donors of blood group O, with no history of gonorrhoea. Percentage positive counts (i.e. cells containing formazan deposits) were made of the preparations and the mean of 10 counts compared statistically by a paired t-test with the unstimulated control cells performed at the same time.

4. Radiolabelling techniques

4.1 Radiolabelling bacteria

E757 gonococci were grown overnight in ANM liquid medium, in an orbital shaker at 37°C.

Radiolabelled bacteria (3×10^{-4} cpm/bacterium) were obtained by inoculating approximately 5×10^5 bacteria into 50 ml of ANM containing 10 µCi/ml of tritium labelled thymidine. After 18h of growth the bacteria were washed three times in PBS and adjusted photometrically to a concentration of 10^8 bacteria/ml. Viable counts of bacteria were performed and stability of colony type was checked.

4.2 Preparation of phagocyte monolayers for radiolabelling experiment

Monocyte and PMN monolayers were prepared as previously described on the day of the experiment. Three days prior to the experiment monocyte monolayers were prepared. These monolayers were prepared in the normal manner and cultured in a humidified incubator with 5-10% CO_2 at 37°C. Daily changes of RPMI minimum essential medium were made. Monocytes were viewed daily using a reverse microscope and the change from monocyte to macrophage monolayer was noted. All phagocyte monolayers were prepared using 1 ml aliquots of 2×10^5 cells/ml.

4.3 Radiolabelled bacteria to phagocyte assays

These assays were carried out essentially in the same manner as the other gonococci-phagocyte association experiments. Four coverslips were used for each determination: two coverslips for the assessment of binding of tritium labelled gonococci and two coverslips for the assessment of binding by microscopy. Coverslips for radioactivity determination were washed repeatedly, removed from the wells and air dried. The dry coverslip was then placed in 3 ml of PPO/POPOP liquid scintillation fluid and the scintillations (cpm) were recorded by a liquid scintillation counter. Coverslips with phagocyte monolayers to which no radiolabelled bacteria had been added were used as background controls.

5. Studies on sugar inhibition of gonococcal binding to phagocytes

Monocyte and PMN monolayers were prepared as described previously. Cell monolayers were preincubated with 0.5 ml of the appropriate sugar solution for 20 min at 4°C. Sugar solutions were: D-galactose; N-acetyl-D-galactosamine; D-galactosamine; N-acetyl-D-glucosamine; D-glucose; L-fucose and D-glucosamine. These sugar solutions were used at 5 mmol l^{-1}, 10 mmol l^{-1} and 20 mmol l^{-1} concentrations in D.PBS with 0.9mM Ca^{++} and 0.5mM Mg^{++} ions. The cells were then washed and the binding assay performed at 4°C for 2 hours. Pilate gonococci were used at a concentration which gave approximately 50% binding for both monocyte and PMN controls..

6. Electron microscopy techniques

6.1 Negative staining

Negative staining was carried out using 2% (w/v) phosphotungstic acid (PTA) buffered at pH 7.0. Equal volumes of gonococcal suspension and PTA were mixed on a clean glass slide. A drop of the suspension was then transferred by means of a fine platinum loop on to the surface of collodion membrane supported on a copper electronmicroscope grid. Excess fluid was removed after 30 seconds by touching the edge of the specimen drop with a piece of filter paper. The specimen was then either left to dry overnight or dried in a dessicator over anhydrous calcium carbonate. All grids were examined in an Hitachi HU12A electron microscope.

6.2 Shadowed preparations

Shadowed preparations were made from gonococcal suspensions by washing and resuspending in 1% (w/v) ammonium acetate and then a drop of the bacterial suspension was transferred to the surface of an electron microscope grid. The preparation was allowed to dry for 5 min in a dessicator over anhydrous calcium carbonate. The grids were then transferred to a special holder and exposed to gold-palladium vapour under vacuum in an Edward's vacuum coater, model 306.

6.3 Thin sections

1 ml of 10^8 cfu/ml E757 P + gonococci were incubated for 30 min at 37°C with 1 ml of PMN or monocyte preparations (10^6 cells/ml).

Tubes were centrifuged at 600g for 10 min. Cells were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer at pH 7.4, washed three times in cacodylate buffer and fixed in 1% osmium in cacodylate buffer for 1 hour. The cells were washed again and blocked in 2% Nobel agar. The blocks were dehydrated by passing them twice through 50% (v/v) ethanol for 10 min followed by absolute alcohol for 15 min.

Cells were cleared and embedded to the following schedule: epoxy propane for 15 min, twice; infiltrated with epoxy propane/ araldite mixture overnight and then embedded in fresh araldite and cured at 60°C overnight.

Sections were cut at 400 Å and stained with saturated uranyl acetate in 50% (v/v) ethanol for 30 min in the dark. Sections were washed in alcohol and counterstained in 2% lead citrate for 2 min. The grids were finally washed 3 times in distilled water and blotted dry before viewing.

7. Statistics

Linear logistic multiple regression, Armitage (1971), was used to compare the UTI with control groups for ABO blood group and secretor status. This method also enables estimation and gives confidence limits for relative risk of UTI among secretors and non-secretors of the 4 blood groups. Chi-squared tests were used for analysis of two-way frequency tables. The relative risk method of Woolf (1954/55) was employed in the analysis of data from the survey of patients attending the STD clinics. Analyses of gonococci-phagocyte association assays

were performed with paired sample *t*-tests. Two factor analysis of variance was used to examine the effect of blood group and other variables on gonococci-monocyte association. When no specific statistical tests were employed, means and standard errors are given. In some bar charts, standard errors are not shown, and the significance tests are given in an accompanying table.

RESULTS

SECTION I

EPIDEMIOLOGICAL STUDIES

1. ABO and secretor status survey of recurrent urinary tract infection patients

This survey was undertaken to determine if there was any correlation between ABO blood group, secretor status and recurrent urinary tract infection (UTI). Table X shows the ABO blood group and secretor status distribution for the two recurrent UTI diagnostic groups: a) pyelonephritis patients; b) uncomplicated UTI patients. No significant differences in the proportion of secretors or the distribution of ABO blood groups were found between the two diagnostic categories of UTI patients; all 319 patients were considered as one group for comparisons with controls.

Table X. Secretor status and ABO blood group distribution in the two diagnostic groups of UTI

Diagnostic group	Secretor status	O	A	B	AB	Total	Totals within each diagnostic group
a	Secretor	23	14	9	3	49	75
	non-secretor	16	7	2	1	26	
b	Secretor	87	48	23	4	162	244
	non-secretor	36	21	16	9	82	
Total		162	90	50	17	319	

Table XI shows the frequencies of different blood groups and secretor status for the UTI patients and for the smaller series of controls for whom secretor status has been determined. For the UTI group, the table shows also the ratios of observed

Table XI Frequencies for UTI patients (n = 319) and controls (n = 334) classified by ABO blood group and secretor status. Figures in brackets for UTI patients are ratios of observed frequencies to those expected from the control data

		Blood Group			
		0	A	B	AB
Controls	Secretor	124	74	35	12
	Non-secretor	49	30	7	3
UTI	Secretor	110 (0.93)	62 (0.88)	32 (0.96)	7 (0.64)
	Non-secretor	52 (1.11)	28 (0.98)	18 (2.69)	10 (3.49)

Table XII Frequencies for controls (n = 6662) and UTI patients (n = 319) classified by ABO blood group

	Blood Group				
	0	A	B	AB	% B + AB
Control	3323	2410	715	214	14%
UTI	162	90	50	17	21%

frequencies to those expected from the pattern found in controls. To test the dependence of UTI risk on ABO blood group or secretor status, a linear logistic multiple regression was carried out on the two samples. This examines the influence of the three main effects (anti-A, anti-B and secretor status) and their interactions on the proportion of subjects with UTI, using approximate chi-squared tests. Presence or absence of anti-A was found not to have a significant effect, but there was a significant interaction between the anti-B and secretor effects; subjects lacking both anti-B and secretor substance i.e. those of blood group B and AB who are non-secretors, ("B/AB, non-S"), had a higher risk of recurrent UTI, whereas subjects lacking only one of these two did not differ significantly in risk from those with both factors present. Thus the data fit extremely well (X^2 = 1.86, 6 d.f.) to a model in which the "B/AB, non-S" group have a highly significant excess risk (X^2 = 9.12, 1 d.f., $P < 0.01$) over all types. The relative risk of UTI for "B/AB, non-S" subjects as compared with other types was estimated as 3.12, with 95% confidence limits of 1.49 and 6.52. This appears to be a genuine example of synergy with absence of both anti-B and secretor substance being necessary to give an increased risk of UTI. The observed/expected ratios in Table XI illustrate this finding clearly, with only those for B non-secretors and AB non-secretors differing substantially from unity.

Although there was no significant difference in the frequency of "B/AB, non-S" subjects between the two diagnostic

categories of UTI patients, only 3 out of the 28 UTI patients who were "B/AB, non-S" subjects were in category 'a'. The incidence rate (4%) for "B/AB, non-S" subjects in category 'a' was not significantly different from that of the controls (3%). Thus while "B/AB, non-S" is more common in category 'b' (incidence rate of 10%) than in controls, the position of category 'a' is equivocal, and a larger study is required to determine whether it is more like category 'b' or controls in terms of "B/AB, non-S" incidence.

The larger series of controls (in whom only ABO group but not secretor status was known) did not differ significantly from the smaller series in their blood group frequencies, but showed a highly significant difference (χ^2 = 16.34, 3 d.f., $P < 0.001$) from the UTI patients (Table XII). This confirms previous findings and is consistent with the suggested model in which "B/AB, non-S" subjects have an increased likelihood of recurrent UTI. The proportion of anti-B negative patients in the UTI sample is inflated by the excess frequency of those who are also non-secretors, even though anti-B negative secretors are not at a higher risk. Thus absence of anti-B, when considered alone, shows a significant risk relative to presence of anti-B (95% confidence limits of 1.24 and 21.7 from Table XII). This level of risk is lower than the three fold increase risk found for "B/AB, non-S" because the anti-B negative group is diluted with anti-B negative secretors.

2. ABO and secretor status survey of new patients attending a sexually transmitted diseases clinic

This survey was undertaken to determine if there was any correlation between ABO blood group, secretor status and susceptibility to gonorrhoea.

The breakdown of all 567 patients into ABO, secretor status and diagnostic groups is presented in Table XIII. As can be seen from this table many patients had more than one species of Neisseria isolated from them.

The distribution of blood groups of patients with and without gonorrhoea and of the controls is given in Table XIV. The blood group frequencies of the controls and the total number of patients attending the clinic showed no significant difference ($\chi^2_{(3)} = 5.864$, $P > 0.1$). The frequency of blood group B in these patients with gonorrhoea was 21.1% compared to 12% in those without. This difference was not, however, significant ($\chi^2_{(3)} = 5.103$, $P > 0.1$). When the frequency of blood group B in the patients with gonorrhoea was compared (21.1%) with that of the controls (10.7%) the difference ($\chi^2_{(3)} = 8.404$, $P < 0.05$) was significant.

The effect of the presence or absence of anti-B on susceptibility to gonorrhoea is shown in Table XV. A higher percentage (20.9%) of patients with no anti-B isohaemagglutinin had gonorrhoea compared with those with anti-B isohaemagglutinin (12.1%) ($\chi^2 = 4.947$, $P < 0.05$). Using the relative risk method of Woolf we found that the relative risk of gonorrhoea was 1.93 for individuals without anti-B isohaemagglutinin. This means

Table XIII Distribution of ABO and secretor status groups among diagnostic categories

Micro-organism isolated	O		A		B		AB		
	S	NS	S	NS	S	NS	S	NS	
N. gonorrhoeae only	18	7	13	5	9	3	2	0	57
N. gonorrhoeae and N. meningitidis	4	5	4	1	4	0	0	0	18
N. gonorrhoeae and N. lactamica	1	0	0	0	0	0	0	0	1
N. meningitidis only	22	9	13	11	11	0	1	0	67
N. lactamica only	2	0	0	0	0	1	0	0	3
Chlamydia	14	10	6	4	8	2	0	0	44
No micro-organism isolated	137	53	101	41	23	14	6	2	377

Table XIV Distribution of ABO blood groups in 567 patients with and without gonorrhoea and controls

Blood group	Culture results for N. gonorrhoeae		Total No. (%) of patients	No.(%) of controls
	No.(%) negative	No.(%) positive		
O	247(50.3)	35(46.1)	282(49.7)	3323(49.9)
A	176(35.8)	23(30.3)	199(35.1)	2410(36.2)
B	59(12.0)	16(21.1)	75(13.2)	715(10.7)
AB	9(1.8)	2(2.6)	11(1.9)	214(3.2)
Total	491(99.9)	76(100.1)	567(99.9)	6662(100.0)

that they are 93% more susceptible to gonorrhoea than persons with anti-B isohaemagglutinin.

The distribution of secretor status for patients with and without gonorrhoea and controls is given in Table XVI. No significant differences were noted between the total patients and the controls ($\chi^2 = 0.917$, $P > 0.1$) nor between those patients with and without gonorrhoea ($\chi^2 = 0.168$, $P > 0.5$).

Table XVII gives the breakdown of the survey results in terms of sex, secretor status, ABO blood group and patients with and without gonorrhoea.

Table XVIII shows the distribution of ABO blood groups among male and female patients with and without gonorrhoea. A chi-squared test reveals no significant heterogeneity within these distributions ($\chi^2_{(7)} = 8.894$, $P > 0.1$).

Table XIX gives the analysis of data from Table XVIII by presence and absence of anti-B isohaemagglutinin. For patients of blood groups B and AB (anti-B absent), there is a higher percentage of females with gonorrhoea (26.1%) than males (15%). Both these frequencies are higher than those found for male and female patients of groups A and O (anti-B present), with gonorrhoea. Multiple logistic regression of culture results on sex and presence and absence of anti-B showed no significant interaction ($\chi^2 = 1.72$, 1 d.f.). Thus we found no significant evidence that differences in susceptibility between patients with and without anti-B were related to sex. The reduced percentage of gonorrhoea infected males without anti-B, 15% as opposed to 26.1% for females, is closer to the 12.7% figure

Table XV Analysis of data from Table XIIIby presence or absence of anti-B isohaemagglutinin

Anti-B	Culture results for N. gonorrhoeae		Total
	No. (%) negative	No. (%) positive	
Present (O and A)	423(87.9)	58(12.1)	481(100)
Absent (B and AB)	68(79.1)	18(20.9)	86(100)
Total	491	76	567

Table XVI Distribution of secretor status for patients with and without gonorrhoea and controls

Secretor status	Culture results for N. gonorrhoeae		Total No.(%) of patients	No.(%) of controls
	No.(%) negative	No.(%) positive		
Secretor	344(70.1)	55(72.4)	399(70.4)	245(73.4)
Non-secretor	147(29.9)	21(27.6)	168(29.6)	89(26.6)
Total	491(100)	76(100)	567(100)	334(100)

Table XVII Distribution of blood group, secretor status and sex among 567 patients with and without gonorrhoea

Culture results for N. gonorrhoeae	Secretor status	O		A		B		AB	
		M	F	M	F	M	F	M	F
Positive	Secretor	10	13	9	8	5	8	1	1
	Non-secretor	8	4	4	2	0	3	0	0
Negative	Secretor	88	87	62	58	21	21	3	4
	Non-secretor	35	37	28	28	9	8	1	1

for males with gonorrhoea who had anti-B in their serum. It is feasible that there is an increased susceptibility to gonorrhoea only for females of groups B and AB and a larger study would be required to show this.

Table XVIII Distribution of ABO blood groups among male and female patients with and without gonorrhoea

Culture results for *N. gonorrhoeae*	O		A		B		AB	
	Male	Female	Male	Female	Male	Female	Male	Female
Positive	18 (48.6%)	17 (43.6%)	13 (35.1%)	10 (25.6%)	5 (13.5%)	11 (28.2%)	1 (2.7%)	1 (2.6%)
Negative	123 (49.4%)	124 (51.2%)	90 (37.8%)	86 (33.9%)	30 (11.2%)	29 (12.8%)	4 (1.6%)	5 (2.1%)

Table XIX Analysis of data from Table VI by presence or absence of anti-B isohaemagglutinin

Anti-B		Culture results for *N. gonorrhoeae*		Total
		No.(%) negative	No.(%) positive	
Present				
(O and A)	Male	213 (87.3)	31 (12.7)	244
	Female	210 (88.6)	27 (11.4)	237
Absent				
(O and A)	Male	34 (85.0)	6 (15.0)	40
	Female	34 (73.9)	12 (26.1)	46
Total		491	76	567

SECTION II

HOST-PARASITE INTERACTION STUDIES

1. Association of Neisseria gonorrhoeae and phagocytes

These assays were set up to examine the binding interactions of non-opsonised,pilate and non-pilate gonococci to PMN, monocytes and macrophages. These experiments were carried out as a necessary preliminary to the examination of the role of ABO blood group characteristics on binding to phagocytes. The association of pilate and non-pilate gonococci with PMN and monocytes was determined. Dose response experiments indicated the optimum concentration of gonococci was 10^8/ml. The binding level varied markedly with concentration and emphasises the importance of using accurately calibrated suspensions. A comparison of the total count (chamber count) and viable count (colony count), revealed a viability of only 14% for gonococci harvested and suspended in PBS. MNYC plates gave a greater percent viability (14%) when compared to GC plates (8%). Spectrophotometric assessment of bacterial concentration, calibrated to standards determined by chamber counts, was rapid and more accurate than viable counts, particularly as the gonococci-phagocyte assays did not involve discrimination between viable and non-viable organisms. Time course experiments suggested that 37°C for 30 min and 4°C for 2 hours, was ideal. Monolayers of 10^5/cells per coverslip facilitated microscopic counting while giving clearly separated cells.

1.1 Association of pilate and non-pilate variants

Figure 8 summarises the results of three experiments comparing the attachment of pilate (T_2) and non-pilate (T_3) gonococci to PMN

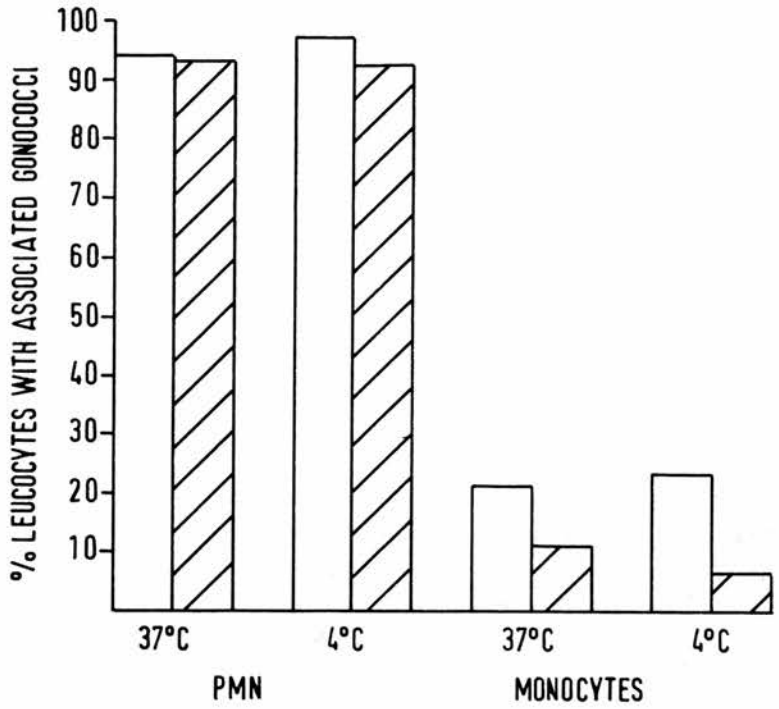


Figure 8 Association of pilate (T_2) and non-pilate (T_3) gonococci with PMN and monocytes. Clear bars = pilate, shaded bars = non-pilate. Each bar represents the mean of three experiments.

and macrophages. There was no significant difference in association of the two types to PMN at either 4°C or 37°C ($P > 0.5$). The association of pilate T_2 cells with monocytes was significantly greater than that observed with non-pilate T_3 cells at both 4°C ($P < 0.02$) and 37°C ($P < 0.01$). The significance tests for the difference in gonococcal association with PMN and monocytes is shown in Table XX. A significantly greater number of PMN than monocytes bound both pilate and non-pilate gonococci ($P < 0.01$).

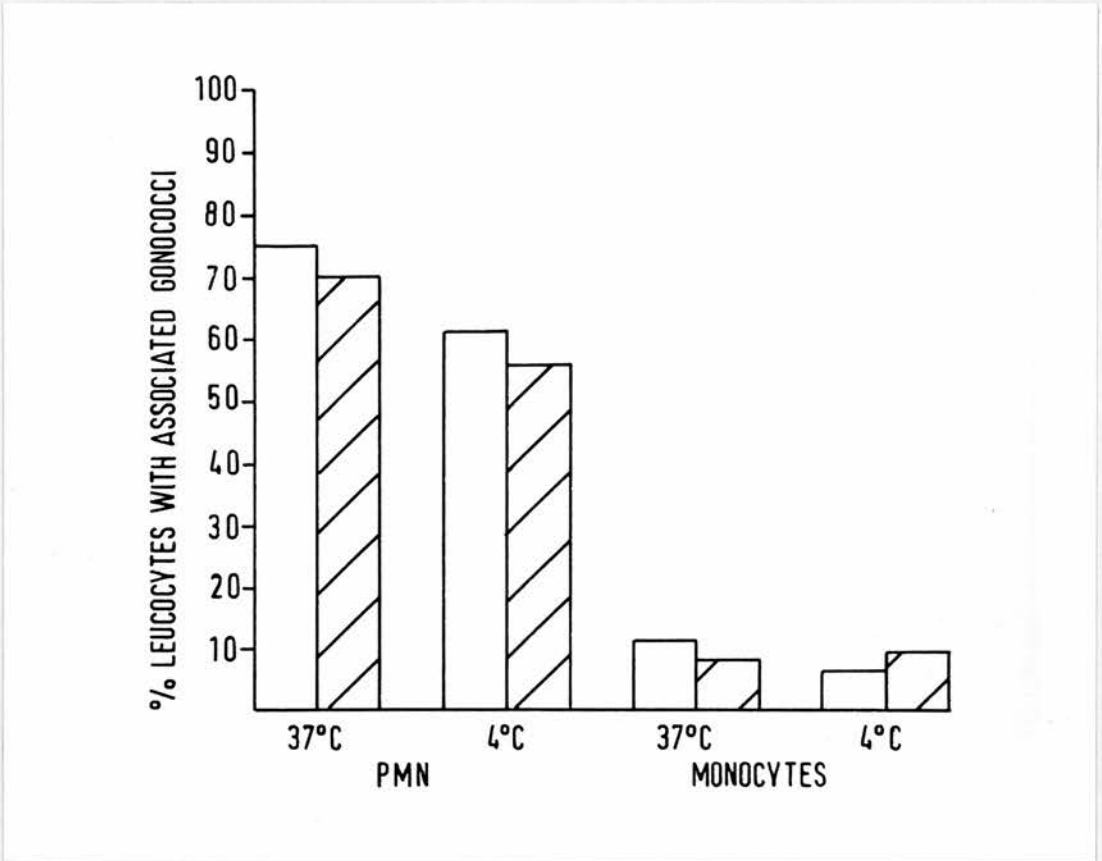


Figure 9 Association of both T_2 and T_3 gonococci to PMN and monocytes following mechanical shearing treatment of the gonococci. Each point represents the mean of three experiments.

1.2 Effect of removal of pili

Figure 9 represents the effect on binding of both T_2 and T_3 cells to PMN and monocytes following vortex treatment of the gonococci. The difference in binding of T_2 and T_3 cells to monocytes illustrated in Figure 8 was abolished by removal of pili from T_2 cells by mechanical shearing. While there was a slight overall reduction in binding of gonococci to the phagocytes following shearing treatment (Table XXI) there was still a significantly greater number of PMN than monocytes binding gonococci ($P < 0.05$).

Table XX Association of pilate (T_2) and non-pilate (T_3) gonococci with PMN and monocytes at 37°C and 4°C with percent differences and significance values

N. gonorrhoeae E757	Temperature	Percentage binding to PMN	Monocytes	% difference	P value
T_2	37°C	94.17	21.17	73.00%	< 0.01
	4°C	97.33	23.67	63.66%	< 0.001
T_3	37°C	93.17	7.00	86.17%	< 0.01
	4°C	92.17	6.50	85.67%	< 0.01

Table XXI Effect of loss of pili on association of gonococci with PMN and monocytes at 37°C and 4°C with percent differences and significance values

N. gonorrhoeae E757	Temperature	Percentage binding to PMN	Monocytes	% difference	P value
T_2 (vortexed)	37°C	75.67	10.25	65.47%	< 0.02
	4°C	61.67	6.33	55.34%	< 0.05
T_3 (vortexed)	37°C	70.25	8.33	61.92%	< 0.05
	4°C	58.58	9.33	49.25%	< 0.05

1.3 Comparison of 4°C and 37°C assays

The results for the assays done at 4°C represent attachment only as the monocytes and PMN do not phagocytose at this temperature. The 37°C assays are for attachment and phagocytosis, i.e. association. Differentiation between phagocytosis and attachment was not done, but the general trends in phagocyte-gonococci association at 4°C and 37°C were consistent and suggest that the type of attachment seen at 4°C also occurs at physiological temperatures at which phagocytosis takes place. Phagocytosis of gonococci by both monocytes and PMN occurred readily at 37°C as determined by thin section electron microscopy (Figures 10 and 11).

1.4 Association of radiolabelled gonococci to phagocytes

Human macrophages are generally located in tissues rather than blood, and are not available for experiments. Human monocytes, when maintained under appropriate conditions, undergo a monocyte to macrophage change after two or three days (W.H. McBride and M. Norval, personal communication). Visual features of this change are increases in cytoplasmic granules and overall size. Association of macrophage monolayers, prepared in this way, could not be assayed microscopically in the same way as monocytes and PMN. This was due to increases in size and numbers of intracellular components that did not permit accurate visual counting. Radiolabelling of gonococci was carried out in an attempt to overcome this problem. Concentrations of bacteria and phagocytes were as described previously. Assays by light

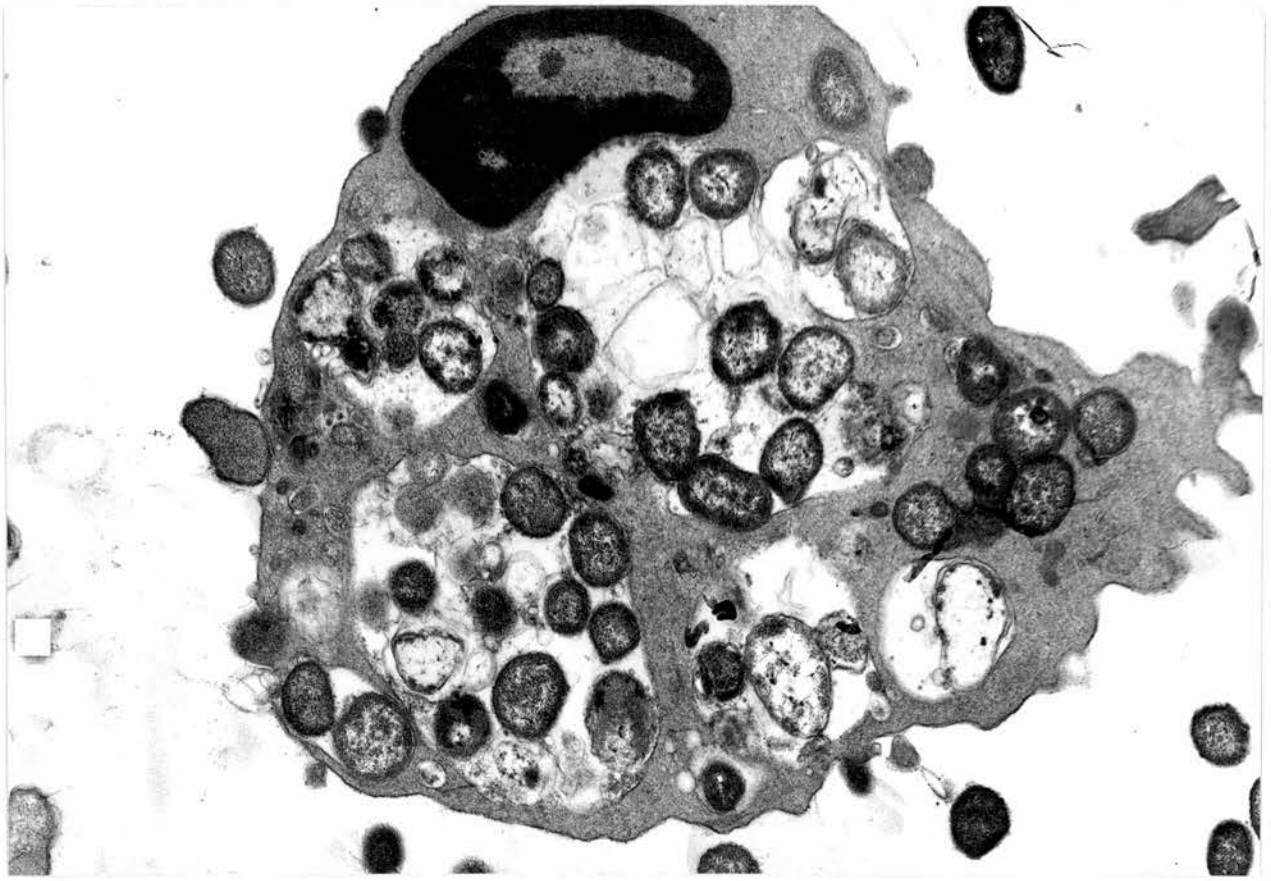


Figure 10 Thin section electronmicrograph of human peripheral blood monocyte with phagocytosed gonococci. X 15,000.

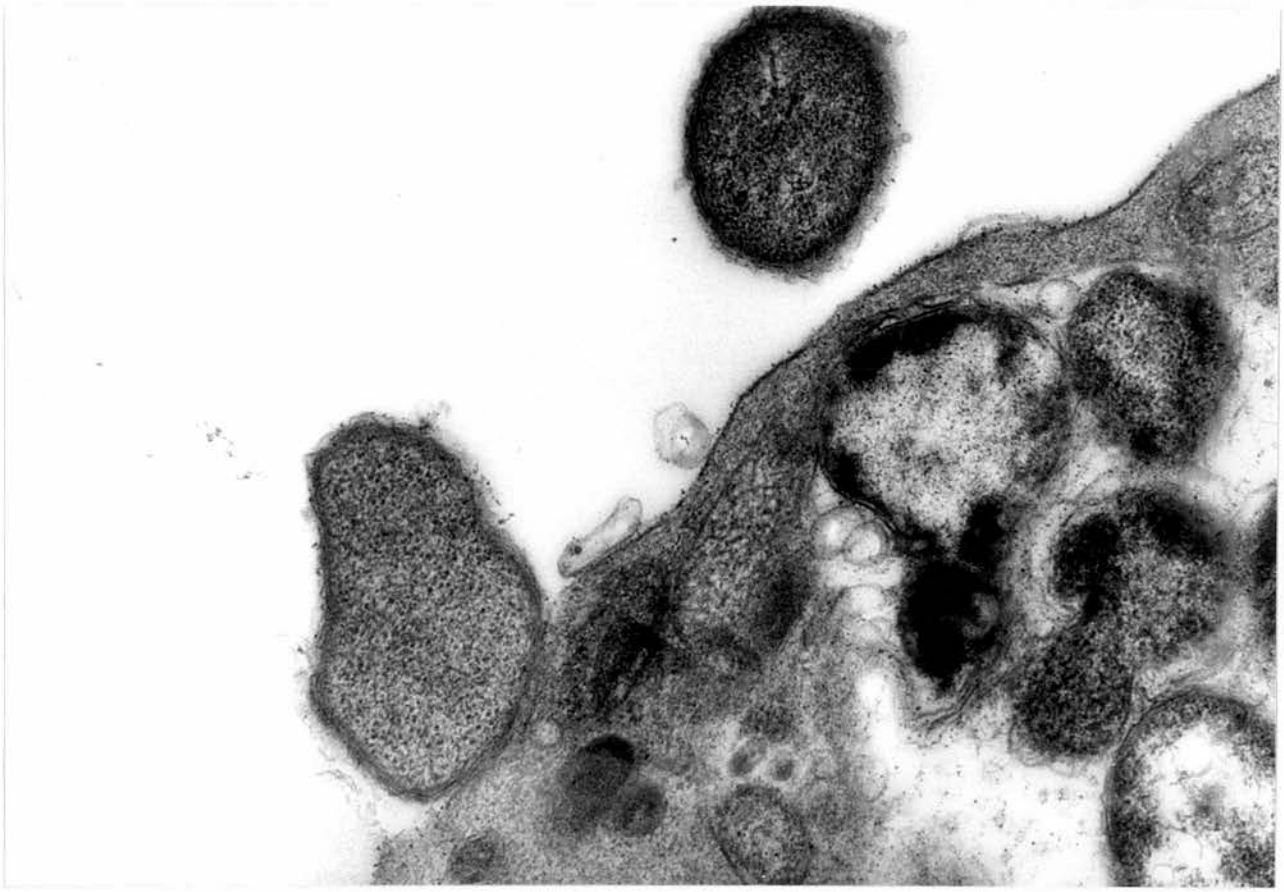


Figure 11 Enlargement of Figure 10 showing attachment of a gonococcus to the monocyte membrane. X 52,500.

microscopy were performed on monocytes and PMN at the same time as radiolabelling assays on all three types of cells. The microscopy assays were carried out to check association of gonococci to phagocytes and to calibrate cpm with microscopic binding counts. Microscope counts gave binding levels of gonococci to monocytes of around 30% which corresponded to approximately 250 cpm; binding of gonococci to PMN gave around 100% binding which corresponded to 550 cpm.

Figure 12 shows the association of gonococci to monocytes, neutrophils and 3 day cultured monocytes. The association of gonococci to macrophages is greater than to PMN which is in turn greater than monocytes. The association of pilate gonococci to PMN and macrophages is not significantly different from that of non-pilate gonococci. The association of pilate gonococci to monocytes however shows an increased level of pilate over non-pilate association but this increase is not statistically significant and the large standard errors demonstrate a rather large variability in the radiolabelling assay. This did not compare well with the technically simpler microscopic assay, and the radiolabelling assay was therefore not used for further studies.

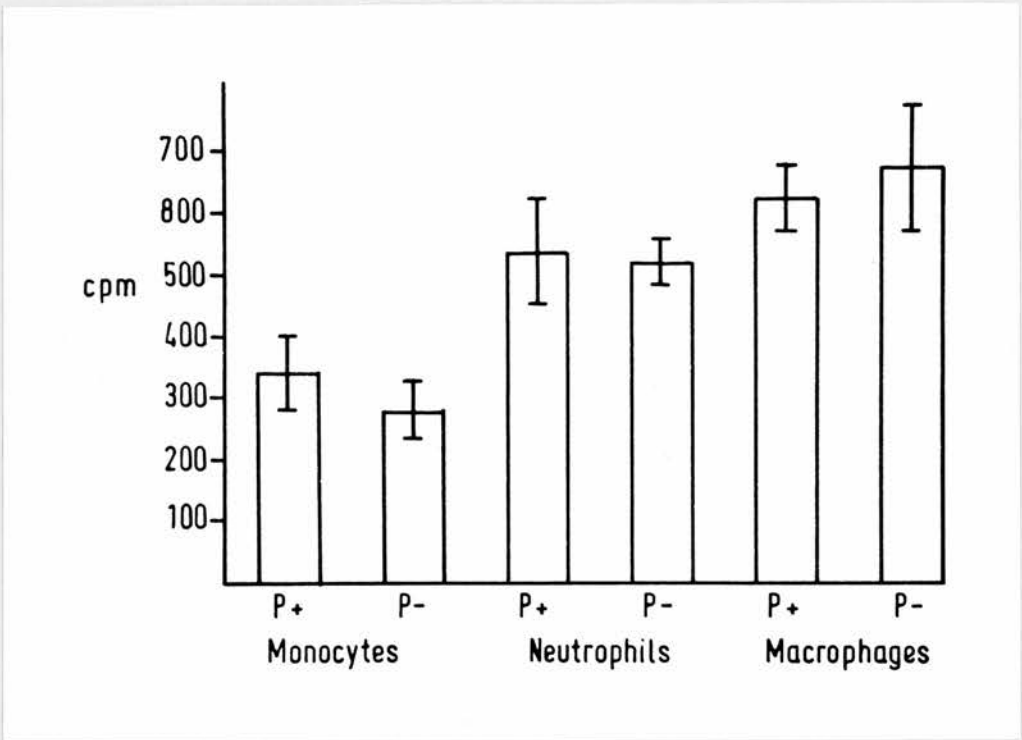


Figure 12 Association of pilate (P+) and non-pilate (P-) gonococci with monocytes, PMN and macrophages. Each bar represents the mean of three experiments, ± 1 SEM.

1.5 Sugar inhibition of gonococcal binding to phagocytes

These studies were undertaken to examine possible lectin-mediated interactions of gonococci with PMN and monocytes. These lectins, present on either bacterium or phagocyte may bind through sugar residues present on either the bacterium or phagocyte. 'Lectin-like' interactions can be demonstrated by binding inhibition after preincubation of cells with simple sugars. As noted previously, various workers using a variety of sugars, have been unable to demonstrate lectins of gonococci that can bind to host cells (Trust et al., 1980; King and Swanson, 1978).

Preliminary experiments with gonococci and leukocytes supported the findings of other workers (Trust et al., 1980; King and Swanson, 1978) and indicated the absence of simple sugar inhibitable lectins on gonococci. Efforts were therefore directed at examining sugar inhibitable lectins on phagocytes that might recognise gonococcal cell wall sugars. That such a mechanism might exist is suggested by the work of Watt et al. (1978) and King and Swanson (1978). Furthermore phagocytes have been shown to express lectins that bind a variety of bacterial species (Weir, 1980).

Figure 13 (a, b, c and d) show the results of sugar inhibition studies on binding of gonococci (pilate, E757) to phagocytes (of group 0) at 4°C for 2 hours. Each Figure shows the effect on binding of preincubation of the phagocytes with sugar at three different concentrations. Gonococcal binding to PMN and monocytes

Figure 13 (a, b, c and d) These figures show the effect of preincubation of phagocytes with the named monosaccharide on the binding of pilate gonococci of strain E757. The assays were carried out with PMN (= X) and monocytes (= ●) of blood group 0 at 4°C for 2 hours. Each point represents the mean of three experiments ± 1 SEM.

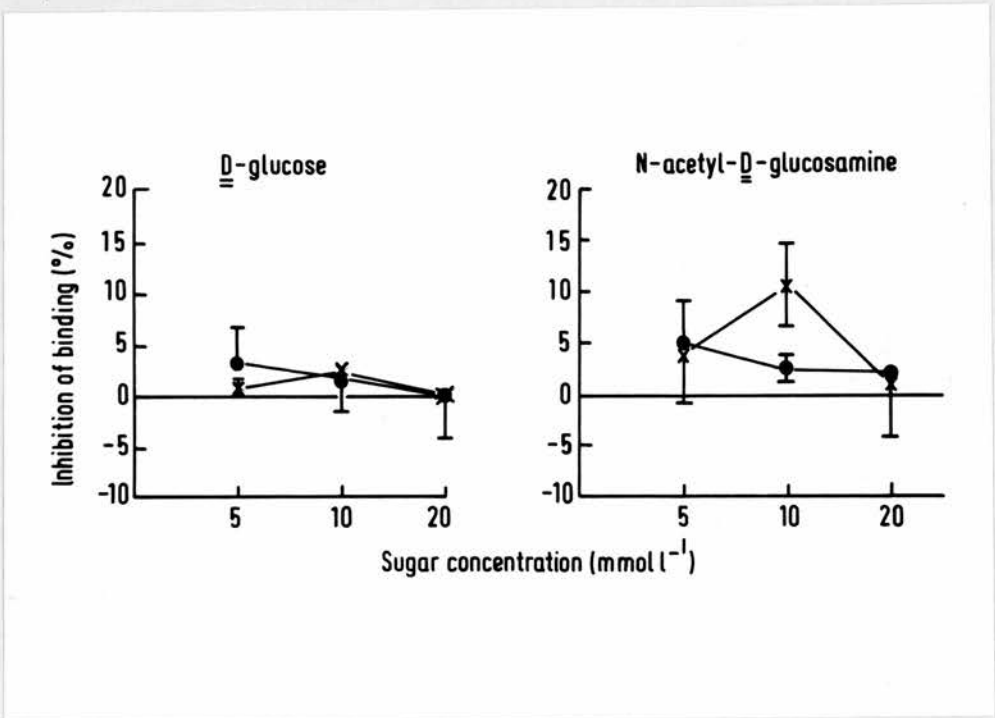


Figure 13(a)

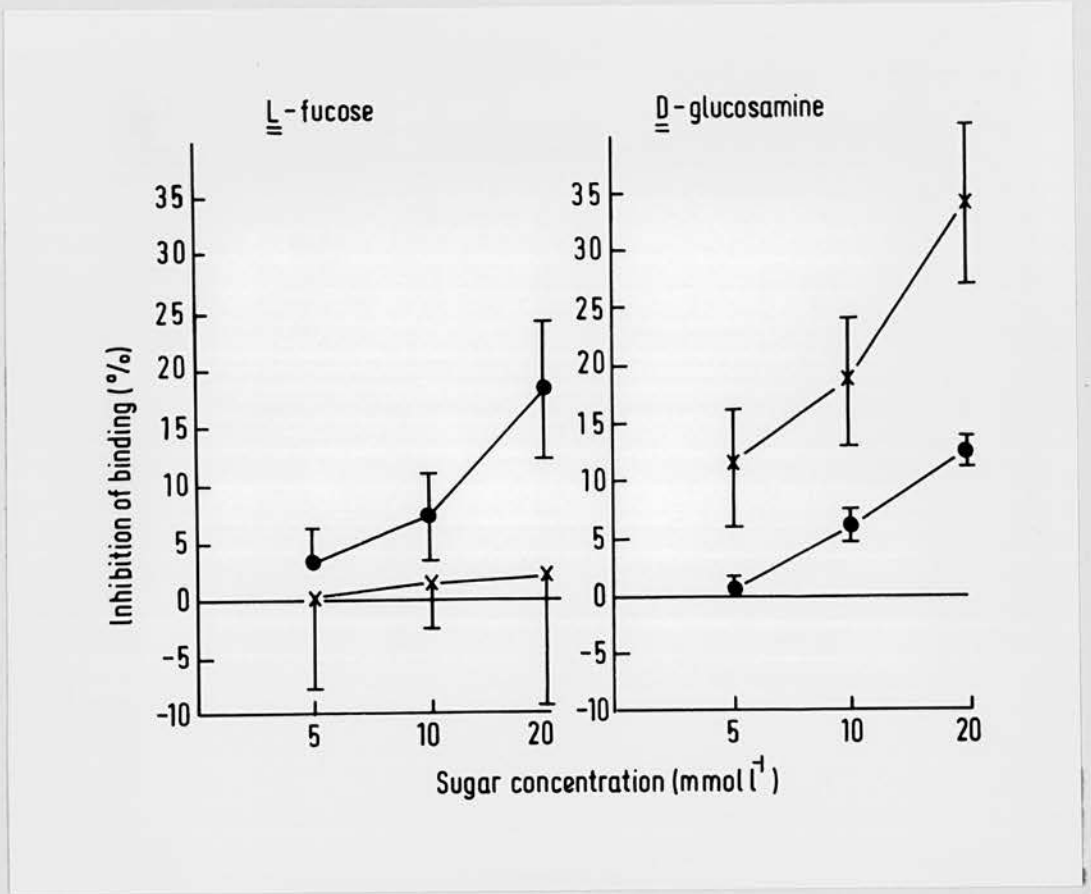


Figure 13(b)

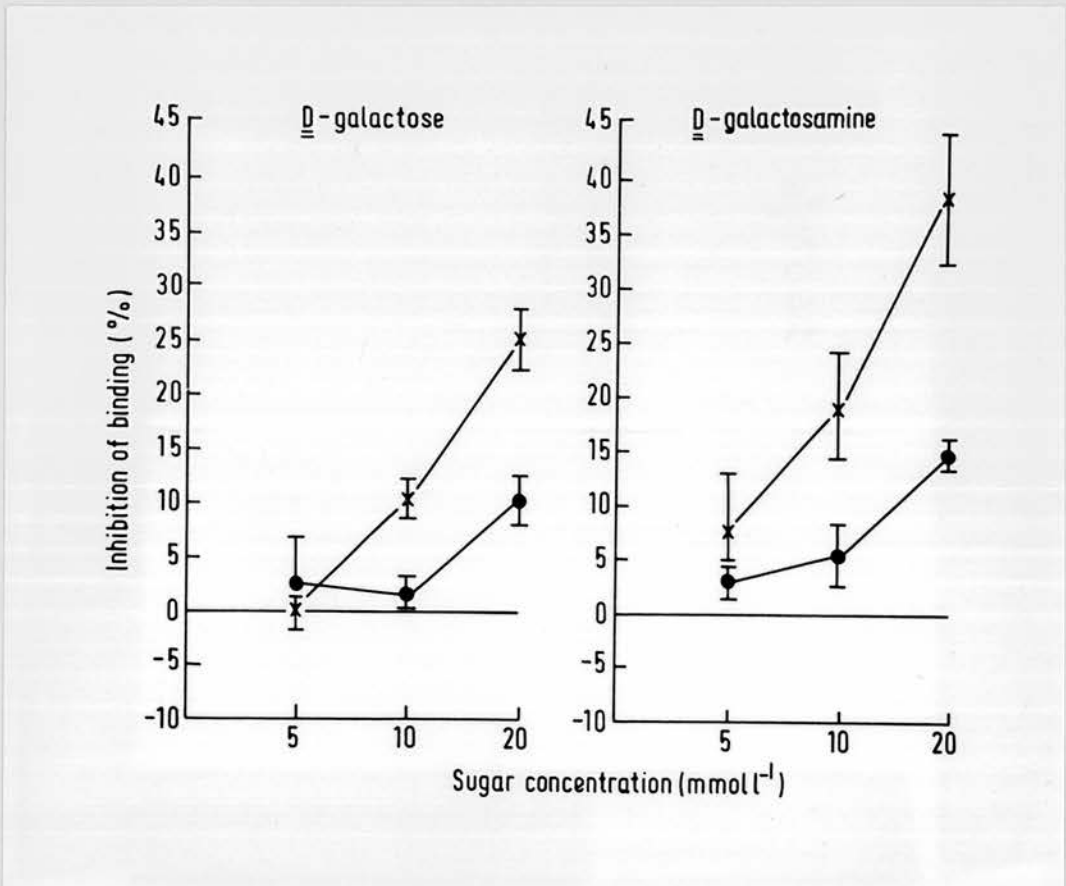


Figure 13(c)

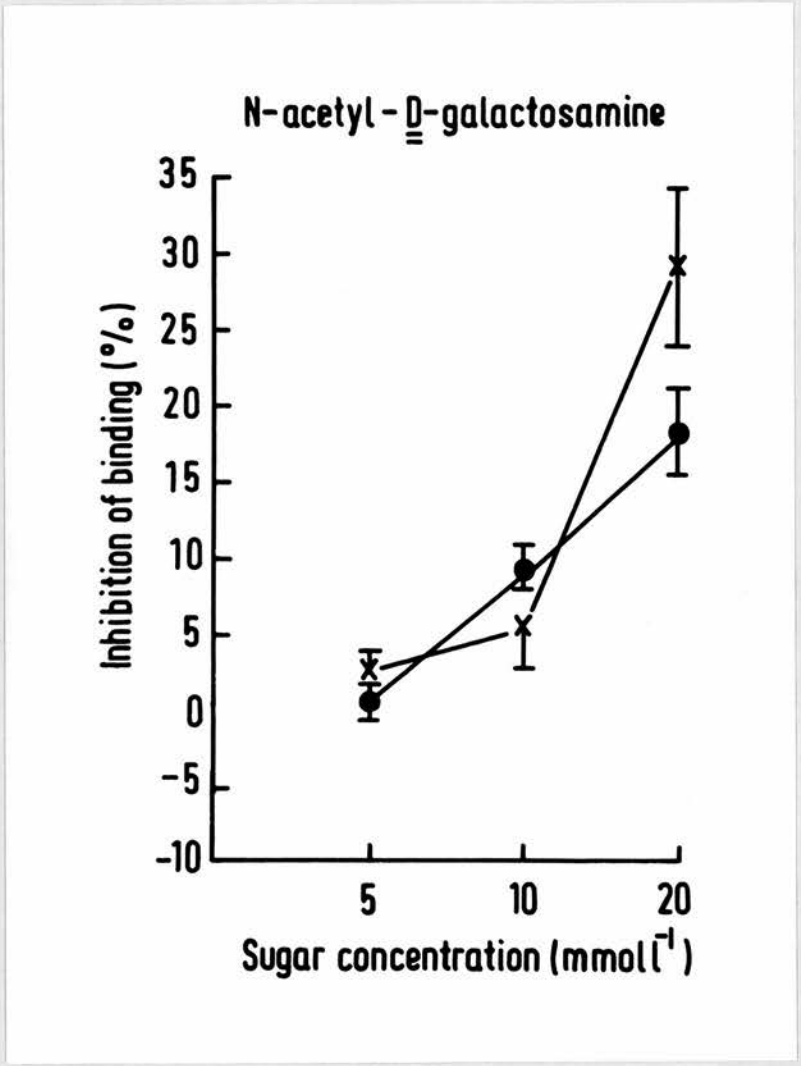

N-acetyl-D-galactosamine
Inhibition of binding (%)
35
30
25
20
15
10
5
0
-5
-10
5
10
20
Sugar concentration (mmol l⁻¹)

Figure 13(d)

can be inhibited by D-galactose, N-acetyl-D-galactosamine, D-galactosamine and D-glucosamine. Each of these inhibitions follows a dose response pattern, with the highest concentration 20 mmol l^{-1} giving the greatest binding inhibition. N-acetyl-D-glucosamine and D-glucose had no effect on the binding of gonococci to either PMN or monocytes. L-fucose inhibited binding of gonococci to monocytes in a dose response fashion but did not inhibit binding of gonococci to PMN. Differences in inhibition for PMN and monocytes were also seen with D-galactosamine, D-galactose and D-glucosamine, with the dose response inhibition of each of these sugars at a lower level for monocytes than PMN.

1.6 Factors affecting nitroblue tetrazolium reduction

These experiments were carried out to determine if differences in LPS among gonococcal strains or differences in pilation within strains affected the activation of intracellular bactericidal activities of PMN. Pilate and non-pilate variants of group I and group II strains were assessed for their potential to stimulate nitroblue tetrazolium (NBT) reductase in PMN. Two strains of group I (M9131 and E757) and 5 strains of group II (F62, M8865, M6967, E760 and 9) were used. No differences in the number of formazan-containing PMN were found in NBT tests with non-pilate variants of the two groups (Table XXII). In contrast for each strain tested, the presence of pili resulted in a significantly lower number of PMN containing formazan deposits compared to results obtained with its non-pilate variant (Table XXIII).

Table XXII Stimulation of NBT reduction by non-pilate (T4) variants of gonococci of groups I and II (percentage PMN containing formazan)

Strain	Number of experiments	Mean percentage PMN containing formazan (SE)	
		Unstimulated (control)	Stimulated (test)
Group I			
M9131	3	3.1 (0.62)	8.87 (2.8)
E757	2	3.8 (0.55)	24.2 (4)
Group II			
F62	3	5.9 (1.1)	24.03 (2.1)
M6967	2	8.4 (2.9)	22.4 (0.5)
9	2	3.9 (1.5)	13.9 (2.1)
M8865	3	3.1 (0.62)	19.3 (2.1)
E760	5	6.0 (2.2)	27.6 (6.6)

Table XXIII The effect of pilation on NBT reduction with gonococci of group I and group II (percentage PMN containing formazan)

Strain	Number of experiments	Mean percentage PMN containing formazan (SE)	
		Unstimulated (control)	Stimulated (test)
Group I			
E757 (T4) non-pilate	2	3.8 (0.55)	24.2 (4)*
E757 (T1) pilate	2	3.8 (0.55)	7 (4.5)†
Group II			
F62 (T4) non-pilate	1	10.6	25.9*
F62 (T1) pilate	1	10.6	10.6†
E760 (T4) non-pilate	5	6 (2.2)	27.6 (6.6)*
E760 (T1) pilate	5	6 (2.2)	11.3 (3)†

* $P < 0.001$

† N.S.

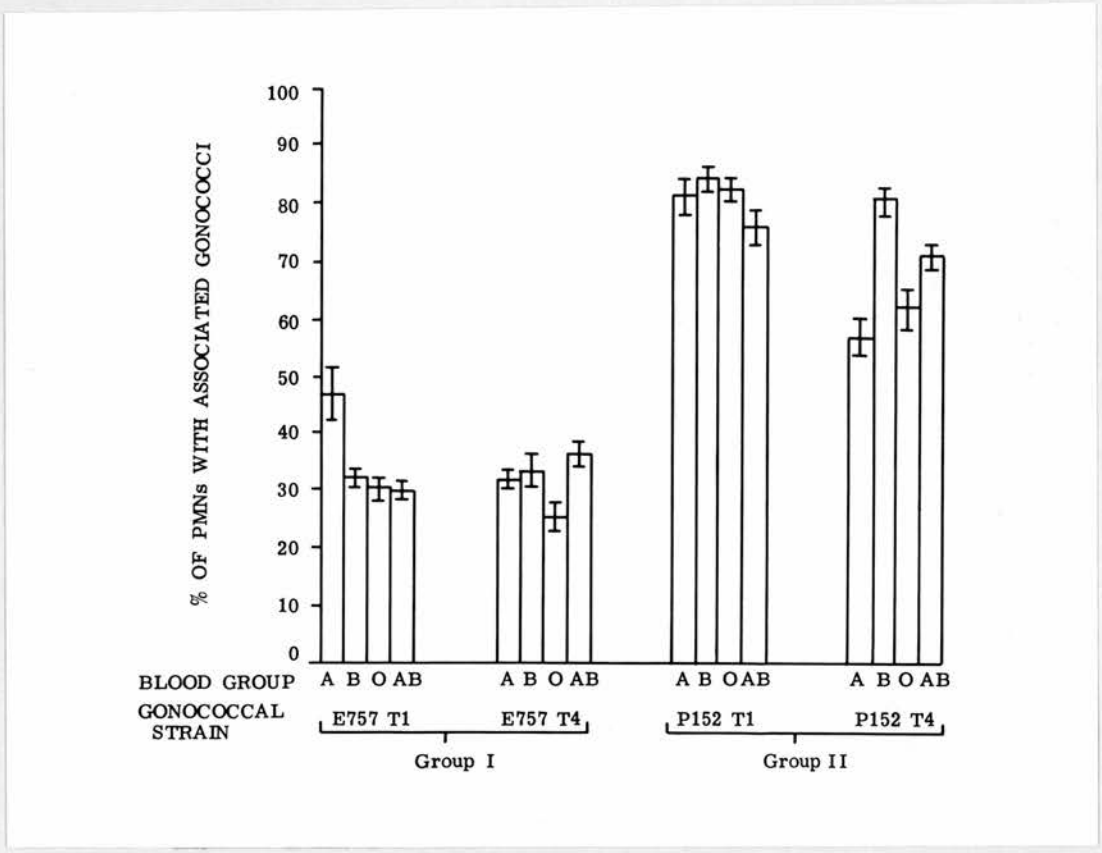


Figure 14 Attachment of gonococci to PMN of different ABO blood groups at 4°C. Each bar represents the mean and standard error of three experiments.

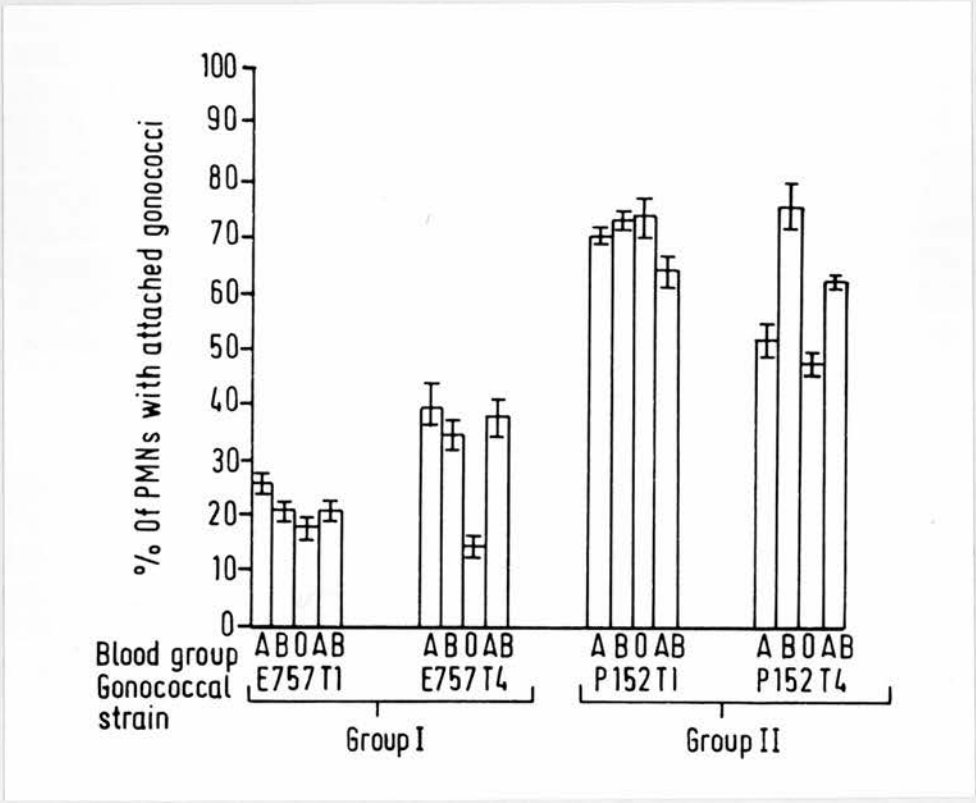


Figure 15 Association of gonococci to PMN of different ABO blood groups at 37°C. Each bar represents the mean and standard error of three experiments.

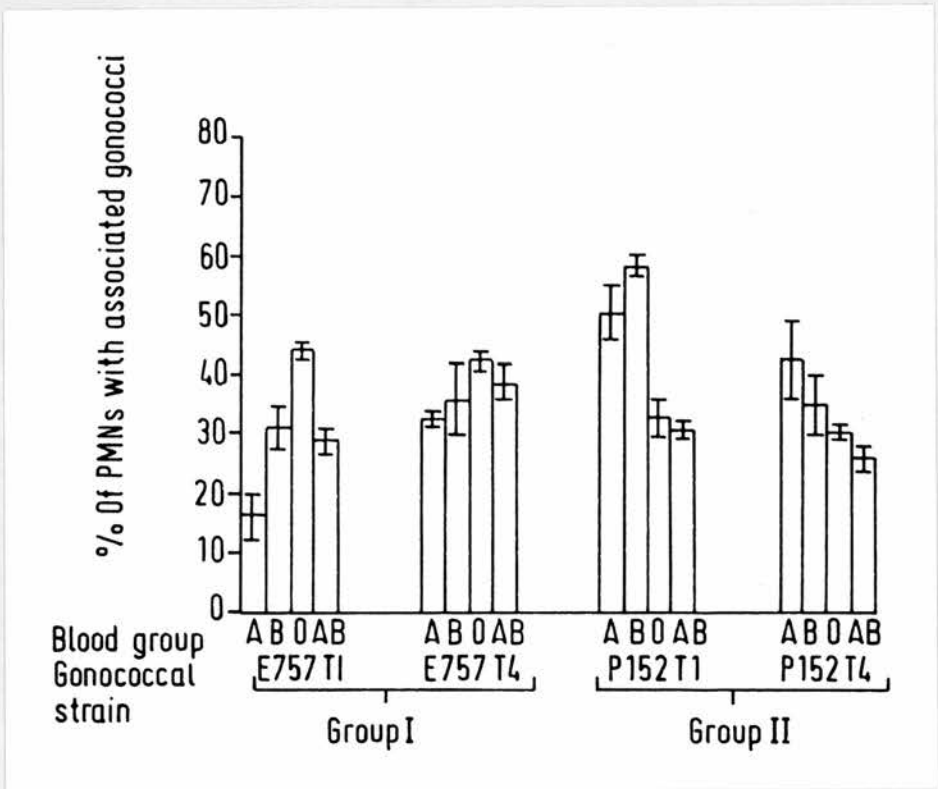


Figure 16 Association of gonococci to PMN of different blood groups at 37°C in the presence of homologous heat inactivated serum. Each bar represents the mean and standard error of three experiments.

2. Association of *Neisseria gonorrhoeae* to phagocytes of the four ABO blood groups

Experiments were undertaken in the absence of serum, that is in D.PBS, to determine if the ABO blood group antigens expressed on the phagocytes influenced binding of gonococci. The gonococcal strains used differed in their LPS (E757 was of pyocin sensitivity group I; P152 was group II) and protein I type (E757 was COA group WII; P152 was COA group WI) (Blackwell *et al.*, 1983; Sandström and Danielsson, 1980). Further gonococcal binding assays were carried out with PMN and monocytes of the four ABO blood groups in their autologous heat inactivated serum (HIS) to examine any opsonising ability of the anti-A and anti-B isohaemagglutinins.

2.1 Association of gonococci to PMN

Gonococcal strains E757 and P152 were examined in their interactions with PMN of the four different ABO blood groups. In D.PBS, similar patterns of association were seen at 4°C (Figure 14) and 37°C (Figure 15), in that gonococci of strain P152 gave higher binding levels with PMN than strain E757.

When the association of gonococci to PMN was examined in the presence of HIS (Figure 16), the previously noted increased binding in D.PBS for strain P152 over E757 was reduced. Heat inactivation of serum was necessary because strain P152 was complement sensitive and gave very low association levels when autologous fresh human serum (FHS) assays were carried out. These FHS assays were not continued. In HIS (Figure 16) as

in D.PBS (Figures 14 and 15), no significant pattern in binding was seen for PMN of the four ABO blood groups, nor were there any significant differences in binding between pilate and non-pilate gonococci.

2.2 Association of gonococci to monocytes

Figure 17 shows the association of pilate gonococci to monocytes of different ABO blood groups under various conditions; Dulbecco's Phosphate Buffered Saline (D.PBS) at 37°C for 30 min, D.PBS at 4°C for 2 hours and autologous heat inactivated serum (HIS) at 37°C for 30 min. Each bar represents the mean of three experiments, different individuals were used for each experiment.

Two factor analysis of variance was used to examine the effects of blood group and of the various conditions on association of gonococci to monocytes for each strain separately.

A significant interaction in this analysis indicates that the effect of blood group on association varied between the different conditions. When D.PBS 37°C and 4°C were compared in this way, no significant interaction was found for either strain, but when HIS was also included there was a significant interaction for both E757 ($P < .001$) and P152 ($P < 0.01$). As Figure 17 shows this is mainly due to the fact that association was greater for group B subjects when HIS was used whereas there was little difference in association between the blood groups when D.PBS was used.

A similar analysis was carried out to examine the effects of blood group and strain on association for each condition

separately. There was a significant interaction for HIS ($P < 0.05$) but not for D.PBS at 37°C or 4°C. In the presence of HIS, strain E757 shows more association with group A subjects than with those of groups O or AB, whereas strain P152 gives little difference in association between these three blood groups (Figure 17). Thus strain may not be important in association of gonococci to monocytes in D.PBS but may be important when serum is present.

Comparing the association in HIS and D.PBS at 37°C there is an overall increase in association for HIS throughout the blood groups with the exception of blood group AB. Blood group AB sera contain no isohaemagglutinins in contrast to A, B and O.

Figure 18 shows the effect of removal of isohaemagglutinins on association of gonococci to monocytes at 37°C. Group O monocytes and sera, from different donors, were used for each of three experiments. Absorption of both anti-A and anti-B isohaemagglutinins from HIS reduces binding levels to that of D.PBS. Absorption of only one isohaemagglutinin also reduced binding levels but not to the same extent as absorption of both isohaemagglutinins suggesting that both anti-A and anti-B isohaemagglutinins may be responsible for the increased binding levels of HIS above D.PBS. It is feasible therefore that although there are differences in binding patterns between strains, the significant interaction or binding pattern mentioned previously for HIS is due to the presence of isohaemagglutinins in the autologous sera.

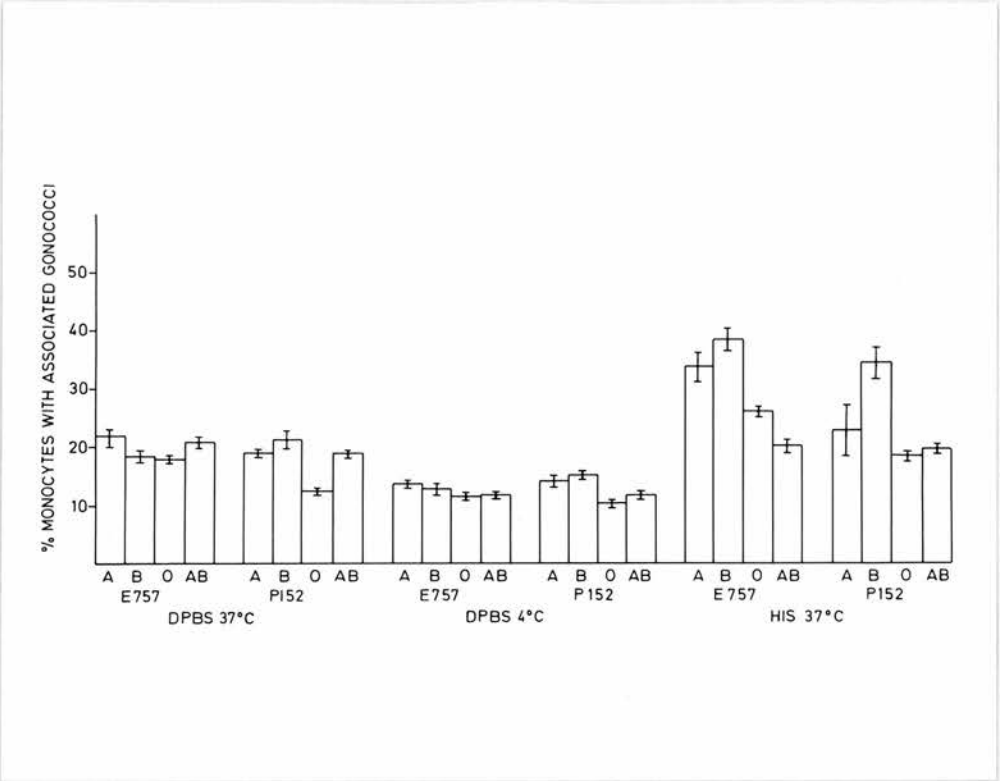


Figure 17 Association of gonococci to monocytes of different ABO blood groups under various conditions. Each bar represents the mean of 3 experiments, different individuals were used for each experiment.

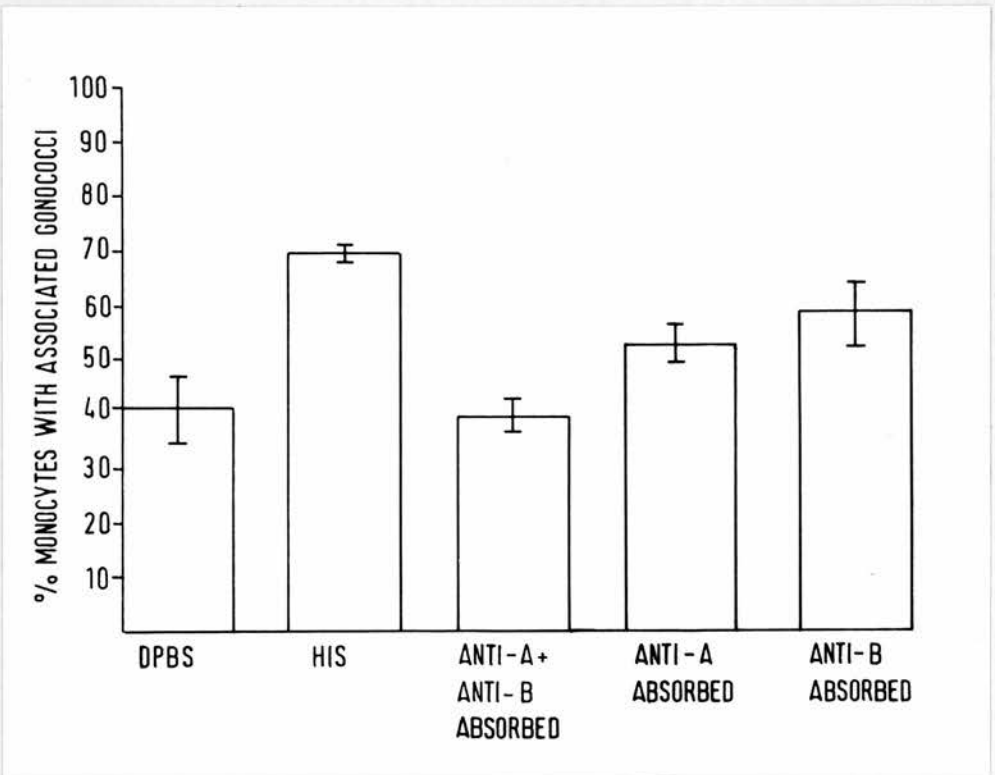


Figure 18 The effect of removal of isohaemagglutinins on association of gonococci to monocytes of blood group O at 37°C. Each bar represents the mean of three experiments ± 1 SEM.

DISCUSSION

1. Recurrent urinary tract infection survey

The highly significant excess of non-secretors of blood groups B or AB in the study group strongly suggests a synergistic link between the absence of anti-B isohaemagglutinin and secretor substances and an increased risk of recurrent urinary tract infection in women. In the controls the prevalence of non-secretors of 26.6% reflects an increased incidence of non-secretors found in Scottish and Irish ethnic groups compared with that reported for other regions in the United Kingdom (Lincoln and Dodd, 1972). This highlights the need to use local control groups in surveys of this nature. Within our controls bias in ABO groups is unlikely, and certainly not specifically in B or AB non-secretors versus other types. The results suggest an aetiological model whereby the presence of either anti-B or secretor substances (or both) is enough to reduce the risk of infection, and conversely, absence of both anti-B and secretor substances synergistically increases the relative risk of recurrent UTI.

Association of infections with ABO blood group and secretor status has led to investigation of the mechanisms concerned (Miler et al., 1977; Springer et al., 1961; Muschel and Osawa, 1959). Many bacteria cross-react with ABO blood groups (Springer, 1971). Drach et al. (1971) found A and B blood group activity in 47% of 34 urinary tract pathogens. Isohaemagglutinins may have an important role against these cross-reacting bacteria (Muschel and Osawa, 1959; Check et al., 1972). The finding that individuals of blood groups B and AB with no anti-B

isohaemagglutinins are more susceptible to urinary tract infection is interesting in that Drach et al. (1971) found antigens like those of blood groups A and B on urinary tract pathogens, often with group A and group B activity on the same bacterium. These workers noted that anti-B titres were sometimes raised in the disease but anti-A ones were not, suggesting that the antigen of blood group A was less immunogenic on the bacteria or that there were deficiencies in recognition of antigens of blood group A. Springer (1970) found E. coli 086:B 7 to be highly blood group active. Muschel and Osawa (1959) have shown anti-B isohaemagglutinin to be bactericidal for E. coli 086. Isohaemagglutinins may also interact with blood group like antigens on bacterial cell walls to inhibit attachment to uroepithelial or periurethral cells. Thus persons capable of producing anti-B isohaemagglutinins may have a greater degree of protection against urinary tract infection. In general, the extent of bacterial cross-reaction with ABO blood groups suggests that a selective advantage linked to ABO blood group might operate in combating infections.

Secretor status is associated with certain differences in immunoglobulin concentrations in that anti-B serum IgA and (in white people) serum IgG concentrations are lower in non-secretors than in secretors (Grundbacher et al., 1970; Grundbacher, 1972). This suggests that non-secretors may have less effective immune protection than secretors and is consistent with our findings that non-secretors were more susceptible to recurrent urinary tract infection. This probably entails the participation of

other factors, and the finding that excretion of blood group substances in urine is an active process in the kidney and not a simple filtration process (Kalinowski, 1972) suggests that these substances may have a protective part to play in secretions. Boat et al. (1978) found that ABH blood group substances of saliva inhibited haemagglutination of influenza B virus and suggested that some of these substances may interfere with access of influenza virus to binding sites. One of the protective roles of these blood group substances may be their ability to occupy or in some way interfere with binding sites either on the bacterium or on the epithelial cell with possible effects on bacterial colonisation and subsequent infection (Figure 19).

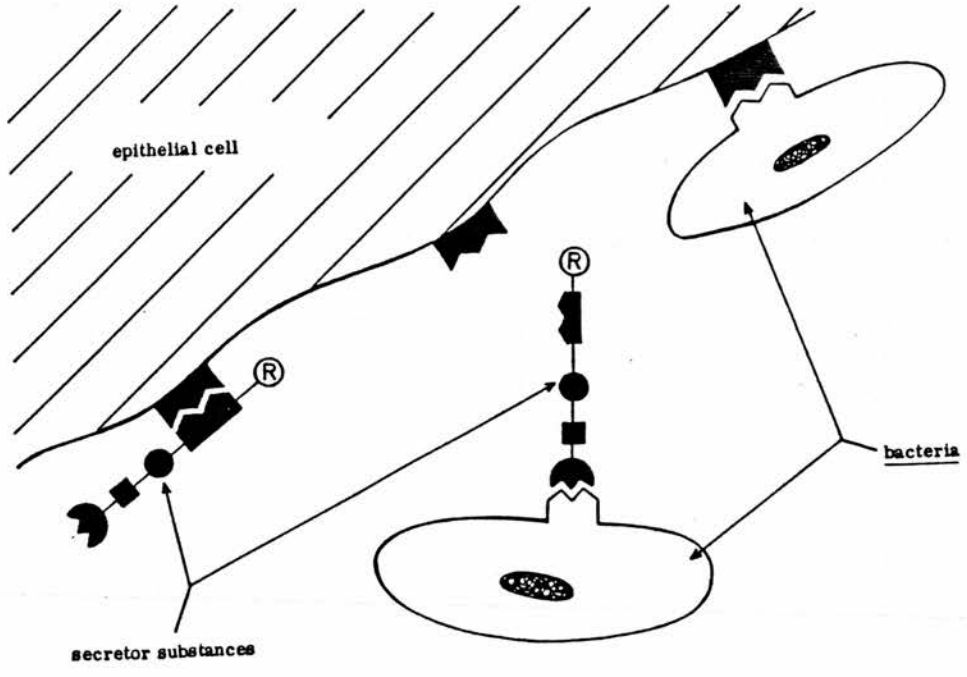


Figure 19 A postulated role for secretor substances in the competitive inhibition of binding of bacteria to epithelial cells.

The clinical value of screening patients by ABO blood group and secretor state, to determine individuals with increased risk, is not great. This screening would miss 91% of UTI patients who are not B or AB, non-secretors. Determination of blood groups and secretor status however, may provide additional information in identifying individuals who are at risk. The importance of UTI is obvious considering its prevalence and the significant risk of renal damage. Although the majority of UTI are undramatic, a large proportion of the population suffer from recurrent UTI and this makes considerable demands on medical resources. Few if any tests are available for providing prognostic information for adults with a normal urinary tract and a history of recurrent infection. Blood group and secretor status may be of relatively minor importance in relation to all other aspects of susceptibility. Nevertheless, it may be worthwhile for the clinician to take this into account in addition to already established factors in the assessment of patients with urinary tract infection when considering their long-term susceptibility and management. Meanwhile, there is a good case for research workers concerned with mechanisms of microbial pathogenicity to add this to an extending list of host factors that bear on host-parasite interactions.

2. Sexually transmitted disease survey

The significant excess of gonorrhoea patients with no anti-B isohaemagglutinin present when compared to the gonorrhoea negative

patients would seem to confirm the findings of others that B individuals are more susceptible to gonorrhoea. These results would further suggest that it is the absence of anti-B isohaemagglutinin that is important in the previously noted susceptibility of group B individuals to gonorrhoea. Johnson et al. (1983) found no increased incidence of blood group B in symptomatic male gonorrhoea persons. Johnson (personal communication) suggested that the differences in our published studies might be accounted for by sex, racial or symptomatic differences in our study populations. Our study was carried out during the non-holiday period. In the Edinburgh area the population is almost completely Caucasian and with the exception of the holiday season and the international Festival period there is a very low proportion of Asians, Negroes and West Indians. The breakdown of patients into symptomatic and asymptomatic attenders was not done; our study group will have been a mixture of both. Data on the sex of our STD clinic attenders was available and the appropriate analyses were carried out and are presented in the results section. Although our percentage of females with gonorrhoea (26.1%) is higher than males (15%), there was no evidence that significant differences in susceptibility between anti-B positive and negative patients were related to sex. However, our results are consistent with Foster and Labrum (1976) in that susceptibility is higher in anti-B negative (mainly group B) females. The reduced percentage of anti-B absent males with gonorrhoea, 15% as opposed to 26.1% for females, is closer to the 12.7% figure for males with gonorrhoea in the anti-B present category. Thus there is a tendency

to agree with Johnson et al. (1983) who found no increased susceptibility to gonorrhoea for group B males. It is feasible that there is an increased susceptibility to gonorrhoea only for females of groups B and AB and a larger study would be required to show this.

There was no significant difference between secretors and non-secretors in the gonorrhoea positive and negative categories and Johnson et al. (1983) have made a similar observation. Although there was a significant increase in the number of B and AB individuals with gonorrhoea, there was no synergy between the absence of anti-B isohaemagglutinin and non-secretion of water soluble blood group antigens as found in patients with urinary tract infections.

The underlying host-parasite interactions responsible for the increased susceptibility of individuals of blood groups B and AB to gonorrhoea are not yet known; they appear, however, to differ from those involved with urinary tract infections with Gram-negative bacteria. These epidemiological results, however, suggest an aetiological model whereby the absence of anti-B isohaemagglutinin increases the likelihood of gonococcal infection. This hypothesis may only apply to females and the epidemiological data on whether this hypothesis also applies to males is equivocal. Host-parasite interactions that would result in females with no anti-B isohaemagglutinin being more susceptible should be considered. Solish et al. (1961) have demonstrated the presence of isohaemagglutinins in the cervical secretions of women. These cervical secretions will be present during intercourse and anti-B isohaemagglutinin might protect against transmission of gonorrhoea. Anti-B

isohaemagglutinin's possible protective role will be dealt with in more detail, subsequently.

3. Interactions of gonococci with phagocytes

The preliminary experiments, employing monocytes and PMN of blood group O in attachment assays with strain E757, were carried out to identify conditions affecting interactions between phagocytes and gonococci. These preliminary experiments revealed differences in phagocyte recognition of gonococci.

The finding that PMN do not appear to discriminate in their ability to bind pilate and non-pilate variants of the strain used, supports observations made by others (Swanson et al., 1975). Monocytes, in contrast, showed an increased binding of pilate gonococci similar to that observed for various epithelial cells (Punsalang and Sawyer, 1973; James-Holmquest et al., 1974; Tramont and Wilson, 1977; Swanson, 1973) and other tissue cell types (Punsalang and Sawyer, 1973; James-Holmquest et al., 1974). The removal of pili from gonococci by mechanical shearing abolished the increased binding of pilate over non-pilate gonococci to monocytes, suggesting pili are important in the increased binding of pilate variants over non-pilate variants.

The overall binding of gonococci to PMN is much greater than that for monocytes indicating that there are other differences in the binding mechanisms of these phagocytes. Although pili enhance association to monocytes, binding still occurs in the absence of pili. Non-pilus components of the gonococcal outer surface, described in the introduction, may have a role in

association to phagocytes, e.g. the 'leukocyte association protein' of King and Swanson (1978). Protein II, present in both the opaque, pilate and non-pilate variants used in this study, has been found to have a role in the binding of gonococci to buccal cells (Lambden et al., 1979; Heckels and James, 1980) and leukocytes (Heckels, 1982). Protein I, in contrast, has been shown not to play a significant role in adhesion to buccal cells (Heckels and James, 1980). Another mechanism may be the 'lectin-like' receptors on phagocytes for gonococcal surface carbohydrates. The terminal sugar groups on the LPS of gonococci may be involved in attachment to receptors present on host cells (Watt et al., 1978) such as the lectin-like receptors of phagocytes (Weir, 1980). Izhar et al. (1982) have demonstrated that binding of Shigella flexneri to guinea pig colonic cells is mediated by a lectin on these epithelial cells. Their results indicated that the surface carbohydrate for their lectin may be in the LPS of the Shigella.

3.1 Sugar inhibition studies

The results presented reveal that phagocytes are capable of binding gonococci by various sugar inhibitable 'lectin-like' interactions. Each of the four sugars giving clear dose response inhibition; D-galactose, D-galactosamine, N-acetyl-D-galactosamine and D-glucosamine are present in the gonococcal outer surface (Wiseman and Caird, 1977; Perry et al., 1975; Stead et al., 1975).

D-glucose has been detected on the gonococcal outer surface, but did not inhibit binding. Work in our laboratory (Glass et al., 1981) has shown that D-glucose did not inhibit binding of Staphylococcus albus to human or guinea pig PMN indicating the absence of a D-glucose specific lectin on the phagocytes. In contrast human monocytes do express a D-glucose specific lectin and the failure to inhibit gonococcal attachment to these cells by D-glucose suggests that the D-glucose present on the gonococcal outer surface was somehow shielded or not exposed exteriorly. There was a notable difference in the ability of L-fucose to inhibit binding of gonococci in that inhibition occurred with monocytes but not with PMN. L-fucose has the same orientation at C_4 as D-glucose and this orientation has been shown to be important in binding of glycoprotein components to rat alveolar macrophages (Stahl et al., 1978; Weir et al., 1983). Thus the differences noted between PMN and monocytes with L-fucose may be due to the absence of the D-glucose-specific lectin on PMN. Further, quantitative differences are seen with D-galactosamine and D-glucosamine in their binding to PMN and monocytes. PMN in both cases were more readily inhibited than monocytes, presumably reflecting the numbers of 'lectin-like' receptors on the two cell types.

D-glucosamine does inhibit binding of gonococci to human PMN in contrast to the lack of inhibition by D-glucose. Thus the amine group on C2 appears to determine recognition. Previous work on the mannosyl, fucosyl, N-acetyl-glucosaminyl receptor

on rat alveolar macrophages had also implicated C2 as a determinant of specificity (Stahl et al., 1978; Weir et al., 1983). The failure of N-acetyl-D-glucosamine to inhibit binding of gonococci to either PMN or monocytes may reflect either the absence of this sugar from the gonococcal cell wall or the inhibition of recognition on adding the acetyl group to D-glucosamine. D-galactose, D-galactosamine and N-acetyl-D-galactosamine in contrast are found in the gonococcal cell wall and inhibit binding of gonococci to both PMN and monocytes.

The details of the specificity of phagocyte lectins for different sugars are not understood. Lectins in general, whilst showing preferential specificity for particular sugars, can also interact to a lesser degree with other sugars e.g. concanavalin A with its main specificity for α-methyl mannoside can also interact with D-mannose, D-fructose, maltose, isomaltose, trehalose and various lipopolysaccharides and bacterial polysaccharides.

The recent finding in our laboratories that there is an association between the mouse phagocyte lectin that recognises cell wall sugars of Staphylococcus albus and Ia antigens of the major histocompatibility complex (Stewart et al., 1982) further suggests that lectins may show a degree of polygamy in their ability to recognise carbohydrate determinants. It is known that only a small number of genes code for the I region antigens and, whilst there is extensive polymorphism in a species, polymorphism in an individual is necessarily limited. The I

region antigens of macrophages are thought to be involved in antigen presentation to T-lymphocytes, to carry out this function the I region must be able to recognise a wide range of determinants (Howie and McBride, 1982).

Little is known of the role of the human major histocompatibility complex (MHC) in determining susceptibility or resistance to gonorrhoea. A recent study in Singapore does however indicate that certain HLA haplotypes (A11 and B15) are associated with resistance to gonorrhoea and syphilis amongst Chinese prostitutes, HLA-B17 being associated with susceptibility. The B17 haplotype was found in 26% of prostitutes with combined infections of syphilis and gonorrhoea compared to 7% in the control population. This haplotype was absent in the prostitutes who were resistant to these infections (Chan and Rajan, 1982). The susceptible group in addition, were found to have poor blastogenic responses in lymphocyte transformation assays to treponemal and gonococcal antigens, compared to the resistant group. So far there is no evidence of any association with the HLA-D region (equivalent to the mouse I region). The importance of the class II MHC antigens in generation of the immune response to bacteria is widely accepted with T-helper cells recognising bacterial antigens in association with class II antigens on macrophages. The findings in the present study point to the need to explore further the recognition of gonococci by phagocytes and possible association with class II MHC antigens as determinants of susceptibility.

3.2 Gonococcal stimulation of the NBT reduction in PMN

The finding that pilate gonococci provoke less stimulation of superoxide production (detected by NBT reduction) in human PMN than non-pilate gonococci agrees with the previous report of Kreiger et al. (1980). They observed a minimal increase in glucose oxidation and oxygen consumption of PMN to which pilate gonococci were attached compared with the marked increase found with non-pilate gonococci. Densen and Mandell (1978) in contrast reported that in the presence of serum non-pilate gonococci gave only a slight increase in oxygen consumption over pilate gonococci. Kreiger et al. suggested that the differences between their study and Densen and Mandell's were due to strain differences. The former workers used DGI strains whereas the latter used F62 a laboratory strain. In the present study a number of strains from localised infections as well as F62 were used. In each case there was a lower percentage of formazan containing PMN in the presence of pilate variants in comparison with non-pilate variants of the same strain. This study suggests a further role for pili as a virulence factor, as decreased stimulation of PMN is likely to result in decreased bactericidal activity.

3.3 The role of pili in gonococcal infection

Two roles have been suggested for pili in the establishment of gonococcal infection: (1) pili assist the organism in attaching to mucosal surfaces (Swanson, 1973; Ward et al., 1974); and (2) pili increases gonococcal resistance to phagocytosis by PMN

(Tramont and Wilson, 1977; Swanson et al., 1971; Thongthai and Sawyer, 1973; Ofek et al., 1974). Support for this second role of pili was given by the NBT reduction assays discussed in the previous section. The finding that pilate gonococci bind better to monocytes suggests a third role for pili in conjunction with Novotny's hypothesis that 'gonorrhoea appears to be a specific disease of human macrophages' (Novotny et al., 1977). If pili increase the binding of gonococci to monocytes, this might increase the probability of gonococci attaching to and being phagocytosed by monocytes, resulting in the formation of 'infectious units'.

PMN bind appreciably more gonococci than monocytes. The large difference shown in binding of gonococci (whether pilate, non-pilate or vortexed) to PMN and monocytes is most striking and contrasts with work done on Staphylococcus albus in our department. There were no differences in the binding of staphylococci to PMN and monocytes (Glass et al., 1981). These results obtained with gonococci may provide one explanation for recurrence of infection in addition to the idea of broad heterogeneity of the gonococcal antigens (Kasper et al., 1977; McMillan et al., 1979b; McMillan et al., 1979c). An ineffective immune response may be explained by the much greater ability of PMN to take up the organism in comparison with the smaller numbers that are recognised and taken up by monocytes. If those that are taken up by macrophages are largely incorporated into 'infectious units' and survive while destroying the macrophage, the presentation of gonococcal antigen by macrophages to lymphocytes

will be reduced.

On the other hand, pili are not readily demonstrated on gonococci in human tissues (Novotny et al., 1975; Evans, 1977). If proteolytic enzymes in pus and tissues remove these from the surface of the organism, the probability of attachment to monocytes would be further reduced. As pili seem to enhance attachment to epithelial cells and macrophages, loss of pili would be likely to limit colonisation and to reduce the possibility of the development of infectious units in macrophages with a consequent reduction in virulence.

4. Association of Neisseria gonorrhoeae with PMN of the four ABO blood groups

These results indicate that there are no significant patterns in binding of gonococci to PMN of different ABO blood groups in the presence or absence of autologous heat inactivated serum (HIS). These findings with PMN do not support the hypothesis of Foster and Labrum (1976) that anti-B isohaemagglutinin is a more effective opsonin than anti-A isohaemagglutinin.

They do however indicate that differences in binding to PMN may be dependent on the strain of gonococci used. In the absence of autologous serum strain P152 bound to a greater proportion of PMN than strain E757, although this increase was not seen in the presence of HIS. Strain P152 has a pyocin type consistent with group II (Blackwell et al., 1983) and a coagglutination pattern, WI (Sandstrom and Danielson, 1980). E757, in contrast, has the pyocin type of a group I strain and

and a coagglutination pattern WII. This increased association of P152 over E757 in D.PBS at 4°C and 37°C may be due to a greater ability to bind on account of its LPS type or protein I type. Heckels and James (1980) pointed out that protein I is not involved to a great extent in the binding of gonococci to buccal epithelial cells. These results suggest that the bacterial surface antigens, and particularly the LPS, may be important in interaction between PMN and gonococci.

This increased binding of group II gonococci to PMN may be a virulence attribute. A mechanism for this has been suggested by Winstanley et al. (submitted for publication) in our laboratory, based on the increased binding of group II gonococci to PMN and the findings of Casey et al. (1983) that viable gonococci had a toxic effect on PMN. The suggested mechanism is that the increased binding of group II gonococci to PMN may result in an enhanced ability to kill PMN by means of this cytotoxin. In addition to the destruction of functional PMN, the lysed PMN may release proteases and other intracellular substances inimical to complement components. These intracellular substances may reduce the ability of complement to kill the complement sensitive group II gonococci and thus these gonococci may be indirectly protected from complement by their increased binding to PMN.

5. Association of Neisseria gonorrhoeae with monocytes of the four ABO blood groups

The results indicate that there are no differences in

gonococcal association to monocytes of different ABO blood groups in the absence of autologous heat inactivated sera. This suggests that differences in ABO blood group antigen expression on monocytes does not influence gonococcal attachment ($4^{\circ}C$) or phagocytosis and/or attachment ($37^{\circ}C$). There were no significant differences between strains P152 and E757 in their binding in D.PBS at both $4^{\circ}C$ and $37^{\circ}C$. This contrasts with work on PMN which shows marked differences in binding between strains.

The association of gonococci to monocytes of different ABO blood groups in homologous heat inactivated sera showed a significant pattern of binding for each strain, P152 ($p < 0.01$) and E757 ($p < 0.001$) with an increased level of association for group B monocytes. A further statistical analysis showed significant differences in pattern of association to different ABO monocytes for P152 and E757 ($p < 0.05$). Thus strain is important in gonococcal association with monocytes in autologous HIS. HIS increases the association of both strains to monocytes of all blood groups except AB, i.e. the blood group with no isohaemagglutinins. Miler et al. (1977) found that the isohaemagglutinins induced by gonococci were predominantly of the immunoglobulin M class as are isohaemagglutinins of normal sera. Haegart (1979) has demonstrated the presence of IgM receptors on human peripheral monocytes. The experiments indicate that isohaemagglutinins were responsible for the increased association of gonococci to monocytes in HIS over that found in D.PBS. This differs from the findings for PMN where various isohaemagglutinins gave no significant increase

in association.

These results would seem to contradict predictions that blood group B individuals may be more susceptible to the disease due to the absence of anti-B isohaemagglutinin in their serum. The possible consequences of increased binding of pilate gonococci to monocytes has been discussed (section 3.3) in relation to Novotny's hypothesis (Novotny et al., 1977). If the increased association of gonococci to group B monocytes resulted in the formation of more "infectious units", this might be part of the explanation for the increased susceptibility of group B individuals to gonorrhoea.

The reasons for this increase in binding to group B monocytes in autologous HIS in comparison with monocytes of other blood groups in autologous HIS may, as has been stated, be due to the isohaemagglutinins present. The results indicate, however, that anti-A and anti-B isohaemagglutinins are both capable of opsonisation and an explanation of how anti-A isohaemagglutinin may result in increased binding is given by Miler et al. (1977). They found that anti-A isohaemagglutinin was absorbed from serum by gonococci much more readily than anti-B. Group O autologous HIS has both anti-A and anti-B isohaemagglutinins present however there is no increased binding seen. The titre of anti-A in both the B sera and O sera used in these assays were similar. The sera of O individuals, "anti-A,B" is not simply a mixture of anti-A and anti-B isohaemagglutinins as there is cross-reactivity of both isohaemagglutinins with the A and B antigens (Race and Sanger, 1975). The reduced binding of

gonococci opsonised with O serum compared to B serum may be due to the anti-A isohaemagglutinin having to compete with the anti-B isohaemagglutinin, either of which may be cross-reacting with A or B antigens of gonococci. This distribution of isohaemagglutinin would also be affected by the representations of A and B-like antigens on the gonococcal surface. If the B-like antigens were situated close to the A-like antigens then opsonisation might be reduced by steric hindrance brought about by the proximity of bound anti-A and anti-B in an analogous fashion to the prozone phenomenon seen with agglutinins (Issitt and Issitt, 1979). Further studies using different strains of gonococci with possible variations in expression of A- and B-like antigens may help to elucidate these issues.

Suggestions for further studies on ABO groups and host-parasite interactions

These studies have not positively identified the link between blood group and susceptibility to gonorrhoea. They have however elucidated a number of aspects of interactions between gonococci and phagocytic cells that may be of importance in the pathogenesis of the disease and indicated areas for future investigation. These studies particularly emphasise that in experimental models of infections characteristics of both the host and parasite must be carefully considered.

From these studies and others in our laboratory the only immunological link between blood group and susceptibility to gonorrhoea appears to be the increased association of gonococci to monocytes of group B. The next stages of the investigation should include:

1. Study of intracellular killing of pilate and non-pilate variants of group I and group II gonococci by monocytes of the various ABO blood groups.

2. Studies on antigen presentation by the monocytes, of these bacteria and Neisseria meningitidis using a lymphocyte proliferation assay.

The first would examine Novotny's assertion that gonococci inactivate intracellular killing within the monocyte with subsequent formation of infectious units. The second study would explore the findings that although gonococci and meningococci are related organisms an effective immune response is produced against meningococci but not gonococci. The response against meningococci is long lived, but that for gonococci is transient. Differences in sites of the disease process may account for these differences in immune protection, but comparison of the ability of monocytes to present gonococcal and meningococcal antigens to lymphocytes may provide evidence for fundamental differences in the host response to these two agents.

Whilst studies so far have not demonstrated lectin-like molecules on gonococci, various studies have suggested there may be lectins capable of binding oligosaccharide, thus the ABO carbohydrate containing antigens could mediate binding of

gonococci to epithelial cell surfaces. Studies on attachment of pilate and non-pilate variants of group I and group II to uroepithelial cells from individuals of different ABO blood may provide evidence for this hypothesis.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr Caroline Blackwell and Professor D.M. Weir for their guidance, encouragement and intellectual stimulation throughout the course of this research. I also thank Professor J.G. Collee for his interest in this study, and for having allowed me to work in the Bacteriology Department of the University of Edinburgh. I wish to thank members of Dr Blackwell's and Professor Weir's research groups; F.P. Winstanley, Sarah Frykenberg, Dr E.J. Glass, Dr J. Stewart, Anne Oliver and all other members of both laboratories. The help and advice of various members of the Bacteriology Department throughout this project has been most valuable. I am grateful to Dr R.P. Brettle of the City Hospital, Edinburgh and Dr D.H.H. Robertson of the Department of Genito-urinary Medicine, Royal Infirmary, Edinburgh, for their valuable assistance in the epidemiological studies. I also wish to thank Dr H. Young for making available diagnostic results and for his useful advice. I thank the Blood Transfusion Service, Royal Infirmary, Edinburgh for undertaking random verification checks on selected saliva and plasma samples, and for the generous supply of reagents. I wish to acknowledge the assistance of Mr M.J. Kowolik of the Department of Oral Medicine, Edinburgh, in carrying out the NBT studies, and Mrs O. Gray for her assistance with thin section electron-micrographs. During the period of this research, Yam Lin Ho Wang Chok, an honours student supervised by the author,

carried out work that contributed to the overall study.
In addition I would like to thank Mrs Vivien McGrath and Mrs C.A. Kinane for their skilful preparation of this typescript and the Medical Illustration Service for their expert help in preparing the figures. Finally I wish to thank the Faculty of Medicine of the University for having granted me a Faculty of Medicine Davidson Research Fellowship from the Faculty of Medicine Scholarship Fund.

REFERENCES

Alexander, W. (1921) An inquiry into the distribution of the blood-groups in patients suffering from 'malignant disease'. Brit. J. Exper. Pathol., 2, 66-69.

Allison, A.C. (1982) Macrophages and neutrophils. In: Lackman, P.L. and Peters, D.K. (ed.), Clinical Aspects of Immunology, Blackwell Scientific Publications. Oxford, London, pp. 101-129.

Angell, M.E., Relman, A.S. and Robbins, S.L. (1978) 'Active' chronic pyelonephritis without evidence of bacterial infection. N. Engl. J. Med., 278, 1303-1308.

Apicella, M.A. and Gagliardi, N.C. (1979) Antigenic heterogeneity of the non-serogroup antigen structure of Neisseria gonorrhoeae lipopolysaccharides. Infect. Immun., 26, 870-874.

Armitage, P. (1971) Statistical methods in medical research. Blackwell, Oxford, pp. 375-384.

Bateson, W. (1909) Mendel's principles of hereditary. Cambridge, Univ. Press., pp. 205-234.

Barr, J. and Danielsson, D. (1971) Septic gonococcal dermatitis. Br. Med. J., 1, 482-485.

Barua, D. and Paguio, A.S. (1977) ABO blood groups and Cholera. Annals of Human Biol., 4, 489-492.

Beardmore, J.A. and Karimi-Booshehri, F. (1983) ABO genes are differentially distributed in socio-economic groups in England. Nature, 303, 522-524.

Bergström, T., Lincoln, K., Redin, B. and Winberg, J. (1968) Studies of urinary tract infections in infancy and childhood. X. Short or long-term treatment in girls with first or second-time urinary tract infections uncomplicated by obstructive urological abnormalities. Acta Paediatr. Scand., 57, 186-194.

Bernstein, F. (1924) Ergebrisse einer biostatischen zusammenfassenden Betrachtung uber die erblichen Blutstrukturen des Menschen. Klin. Wschr., 3, 1495-1497.

Blackwell, C.C., Young, H. and Andersen, I. (1979) Sensitivity of Neisseria gonorrhoeae to partially purified R-type pyocins and a possible approach to epidemiological typing. J. Med. Microbiol., 12, 321-335.

Blackwell, C.C., Kowolik, M., Winstanley, F.P., Kinane, D.F., Weir, D.M., Law, J.A. and Ho Wang Chok, Y.L. (1983) ABO blood group and susceptibility to gonococcal infection. I. Factors affecting phagocytosis of Neisseria gonorrhoeae. J. Clin. Lab. Immunol., 10, 173-178.

Blake, M. and Swanson, J. (1975) Studies on gonococcus infection. IX. In vitro decreased association of pilated gonococci with mouse peritoneal macrophages. Infect. Immun., 11, 1402-1404.

Boat, T.F., Davis, J., Stern, R.C. and Cheng, P.W. (1978) Effect of blood group determinants on binding of human salivary mucous glycoproteins to influenza virus. Biochem. Biophys. Acta., 540, 127-133.

Boswell, J. (1980) Life of Johnson. Oxford Univ. Press, Oxford, New York, Toronto, Melbourne. 1492 pages.

Boyle, W. (1968) An extension of the ^{51}Cr release assay for the estimation of mouse cytotoxins. Transplantation, 67, 761-764.

Böyum, A. (1968) Isolation of leucocytes from human blood. Further observation. Methyl cellulose dextran and Ficoll as erythrocyte-aggregating agents. J. Clin. Lab. Invest., 21, Suppl. 97, 31-50.

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.

Brooker, B.E. and Fuller, R. (1975) Adhesion of lactobacilli to the chicken crop epithelium. J. Ultrastruct. Res., 52, 21-31.

Brown, W.J., Lucas, C.T. and Kuhn, U.S.G. (1972) Gonorrhoea in the chimpanzee. Infection with laboratory-passed gonococci and by natural transmission. Br. J. Vener. Dis., 48, 177-178.

Buchanan, J.A. and Higley, E.T. (1921) The relationship of blood groups to disease. Brit. J. exp. Pathol., 2, 247-255.

Buchanan, T.M. (1978) Antigen-specific serotyping of Neisseria gonorrhoeae. I. Use of an enzyme-linked immunosorbent assay to quantitate pilus antigens on gonococci. J. Inf. Dis., 138, 319-325.

Buchanan, T.M. and Gotschlich, E.C. (1973) Studies on gonococcus infection. III. Correlation of gonococcal morphology with infectivity for the chick embryo. J. Exp. Med., 137, 196-200.

Buchanan, T.M. and Hildebrandt, J.F. (1981) Antigen-specific serotyping of Neisseria gonorrhoeae characterisation based upon principal outer membrane protein. Infect. Immun., 32, 985-994.

Buchanan, T.M. and Pearce, W.A. (1976) Pili as a mediator of the attachment of gonococci to human erythrocytes. Infect. Immun., 13, 1483-1489.

Buchanan, T.M., Chen, K.C.S., Jones, R.B., Hildebrandt, J.F., Pearce, W.A., Hermodsen, M.A., Newland, J.C. and Luchtel, D.L. (1978) Pili and principal outer membrane protein of Neisseria gonorrhoeae: immunochemical structural and pathogenic aspects. In: Brooks, G.F., Gotschlich, E.C., Holmes, K.K., Sawyer, W.D. and Young, F.E. (ed.), Immunobiology of Neisseria gonorrhoeae, Am. Soc. Microbiol., Washington D.C., pp. 145-154.

Buchanan, T.M., Eschenbach, D.A., Knapp, J.S. and Holmes, K.K. (1980) Gonococcal salpingitis is less likely to recur with Neisseria gonorrhoeae of the same principal outer membrane protein antigenic type. Am. J. Obs. Gyn., 138, 978-980.

Casey, S.G., Veale, D.R. and Smith, H. (1983) Cytotoxicity of Neisseria gonorrhoeae for human peripheral blood phagocytes. J. Gen. Microbiol., 129, 1097-1102.

Cattell, R.B., Boutourline Young, H. and Hundleby, J.D. (1964) Blood groups and personality traits. Amer. J. Hum. Gen., 16, 397-402.

Chan, K. and Wiseman, G.M. (1975) A new colonial type of N. gonorrhoeae. Br. J. Vener. Dis., 51, 251-256.

Chan, S.H. and Rajan, V.S. (1982) HLA-resistance and susceptibility to venereal disease. In: Dawkins, R.L., Christiansen, F.T. and Zilko, P.J. (ed.), Immunogenetics in rheumatology, Excerpta Medica, Amsterdam, Oxford, Princeton, pp. 20-23.

Chaudhuri, A. and DasAdhikary, C.R. (1978) Possible role of blood group secretory substances in the aetiology of cholera. Trans. R. Soc. Trop. Med. Hyg., 72, 664-665.

Check, J.H., O'Neill, E.A., O'Neill, K.E. and Fuscaldo, K.E. (1972) Effect of anti-B antiserum on the phagocytosis of Escherichia coli. Infect. Immun., 6, 95-96.

Cohen, I.R. (1967) Natural and immune human antibodies reactive with antigens of virulent Neisseria gonorrhoeae: immunoglobulins G, M and A. J. Bact., 94, 141-148.

Cruz-Coke, R., Paredes, L. and Montenegro, A. (1965) Blood groups and urinary microorganisms. J. Med. Genet., 2, 185-188.

Decastello, A. and Sturli, A. (1902) Über die Isoagglutinine im Serum gesunder und kranker Menschen. München. Med. Wchnschr., 1090-1095.

Demarco de Hormaeche, R., Thornley, M.J. and Holmes, A. (1983) Surface antigens of gonococci: correlation with virulence and serum resistance. J. Gen. Microbiol., 129, 1559-1567.

Demarco de Hormaeche, R., Thornley, M.J. and Glauert, A.M. (1978) Demonstration by light and electron microscopy of capsules on gonococci recently grown in vivo. J. Gen. Microbiol., 106, 81-91.

Densen, P. and Mandell, G.L. (1978) Gonococcal interaction with polymorphonuclear neutrophils. Importance of the phagosome for bactericidal activity. J. Clin. Invest., 62, 1161-1171.

Diaz, J.L. and Heckels, J.E. (1982) Antigenic variation of outer membrane protein. II. In colonial variants of Neisseria gonorrhoeae P9. J. Gen. Microbiol., 128, 585-593.

Diena, B.B., Ashton, F.E., Ryan, A., Wallace, R. and Perry, M.B. (1978) The lipopolysaccharide (R-type) as a common antigen of Neisseria gonorrhoeae. I. Immunising properties. Can. J. Microbiol., 24, 117-123.

Dilworth, J.A., Hendley, J.O. and Mandell, G.L. (1975) Attachment and ingestion of gonococci by human neutrophils. Infect. Immun., 11, 512-516.

Douglas, J.T., Lee, M.D. and Nikaido, H. (1981) Protein 1 of Neisseria gonorrhoeae is a porin. FEMS Microbiol. Lett., 12, 305-309.

Drach, G.W. (1971) Antigens common to human and bacterial cells: Tolerance or resistance to urinary and surgical infections. Surg. Forum, 22, 67-68.

Drach, G.W., Reed, W.P. and Williams, R.C. (1971) Antigens common to human and bacterial cells: Urinary tract pathogens. J. Lab. Clin. Med., 78, 725-735.

Draper, D.L., Donegan, E.A., James, J.F., Swat, R.L. and Brooks, G.F. (1980) In vitro modeling of acute salpingitis caused by Neisseria gonorrhoeae. Am. J. Obstet. Gynecol., 138, 996-1002.

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.

Dungern, von, E. and Hirschfeld, L. (1910) Ueber Vererbung gruppenspezifischer strukturen des Blutes. Z. Immun. Forsch., 6, 284-292.

Dungern, von, E. and Hirschfeld, L. (1911) Über gruppenspezifische strukturen des Blutes III. Zschr. Immunit. forsch., 8, 526-562.

Eisenstein, B.I., Lee, T.J. and Sparling, P.F. (1977) Penicillin sensitivity and serum resistance are independant attributes of strains of Neisseria gonorrhoeae causing disseminated gonococcal infection. Infect. Immun., 15, 834-841.

Evans, B.A. (1977) Ultrastructural study of cervical gonorrhoea. J. Infect. Dis., 136, 248-255.

Extract from the report of the chief medical officer of the department of health and social security for the year 1981. (1983) Sexually transmitted diseases. Br. J. Vener. Dis., 59, 206-210.

Falk, V. (1965) Treatment of acute non-tuberculous salpingitis with antibiotics alone and in combination with glucocorticoids. Acta Obstet. Gynecol. Scand., 44 (suppl. 6), 13-14.

Faur, Y.C., Weisburd, M.H., Wilson, M.E. and May, P.S. (1973) A new medium for the isolation of pathogenic Neisseria (NYC medium). I. Formulation and comparisons with standard media. Health Lab. Sci., 10, 44-54.

Foster, M.T. and Labrum, A.H. (1976) Relation of infection with Neisseria gonorrhoeae to ABO blood groups. J. Inf. Dis., 133, 329-330.

Frasch, C.E. (1980) Role of lipopolysaccharide in wheat germ agglutinin-mediated agglutination of Neisseria gonorrhoeae. J. Clin. Microbiol., 12, 498-501.

Friedenreich, V. and Hartmann, G. (1938) Über die verteillung der Gruppenantigene im Organisms der sogenannten 'Ausscheider' und 'Nichtausscheider'. Z. Immun. Forsch., 92, 141-151.

Freimer, N.B., Ögmundsdóttir, H.M., Blackwell, C.C., Sutherland, I.W., Grahame, L. and Weir, D.M. (1978) The role of cell wall carbohydrates in binding of microorganisms to mouse peritoneal exudate macrophages. Acta Path. Microbiol. Scand. Sect. B., 86, 53-57.

Furakawa, T. (1930) A study of temperament and blood-groups. J. Soc. Psych., 1, 494-509.

Gallin, J. (1980) Disorders of phagocyte chemotaxis. Ann. intern. Med., 92, 520-538.

Gibbons, R.J. and Houte, van, J. (1971) · Selective bacterial adherence to oral epithelial surfaces, and its role as an ecological determinant. Infect. Immun., 3, 567-573.

Glass, E.J., Stewart, J. and Weir, D.M. (1981) Presence of 'lectin-like' receptors on phagocytes. Immunol., 44, 529-534.

Glynn, A.A. and Ward, M.E. (1970) Nature and heterogeneity of the antigens of Neisseria gonorrhoeae involved in the serum bactericidal reaction. Infect. Immun., 2, 162-168.

Glynn, A.A., Glynn, L.E. and Holborow, E.J. (1956) The secretor status of rheumatic-fever patients. Lancet, 2, 759-762.

Glynn, A.A., Glynn, L.E. and Holborow, E.J. (1959) Secretion of blood group substances in rheumatic fever - a genetic requirement for susceptibility? Brit. Med. J., 2, 266-270.

Govan, J.R.W. (1974) Studies on the pyocins of Pseudomonas aeruginosa: morphology and mode of action of contractile pyocins. J. Gen. Microbiol., 80, 1-15.

Gregg, C.R., Melly, M.A., Hellerqvist, C.G., Coniglo, J.G. and McGee, Z.A. (1981) Toxic activity of purified lipopolysaccharide of Neisseria gonorrhoeae for human fallopian tube mucosa. J. Inf. Dis., 143, 432-439.

Grubb, R. (1948) Correlation between Lewis blood group and secretor character in man. Nature, 162, 933.

Grubb, R. (1950) Quelque aspects de la complexité des groupes ABO. Rev. hemat., 5, 268-275.

Grundbacher, F.J. (1972) Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart diseases. Hum. Hered., 25, 399-404.

Grundbacher, F.J. and Shreffler, D.C. (1970) Effects of secretor, blood and serum groups on isoantibody and immunoglobulin levels. Am. J. Hum. Genet., 22, 194-202.

Gupta, M. and Chowdhuri, A.N.R. (1980) Relationship between ABO blood groups and malaria. Bull. W.H.O. 58, 913-915.

Haegart, D.G. (1979) Demonstration of IgM receptors on human peripheral monocytes using a direct plaque forming cell (PFC) assay. J. Immunol., Meth., 28, 97-104.

Hakomori, S. and Strycharz, G.D. (1968) Investigations on cellular blood-group substances. I. Isolation and chemical composition of blood-group ABH and Le^b isoantigens of sphingoglycolipid nature. Biochemistry, 7, 1279-1286.

Handsfield, H.H., Hodson, W.A. and Holmes, K.K. (1973) Neonatal gonococcal infection, 1. Orogastric contamination with Neisseria gonorrhoeae. J. Am. Med. Assoc., 225, 697-701.

Harkness, A.H. (1948) The pathology of gonorrhoea. Br. J. Vener. Dis., 24, 137-147.

Haverkorn, M.J. and Goslings, W.R.O. (1969) Streptococci, ABO blood groups and secretor status. Amer. J. Hum. Genet., 21, 360-375.

Heckels, J.E. (1978) The surface properties of Neisseria gonorrhoeae: topographical distribution of the outer membrane protein antigens. J. Gen. Microbiol., 108, 213-219.

Heckels, J.E. (1979) The outer membrane of Neisseria gonorrhoeae: evidence that protein I is a transmembrane protein. FEMS Microbiol. Lett., 6, 325-327.

Heckels, J.E. (1982) Role of surface proteins in the adhesion of Neisseria gonorrhoeae. In: Schlessinger, D. (ed.), Microbiology 1982, Washington, Am. Soc. Microbiol., pp. 301-304.

Heckels, J.E. and James, L.T. (1980) The structural organisation of the gonococcal cell envelope and its influence on pathogenesis. In: Danielsson, D. and Normark, S. (ed.), Genetics and immunobiology of pathogenic Neisseria, Umea, Sweden, University of Umea, pp. 25-28.

Heckels, J.E., Blackett, B., Everson, J.S. and Ward, M.E. (1976) The influence of surface charge on the attachment of Neisseria gonorrhoeae to human cells. J. Gen. Microbiol., 96, 359-364.

Hendley, J.O., Powell, K.R., Rodewald, R., Holzgrefe, H.H. and Lyles, R. (1977) Demonstration of a capsule on Neisseria gonorrhoeae. New Eng. J. Med., 296, 608-611.

Hermodsen, M.A., Chen, K.C.B. and Buchanan, T.M. (1978) Neisseria pili proteins: amino-terminal amino acid sequences and identification of an unusual amino acid. Biochemistry, 17, 442-445.

Hildebrandt, J.F., Mayer, L.W., Wang, S.P. and Buchanan, T.M. (1978) Neisseria gonorrhoeae acquire a new principal-outer membrane protein when transformed to resistance to serum bactericidal activity. Infect. Immun., 20, 267-273.

Hirschfeld, L. and Hirschfeld, H. (1919) Serological differences between the blood groups of different races. The result of researches on the Macedonian front. Lancet, ii, 675-679.

Holmes, K.K., Counts, G.W. and Beatty, H.N. (1971) Disseminated gonococcal infection. Annals Int. Med., 74, 979-993.

Holmes, K.K., Johnson, D.W. and Throstle, H.J. (1970) An estimate of the risk of men acquiring gonorrhoea by sexual contact with infected males. Am. J. Epidemiol., 91, 170-174.

Holmgren, J. and Smith, J.W. (1975) Immunological aspects of urinary tract infections. Progr. Allergy, 18, 289-352.

Howie, S. and McBride, W.H. (1982) Cellular interactions in thymus-dependant antibody responses. Immunol. Today, 3, 273-278.

Ingwer, I., Petersen, B.H. and Brooks, G. (1978) Serum bactericidal action and activation of the classic and alternate complement pathways by Neisseria gonorrhoeae. J. Lab. Clin. Med., 92, 211-220.

Iseki, S., Masaki, S. and Shubasaki, K. (1957)$_c$ Studies on the Lewis blood group system. I. Le^c blood group factor. Proc. Imp. Acad. Japan., 33, 492-497.

Issitt, P.D. and Issitt, C.H. (1979) Applied blood group serology. Spectra Biologicals, Calif. pp. 91-101; 30.

Izhar, M., Nuchamowitz, Y. and Mirelman, D. (1982) Adherence of Shigella flexneri to guinea pig intestinal cells is mediated by a mucosal adhesin. Infect. Immun., 35, 1110-1118.

James, A.N. and Williams, R.P. (1978) Chemotactic effect of Neisseria gonorrhoeae on polymorphonuclear leukocytes. In: Brooks, G.F., Gotschlich, E.C., Holmes, K.K., Sawyer, W.D. and Young, F.E. (ed.), Immunobiology of Neisseria gonorrhoeae, Am. Soc. Microbiol., Washington D.C. pp. 338-343.

James-Holmquest, A.N., Swanson, J., Buchanan, T.M., Wende, R.D. and Williams, R.P. (1974) Differential attachment by pilated and nonpilated Neisseria gonorrhoeae to human sperm. Infect. Immun., 9, 897-902.

James, J.F. and Swanson, J. (1977) The capsule of the gonococcus. J. Exp. Med., 145, 1082-1086.

James, J.F. and Swanson, J. (1978) Studies on gonococcus infection. XIII. Occurence of color/opacity variants in clinical cultures. Infect. Immun., 19, 332-340.

James, J.F., Lammel, C.J., Draper, D.L. and Brooks, G.F. (1980) Attachment of N. gonorrhoeae colony phenotype variants to eukaryotic cells and tissues. In: Danielsson, D. and Normark, S. (ed.), Genetics and Immunobiology of pathogenic Neisseria, Umea, Sweden, University of Umea, pp. 213-216.

Jasin, H.E. (1972) Human heat labile opsonins: Evidence for their mediation via the alternate pathway of complement activation. J. Immunol., 109, 26-31.

Jephcott, A.E. and Reyn, A. (1971) Neisseria gonorrhoeae. Colony variation I. Acta path. microbiol. scand. Sec. B., 79, 609-614.

Johnson, A.P., Osborn, M.F., Hanna, N.F., Dawson, S.G., McManus, T.J. and Taylor-Robinson, D. (1983) A study of the relationship between ABO blood groups, secretor status and infection with Neisseria gonorrhoeae. J. Infect., 6, 171-174.

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.

Johnston, K.H., Holmes, K.K. and Gotschlich, E.C. (1976) I. The serological classification of Neisseria gonorrhoeae. J. Exp. Med., 143, 741-758.

Kageyama, M. (1975) Bacteriocins and bacteriophages in Pseudomonas aeruginosa. In: Mitsuhasi, S. and Hashimoto, H. (ed.), Microbial drug resistance, Baltimore, Univ. Park Press, pp. 291-305.

Kalinowski, M. (1972) Urinary elimination of group B substances ABO (H) in different renal diseases. Folia Haematol. (Leipz), 100, 144-163.

Kallenius, G., Mollby, R., Svenson, S.B., Helin, I., Hulteberg, H., Cedergren, B. and Winberg, J. (1981) Occurrence of P-fimbriated Escherichia coli in urinary tract infections. Lancet, 2, 1369-1372.

Kasper, D.L., Rice, P.A. and McCormack, W.M. (1977) Bactericidal antibody in genital infection due to Neisseria gonorrhoeae. J. Infect. Dis., 135, 243-251.

Kearns, D.H., O'Reilly, R.J., Lee, L. and Welch, B.G. (1973a) Secretory IgA antibodies in the urethral exudate of men with uncomplicated urethritis due to Neisseria gonorrhoeae. J. Inf. Dis., 127, 99-101.

Kearns, D.H., Seibert, G.B., O'Reilly, R., Lee, L. and Logan, L. (1973b) Paradox of the immune response to uncomplicated gonococcal urethritis. New Eng. J. Med., 289, 1170-1174.

Kellogg, D.S., Jr., Peacock, W.L., Jr., Deacon, W.E., Brown, L. and Pirkel, C.I. (1963) Neisseria gonorrhoeae. I. Virulence genetically linked to clonal variation. J. Bacteriol., 85, 1274-1279.

King, G.J. and Swanson, J. (1978) Studies on gonococcus infection. XV. Identification of surface proteins of Neisseria gonorrhoeae correlated with leukocyte association. Infect. Immun., 21, 575-584.

Knapp, J.S. and Holmes, K.K. (1975) Disseminated gonococcal infections caused by Neisseria gonorrhoeae with unique nutritional requirements. J. Infect. Dis., 132, 204-208.

Kopeć, A.C. (1970) The distribution of the blood groups in the United Kingdom. London, Oxford University Press, p. 26.

Koval, S.F. and Meadow, P.M. (1977) The isolation and characterisation of the lipopolysaccharide-defective mutants of Pseudomonas aeruginosa PAC 1. J. Gen. Microbiol., 98, 387-398.

Kowolik, M.J. and Moody, G.H. (1979) A simplified technique for the NBT test. IRCS Med. Sci., 7, 100.

Kreiger, A.G., Schiller, N.L. and Roberts, R.B. (1980) Gonococci-human polymorphonuclear leucocyte interactions: metabolic studies associated with attachment and ingestion. Infect. Immun., 28, 991-1000.

Kuusela, P. (1978) Fibronectin binds to Staphylococcus aureus. Nature, 276, 718-720.

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. J. Bacteriol., 141, 393-396.

Lambden, P.R., Robertson, J.N. and Watt, P.J. (1981) The preparation and properties of α and β pili from variants of Neisseria gonorrhoeae P9. J. Gen. Microbiol., 124, 109-117.

Landsteiner, K. (1900) Zur Kenntnis der antifermentativen, lytischen und agglutinierenden Wirkungen des Blutserums und der Lymphe. Zbl. Bakt., 27, 357-362.

Landsteiner, K. (1901) Über Agglutinationser-scheinungen normalen menschlichen Blutes. Wein. klin. Wschr., 14, 1132-1134.

Lee, T.J., Schmoyer, A., Synderman, R., Yount, W.J. and Sparling, P.F. (1978) Familial deficiency of the sixth and seventh components of complement associated with bacteremic Neisseria infections. In: Brooks, G.F., Gotschlich, E.C., Holmes, K.K., Sawyer, W.D. and Young, F.E. (ed.), Immunobiology of Neisseria gonorrhoeae, Am. Soc. Microbiol., Washington D.C., pp. 204-206.

Leffler, H. and Svanborg-Edén, C. (1980) Chemical identification of a glycosphingolipid receptor for Escherichia coli attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. FEMS Microbiol. Lett., 8, 127-134.

Levine, P., Robinson, E., Celano, M., Briggs, O. and Falkinburg, L. (1955) Gene interaction resulting in suppression of blood group substance B. Blood, 102, 1100-1108.

Levine, P., Uhlir, M. and White, J. (1961) A_h, an incomplete suppression of A resembling O_h. Vox Sang, 6, 561-567.

Lincoln, P.J. and Dodd, B.E. (1972) Variation in secretor and Lewis type frequencies within the British Isles. J. Med. Gen., 9, 43-45.

Mackenzie, J.S. and Fimmel, P.J. (1978) The effect of ABO blood groups on the incidence of epidemic influenza and on the response to live, attenuated and detergent split influenza vaccines. J. Hyg., (Camb.), 80, 21-30.

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

Mardh, P.A., Baldetorp, B., Hakansson, C.H., Fritz, H. and Weström, L. (1979) Studies of ciliated epithelia of the human genital tract. 3: Mucociliary wave activity in organ cultures of human fallopian tubes challenged with Neisseria gonorrhoeae and gonococcal endotoxin. Br. J. Vener. Dis., 55, 256-264.

McDonald, I.J. and Adams, G.A. (1971) Influence of cultural conditions on the lipopolysaccharide composition of Neisseria sicca. J. Gen. Microbiol., 65, 201-207.

McDonald, J.C. and Zuckerman, A.J. (1962) ABO blood groups and acute respiratory virus disease. Brit. Med. J. (11), 89-90.

McMillan, A., McNeillage, G. and Young, H. (1979a) Antibodies to Neisseria gonorrhoeae: a study of the urethral exudates of 232 men. J. Inf. Dis., 140, 89-95.

McMillan, A., McNeillage, G., Young, H. and Bain, S.S.R. (1979b) Secretory antibody response of the cervix to infection with Neisseria gonorrhoeae. Br. J. Vener. Dis., 55, 265-270.

McMillan, A., McNeillage, G., Young, H. and Bain, S.S.R. (1979c) Serum immunoglobulin response in uncomplicated gonorrhoea. Br. J. Vener. Dis., 55, 5-9.

Melly, M.A., Gregg, C.R. and McGee, Z.A. (1981) Studies of toxicity of Neiseria gonorrhoeae for human fallopian tube mucosa. J. Infect. Dis., 143, 423-431.

Melly, M.A., McGee, Z.A., Horn, R.G., Morris, F. and Glick, A.D. (1979) An electron microscopic India ink technique for demonstrating capsules on microorganisms: studies with Streptococcus pneumoniae, Staphylococcus aureus and Neisseria gonorrhoeae. J. Infect. Dis., 140, 605-609.

Metchnikoff, E. (1905) Immunity in infective diseases. Cambridge, University Press, p.572.

Michael, J.G. and Rosen, F.S. (1963) Association of "natural" antibodies to Gram-negative bacteria with the -macroglobulins. J. Exp. Med., 118, 619-626.

Miler, J.J., Novotny, P., Walker, P.D., Harris, J.R.W. and Maclennan, I.P.B. (1977) Neisseria gonorrhoeae and ABO isohemagglutinins. Infect. Immun., 15, 713-719.

Mollison, P.L. (1979) Blood transfusion in clinical medicine. Blackwell, Oxford. pp. 414-482.

Mourant, A.E., Kopec, A.C. and Domaniewska-Sobczak, K. (1976) The distribution of the human blood groups and other polymorphisms. Oxford Univ. Press. Oxford, New York, Toronto, pp. 126, 1016 pages.

Mourant, A.E., Kopec, A.C., Domaniewska-Sobczak, K. (1978) Blood groups and diseases. A study of associations with blood groups and other polymorphisms. Oxford Univ. Press, Oxford, New York, Toronto, pp. 1, 328 pages.

Muschell, L.H. (1966) Blood groups, disease, and selection. Bact. Rev., 30, 427-441.

Muschell, L. and Osawa, E. (1959) Human blood group substance B and Escherichia coli 086. Proc. Soc. Exp. Biol. Med., 101, 614-617.

Newhall, W.J., Sawyer, W.D. and Haak, R.A. (1980) Cross-linking analysis of the outer membrane proteins of Neisseria gonorrhoeae. Infect. Immun., 28, 785-791.

Noble, R.C., Cooper, R.M. and Miller, B.R. (1979) Pharyngeal colonisation by Neisseria gonorrhoeae and Neisseria meningitidis in black and white patients attending a venereal disease clinic. Br. J. Vener. Dis., 55, 14-19.

Novotny, P., Short, J.A. and Walker, P.D. (1975) An electron-microscope study of naturally occurring and cultured cells of Neisseria gonorrhoeae. J. Med. Microbiol., 8, 413-427.

Novotny, P., Short, J.A., Hughes, M., Miler, J.J., Syrett, C., Turner, W.H., Harris, J.R.W. and MacLennan, J.P.B. (1977) Studies on the mechanism of pathogenicity of Neisseria gonorrhoeae. J. Med. Microbiol., 10, 347-365.

Ofek, I., Beachey, E.H. and Bisno, A.L. (1974) Resistance of Neisseria gonorrhoeae to phagocytosis: relationship to colonial morphology and surface pili. J. Infect. Dis., 129, 310-316.

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.

Ögmundsdóttir, H.M., Weir, D.M. and Marmion, B.P. (1978) Binding of micro-organisms to the macrophage plasma membrane; effects of enzymes and periodate. Brit. J. exp. Path., 59, 1-7.

O'Reilly, R.J., Lee, L. and Welch, B.G. (1976) Secretory IgA antibody responses to Neisseria gonorrhoeae in the genital secretions of infected females. J. Inf. Dis., 133, 113-125.

Payne, S.M. and Finkelstein, R.A. (1975) Pathogenesis and immunology of experimental gonococcal infection: Role of iron in virulence. Infect. Immun., 12, 1313-1318.

Perry, M.B., Diena, B.B. and Ashton, F.E. (1978) Lipopolysaccharides of Neisseria gonorrhoeae. In: Roberts, R.B. (ed.), The gonococcus, John Wiley and sons, New York, Chichester, Brisbane, Toronto, pp. 285-301.

Perry, M.B., Daoust, V., Diena, B.B., Ashton, F.E. and Wallace, R. (1975) The lipopolysaccharides of Neisseria gonorrhoeae colony types 1 and 4. Can. J. Biochem., 53, 623-629.

Petersen, B.H., Graham, J.A. and Brooks, G.F. (1976) Human deficiency of the eighth component of complement. The requirement of C8 for serum Neisseria gonorrhoeae bactericidal activity. J. Clin. Invest., 57, 283-290.

Pettenkofer, H.J. and Bickerich, R. (1960) Über Antigengemeinschaften zwischen den menschlichen Blutgruppen ABO und den Erregren gemeingefährlicher Krankheiten. Zb. Bakt., I, 179-433.

Pettenkofer, H.J., Stöss, B., Helmbold, W. and Vogel, F. (1962) Alleged causes of the present-day world distribution of the human ABO blood groups. Nature, 193, 445-446.

Plaut, A.G., Gilbert, J.V., Artenstein, M.S. and Capra, J.D. (1975) Neisseria gonorrhoeae and Neisseria meningitidis: extracellular enzyme cleaves human immunoglobulin A. Science, 190, 1103-1105.

Potapov, M.I. (1970) Detection of the antigen of the Lewis system characteristic of the erythrocytes of the secretory characteristic group Le (a- b-). Probl. Haematol., Moscow., 15, 45-49.

Potter, C.W. (1969) Haemagglutination inhibition antibody to various influenza viruses and adenovirus in individuals of blood group A and O. J. Hyg., (Camb.), 67, 67-74.

Pradhan, A.C., Chawla, T.N., Samuel, K.C. and Pradhan, S. (1971) The relationship between periodontal disease and blood groups and secretor status. J. Periodont. res., 6, 294-300.

Prokop, O. and Uhlenbruck, G. (1969) Human blood and serum groups. English edition. Maclaren, London, pp. 690-722.

Punsalang, A.P., Jr. and Sawyer, W.D. (1973) Role of pili in the virulence of Neisseria gonorrhoeae. Infect. Immun., 8, 355-263.

Race, R.R. and Sanger, R. (1975) Blood groups in man. Blackwell Scientific Publications, Oxford, pp. 48-50.

Rice, P.A. and Kasper, D.L. (1977) Characterisation of gonococcal antigens responsible for induction of bactericidal antibody in disseminated infection. J. Clin. Invest., 60, 1149-1158.

Richardson, W.P. and Sadoff, J.C. (1977) Production of a capsule by Neisseria gonorrhoeae. Infect. Immun., 15, 663-664.

Robinson, M.G., Tolchin, D. and Halpern, C. (1971) Enteric bacterial agents and the ABO blood groups. Am. J. Hum. Genet., 23, 135-145.

Robertson, J.N., Vincent, P. and Ward, M.E. (1977) The preparation and properties of gonococcal pili. J. Gen. Microbiol., 102, 167-177.

Rosenthal, R.S., Fulbright, R.S., Eads, M.E. and Sawyer, W.D. (1977) Ethylenediaminetetracetic acid-sensitive antiphagocytic activity of Neisseria gonorrhoeae. Infect. Immun., 15, 817-827.

Sadoff, J.C., Zollinger, W.D. and Sidberry, H.D. (1978) Cell surface structures of Neisseria gonorrhoeae. In: Brooks, G.F., Gotschlich, E.C., Holmes, K.K., Sawyer, W.D. and Young, F.E. (ed.), Immunobiology of Neisseria gonorrhoeae. Am. Soc. Microbiol., Washington, D.C., pp. 93-100.

Salit, I.E., Blake, M. and Gotschlich, E.C. (1980). Intra strain heterogeneity of gonococcal pili is related to opacity colony variance. J. Exper. Med., 151, 716-725.

Sandström, E. and Danielsson, D. (1980) Serology of Neisseria gonorrhoeae: Classification with co-agglutination. Acta path. microbiol. scand. Sect. B, 88, 27-38.

Sandström, E.G., Knapp, J.S. and Buchanan, T.B. (1982b) Serology of Neisseria gonorrhoeae: W-antigen serogrouping by co-agglutination and protein 1 serotyping by enzyme linked immunosorbent assay both detect protein 1 antigens. Infect. Immun., 35, 229-239.

Sandström, E.G., Chen, K.S. and Buchanan, T.M. (1982b) Serology of Neisseria gonorrhoeae: coagglutination serogroups WI and WII/WIII correspond to different outer membrane proteins. Infect. Immun., 38, 462-470.

Santoro, J. and Kaye, D. (1978) Recurrent urinary tract infections. Pathogenesis and management. Med. Clin. N. Am., 62, 1005-1020.

Schiff, F. (1924) Über gruppenspezifische Serumpräzipitine. Klin. Wschr., 3, 679-680.

Schiff, F. and Sasaki, H. (1932) Der Ausscheidungstypus, ein auf serologischen Wege nachweisbares mendelnes Merkmal. Klin. Wschr., 11, 1426-1429.

Schiller, N.L., Friedmann, G.L. and Roberts, R.B. (1979) The role of natural IgG and complement in the phagocytosis of type 4 gonococci by human polymorphonuclear leukocytes. J. Infect. Dis., 140, 698-707.

Schneider, H.J., Griffiss, J.M., Williams, G.D. and Pier, G.B. (1982) Immunological basis of serum resistance of Neisseria gonorrhoeae. J. Gen. Microbiol., 128, 13-22.

Schoolnik, G.K., Buchanan, T.M. and Holmes, K.K. (1976) Gonococci causing disseminated gonococcal infection are resistant to the bactericidal action of normal human sera. J. Clin. Invest., 58, 1163-1173.

Schoolnik, G.K., Ochs, H.D. and Buchanan, T.M. (1979) Immunoglobulin class responsible for gonococcal bactericidal activity of normal human sera. J. Immunol., 122, 1771-1779.

Shands, J.W. (1965) Localisation of somatic antigen on Gram-negative bacteria by electron-microscopy. J. Bacteriol., 90, 266-270.

Sneath, J.S. and Sneath, P.H.A. (1955) Transformation of the Lewis groups of human red cells. Nature, 176, 172.

Socha, W., Bilinska, M., Kaczera, Z., Pajdak, E. and Stankiewicz, D. (1969) Escherichia coli and ABO blood groups. Folia. Biol. (Krakow), 17, 259-269.

Solish, G.I., Gershowitz, H. and Behrman, S.J. (1961) Occurrence and titre of isohaemagglutinins in secretions of the human uterine cervix. Proc. Soc. Exp. Biol. Med., 108, 645-649.

Sparling, P.F. and Yobs, A.R. (1967) Colonial morphology of Neisseria gonorrhoeae isolated from males and females. J. Bact., 93, 513.

Sparling, P.F., Biswas, G., Graves, J. and Blackman, E. (1980) Mechanisms of gene exchange in the gonococcus. In: Danielsson, D. and Normark, S. (ed.), Genetics and immunobiology of pathogenic Neisseria, Umea, Sweden, University of Umea, pp. 123-126.

Springer, G.F. (1966) Relation of microbes to blood-group active substances. Angew. Chem. internat. Edit., 5, 909-920.

Springer, G.F. (1970) Importance of blood group substances in interactions between man and microbes. Ann. N.Y. Acad. Sci., 169, 134-152.

Springer, G.F. (1971) Blood group and Forssman antigenic determinants shared between microbes and mammalian cells. Prog. Allergy, 15, 9-77.

Springer, G.F. and Weiner, A.S. (1962) Alleged causes of the present-day world distribution of the human ABO blood groups. Nature, 193, 444-445.

Springer, G.F., Williamson, P. and Brandes, W.C. (1961) Blood group activity of Gram-negative bacteria. J. Exp. Med., 113, 1077-1093.

Stahl, P.D., Rodman, J.S., Miller, M.J. and Schlesinger, P.H. (1978) Evidence for receptor mediated binding of glycoproteins, glycoconjugate, and lysosomal glycosidases by alveolar macrophages. Proc. Nat. Acad. Sci. (Washington), 75, 1399-1403.

Stead, A., Main, J.S., Ward, M.E. and Watt, P.J. (1975) Studies on lipopolysaccharides isolated from strains of Neisseria gonorrhoeae. J. Gen. Microbiol., 88, 121-131.

Stewart, J., Glass, E.J. and Weir, D.M. (1982) Macrophage binding of Staphylococcus aureus is blocked by anti I-region alloantibody. Nature, 298, 852-854.

Smith, H. (1976) Survival of vegetative bacteria in animals. Symp. Soc. Gen. Microbiol., 26, 299-326.

Smith, H. (1977) Microbial surfaces in relation to pathogenicity. Bacteriol. Rev., 41, 465-500.

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

Swanson, J. (1978a) Studies on gonococcus infection. XII. Colony color and opacity variants of gonococci. Infect. Immun., 19, 320-331.

Swanson, J. (1978b) Studies on gonococcus infection. XIV. Cell wall protein differences among color/opacity colony variants of Neisseria gonorrhoeae. Infect. Immun., 21, 292-302.

Swanson, J. (1980) Adhesion and entry of bacteria into cells. A model of the pathogenesis of gonorrhoea. Molecular Basis of Microb. Path., 16, 17-40.

Swanson, J. (1981) Surface-exposed protein antigens of the gonococcal outer-membrane. Infect. Immun., 34, 804-816.

Swanson, J. and Heckels, J.E. (1980) Proposal: Nomenclature of gonococci outer membrane proteins. In: Danielsson, D. and Normark, S. (ed.), Genetics and immunobiology of pathogenic Neisseria, Umea, Sweden, University of Umea, pp. XXI-XXVI.

Swanson, J., King, G. and Zeligs, B. (1975) Studies on the gonococcus infection, VIII. ^{125}Iodine labelling of gonococci and studies on their in vitro interactions with eukaryotic cells. Infect. Immun., 11, 453-459.

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., Zeligs, B., Siam, M. and Parrot, C. (1974) Studies on gonococcus infection. V. Observations on in vitro interactions of gonococci and human neutrophils. Infect. Immun., 10, 633-644.

Taylor-Robinson, D., Whytock, S., Green, C.J. and Carney, F.E. Jr. (1974) Effect of Neisseria gonorrhoeae on human and rabbit oviducts. Br. J. Vener. Dis., 50, 279-288.

Thomas, D.W., Hill, J.C. and Tyeryar, F.J. Jr. (1973) Interaction of gonococci with phagocytic leukocytes from men and mice. Infect. Immun., 8, 98-104.

Thomsen, O., Friedenreich, V. and Worsaae, E. (1930) Über die Möglichkeit der Existenz zweier neuer Blutgruppen; auch ein Beitrag zur Beleuchtung sogennanter Untergruppen. Acta path. microbiol. scand., 7, 157-190.

Thongthai, C. and Sawyer, W.D. (1973) Studies on the virulence of Neisseria gonorrhoeae. 1. Relation of colonial morphology and resistance to phagocytosis by polymorphonuclear leukocytes. Infect. Immun., 7, 373-379.

Today's V.D. control problem 1975. Joint statement committee, chaired by Fleming, W.L. (1975) Amer. Social Health Assoc., New York, 63 pages.

Tramont, E.C. and Wilson, C. (1977) Variations in buccal cells adhesion of N. gonorrhoeae. Infect. Immun., 16, 709-711.

Tramont, E.C., Sadoff, J.C. and Artenstein, M.S. (1974) Cross reactivity of Neisseria gonorrhoeae and Neisseria meningitidis and the nature of antigens involved in the bactericidal reaction. J. Inf. Dis., 130, 240-247.

Trust, T.J., Lambden, P.R. and Watt, P.J. (1980) The cohesive properties of variants of Neisseria gonorrhoeae strain P9: specific pilus-mediated and non-specific interactions. J. Gen. Microbiol., 119, 179-187.

Veale, D.R., Penn, C.W. and Smith, H. (1977) The resistance of gonococci to killing by human phagocytes. In: Skinner, F.A., Walker, P.D. and Smith, H. (ed.), Gonorrhoea: Epidemiology and pathogenesis, Acad. Press, London, New York, San Francisco, pp. 97-115.

Veale, D.R., Smith, H. and Witt, K. (1975) Penetration of penicillin into human phagocytes containing gonococci. Lancet, 1, 306-308.

Viskum, K. (1975) The ABO and Rhesus blood groups in patients with pulmonary tuberculosis. Tubercle, 56, 329-334.

Vogel, F. and Motulsky, A.G. (1982) Human Genetics. Problems and Approaches. Springer-Verlag, Berlin, Heidleberg, New York, pp. 13-14.

Vogel, F., Pettenkofer, H.J. and Helmbold, W. (1960) Über die Populationsgenetik der ABO-Blutgruppen. 2. Mitteilung Genhäufigkeit und epidemische Erkrankungen. Acta genet. Stat. med., 10, 267-294.

Wallace, R., Ashton, F.E., Ryan, A., Diena, B.B., Malysheff, C. and Perry, M.B. (1978) The lipopolysaccharide (R-type) as a common antigen of Neisseria gonorrhoeae. II. Use of hen antiserum to gonococcal lipopolysaccharide in a rapid slide test for the identification of N. gonorrhoeae from primary isolates and secondary cultures. Can. J. Microbiol., 24, 124-128.

Wang, S.P., Holmes, K.K., Knapp, J.S., Ott, S. and Kyser, D.D. (1977) Immunological classification of Neisseria gonorrhoeae with micro-immunofluorescence. J. Immunol., 119, 795-803.

Ward, M.E. and Watt, P.J. (1972) Adherence of Neisseria gonorrhoeae to urethral mucosal cells: an electron-microscopic study of human gonorrhoea. J. Inf. Dis., 126, 601-605.

Ward, M.E., Watt, P.J. and Robertson, J.N. (1974) The human fallopian tube: a laboratory model for gonococcal infection. J. Inf. Dis., 129, 650-659.

Ward, M.E., Lambden, P.R., Heckels, J.E. and Watt, P.J. (1978) The surface properties of Neisseria gonorrhoeae: Determinants of susceptibility to antibody complement killing. J. Gen. Microbiol., 108, 205-212.

Watkins, W.M. (1972) The biochemical basis of human blood group ABO and Lewis polymorphism. Rev. Annual report, Lister Inst. of Prev. Medicine, 12-29.

Watt, P.J. and Heckels, J.E. (1982) Prospects and strategy for immunisation against gonococcal infection. WHO/VDT/RES/GON/82. 429, 20 pages.

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. (ed.), Immunobiology of Neisseria gonorrhoeae, Am. Soc. Microbiol., Washington D.C., pp. 253-257.

Weinberg, E.D. (1974) Iron and susceptibility to infectious disease. Science, 184, 952-956.

Weiner, A.S. (1970) Blood groups and disease. Amer. J. Hum. Genet., 22, 476-483.

Weir, D.M. (1980) Surface carbohydrates and lectins in cellular recognition. Immunol. Today, 1, 45-51.

Weir, D.M., Stewart, J., Glass, E.J. and Blackwell, C.C. (1983) Phagocyte lectin receptors. In: Proceedings of ETCS and EURES congress, Budapest, Academiai Kiado. Publishing house of the Hungarian Acad. Sci., Budapest. In press.

Weistreich, G.A. and Baker, R.F. (1971) The presence of fimbria (pili) in three species of Neisseria. J. Gen. Microbiol., 65, 167-173.

Weström, L. (1980) Incidence, prevalence and trends of acute pelvic inflammatory disease and its consequences in industrialised countries. Am. J. Obstet. Gynecol., 138, 880-892.

Wilkinson, P.C. (1976) Recognition and response in mononuclear and granular phagocytes. Clin. exp. Immunol., 25, 355-366.

Winberg, J., Anderson, H.J., Bergström, T., Jacobsson, B., Larson, H. and Lincoln, K. (1974) Epidemiology of symptomatic urinary tract infection in childhood. Acta Paediatr. Scand. (Suppl.), 252, 1-20.

Winstanley, F.P., Blackwell, C.C., Weir, D.M. and Kinane, D.F. (1983) ABO blood groups and susceptibility to gonococcal infection. II. The relationship of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. J. Clin. Lab. Immunol., 11, 27-32.

Winstanley, F.P., Blackwell, C.C., Weir, D.M. and Kinane, D.F. (1983) Host parasite interactions influencing gonococcal infection - A paradox resolved? (submitted for publication).

Wiseman, G.M. and Caird, J.D. (1977) Composition of the lipopolysaccharide of Neisseria gonorrhoeae. Infect. Immun., 16, 550-556.

Woolf, B. (1954/55) On estimating the relation between blood group and disease. Ann. Human Genet., 19, 251-253.

World Health Organisation (1973) Neisseria gonorrhoeae and gonococcal infections. Techn. Rep. Series 616, Geneva, 144 pages.

Yamakami, K. (1926) The individuality of semen, with reference to its property of inhibiting specifically isohaemagglutination. J. Immunol., 12, 185-189.

APPENDIX

PUBLICATIONS

The following work connected with this thesis has been or is about to be published.

Kinane, D.F., Blackwell, C.C., Brettle, R.P., Weir, D.M., Winstanley, F.P. and Elton, R.A. (1982) ABO blood groups, secretory status and susceptibility to recurrent urinary tract infection in women. Br. Med. J., 285, 7-9.

Kinane, D.F., Blackwell, C.C., Weir, D.M. and Winstanley, F.P. (1982) Phagocyte recognition of Neisseria gonorrhoeae. J. Clin. Lab. Immunol., 9, 169-172.

Kinane, D.F., Blackwell, C.C., Winstanley, F.P. and Weir, D.M. (1983) Blood group and secretor status and susceptibility to infection by Neisseria gonorrhoeae. Br. J. Vener. Dis., 59, 44-46.

Blackwell, C.C., Kowolik, M., Winstanley, F.P., Kinane, D.F., Weir, D.M., Law, J.A. and Ho Wang Chok, Y.L. (1983) ABO blood group and susceptibility to gonococcal infection. I. Factors affecting phagocytosis of Neisseria gonorrhoeae. J. Clin. Lab. Immunol., 10, 173-178.

Winstanley, F.P., Blackwell, C.C., Weir, D.M. and Kinane, D.F. (1983) Blood groups and susceptibility to gonococcal infection. II. The relations of lipopolysaccharide type to gonococcal sensitivity to the bactericidal activity of normal human serum. J. Clin. Lab. Immunol., 11, 27-32.

Kinane, D.F., Blackwell, C.C., Weir, D.M., Winstanley, F.P. and Elton, R.A. (1983) ABO blood group and susceptibility to gonococcal infection. III. Role of isohaemagglutinins in increased association of Neisseria gonorrhoeae to monocytes from blood group B individuals. J. Clin. Lab. Immunol. In press.

Winstanley, F.P., Blackwell, C.C., Weir, D.M. and Kinane, D.F. (1983) Host parasite interactions influencing gonococcal infection - A paradox resolved? (submitted).

Reprinted from the BRITISH MEDICAL JOURNAL
3 July 1982, 285, 7 - 9

ABO blood group, secretor state, and susceptibility to recurrent urinary tract infection in women

D F KINANE, C CAROLINE BLACKWELL, R P BRETTLE, D M WEIR, F P WINSTANLEY, R A ELTON

Abstract

ABO blood group and secretor state was determined in 319 women with recurrent urinary tract infection and compared with those of a control group of 334 women of similar age ranges. Women of blood groups B and AB who are non-secretors of blood group substances showed a significant relative risk of recurrent urinary tract infection of 3·12 (95% confidence limits, 1·49 and 6·52) in comparison with other types. This appears to be a genuine example of synergy in which absence of anti-B isohaemagglutinin and secretor substances combines to give an increased risk of recurrent urinary tract infection.

Determination of blood group and secretor state may provide additional information in identifying those at risk.

Department of Bacteriology, University of Edinburgh Medical School, Edinburgh EH8 9AG

D F KINANE, BDS, research fellow
C CAROLINE BLACKWELL, PHD, lecturer
D M WEIR, MD, FRCP, reader
F P WINSTANLEY, BSC, research associate

Department of Infectious Diseases, City Hospital, Edinburgh EH10 5SB

R P BRETTLE, MRCP, senior registrar

Medical Computing and Statistics Unit, University of Edinburgh Medical School, Edinburgh EH8 9AG

R A ELTON, PHD, lecturer

Introduction

Correlation between ABO blood group and susceptibility to certain infectious diseases is well documented.[1-6] In studies of a Chilean population, patients of blood group B had 50% greater probability than those of other blood groups of contracting *Escherichia coli* urinary tract infections.[4]

The antigens of ABO blood groups are present in two forms: (*a*) alcohol-soluble in tissues of all subjects, and (*b*) water-soluble in most body fluids and organs of secretors. The non-secretor state has been linked with the susceptibility to certain infectious agents.[7-9] Haverkorn and Goslings[7] found that patients in blood groups other than O and non-secretor patients were most susceptible to rheumatic fever and rheumatic heart disease, and carriage of group A streptococci was observed more often in non-secretors.

We analysed the distribution of ABO blood groups and secretor state among women with recurrent urinary tract infections in Edinburgh.

Subjects

Regular attenders at the pyelonephritis clinic of the City Hospital, Edinburgh, were asked to provide a specimen of blood and saliva for determination of blood group and secretor state. All patients in this survey were girls and women aged 10-80 (mean 49) years. None had predisposing factors such as stones.

The patients were divided prospectively into two categories:

Patients with pyelonephritis—Those with unequivocal radiological evidence of chronic pyelonephritis—that is, focal loss of parenchyma associated with distortion of calyces—or those with a history and clinical and laboratory findings consistent with acute pyelonephritis.

Patients with uncomplicated urinary tract infections—Those patients with normal appearance on intravenous pyelograms, but with appreciable bacteriuria (10^5 organisms/ml) and a history of recurrent urinary tract infections.

Controls—Control data were produced by testing 334 samples of plasma from female donors (age range 18-60) from the survey area for the presence of secretor substances. Further control data for distribution of blood groups were available for 6662 donors from the same area as that from which our patients were drawn.[10]

Methods

Blood from each patient was collected at the clinic and stored in heparinised tubes. The saliva from each patient was boiled for 20 min, centrifuged at $5000 \times g$ for 10 min, and the supernate stored briefly at 4°C until tested for the presence of blood group antigen. Blood group was determined by agglutination tests in plastic wells. Secretor state was determined as described by Mollinson[11] with saliva or

plasma samples. Saliva or plasma from secretors and non-secretors representative of the four blood groups were used as controls for each experiment. Agglutinins used were anti-A and anti-B sera and *Ulex europaeus* lectin. The red blood cells used were A_2, B, and O.

Linear logistic multiple regression[12] was used to compare the ABO blood group and secretor state categories of the study and control groups. This also gave estimates and confidence limits for relative risk of urinary tract infection between categories. χ^2 tests were used for analysis of two-way frequency tables.

Results

No significant differences in the proportion of secretors or the distribution of ABO blood groups were found between the two diagnostic categories of patients with urinary tract infections; all 319 patients were considered as one group for comparisons with controls. The table shows the distribution of different blood groups and secretor state for the study group and for the smaller series of controls in whom secretor status had been determined. For the study group, the table shows also the ratios of observed prevalences to those expected from the pattern found in controls.

Distribution of blood groups in study group and in two groups of controls, those in whom secretor state was determined (group 1) and those in whom it was unknown (group 2) (numbers in parentheses are ratios of distribution found in study group to that expected from control data)

	Blood group			
	O	A	B	AB
Control group 1 (n = 334):				
Secretors	124	74	35	12
Non-secretors	49	30	7	3
Study group (n = 319):				
Secretors	110 (0·93)	63 (0·88)	32 (0·96)	7 (0·64)
Non-secretors	52 (1·11)	28 (0·98)	18 (2·69)	10 (3·49)
Control group 2 (n = 6662)	3323	2410	715	214

To test the dependence of risk of urinary tract infection on ABO blood group or secretor state, a linear logistic multiple regression was carried out on the two samples. This examines the influence of the three main effects (anti-A, anti-B, and secretor state) and their interactions on the proportion of subjects with urinary tract infection, using approximate χ^2 tests. Presence or absence of anti-A was found not to have a significant effect, but there was a significant interaction between the anti-B and secretor effects; subjects lacking both anti-B and secretor substance—that is, those of blood groups B and AB who are non-secretors—had a higher risk of recurrent urinary tract infection, whereas subjects lacking only one of these two did not differ signifi-

cantly in risk from those with both factors present. Thus the data fit extremely well ($\chi^2 = 1{\cdot}86$, 6 df) to a model in which the non-secretor in blood groups B and AB have a highly significant excess risk ($\chi^2 = 9{\cdot}12$, 1 df, $p < 0{\cdot}01$) over all other types. The relative risk of urinary tract infection for these subjects as compared with other types was estimated as 3·12, with 95% confidence limits of 1·49 and 6·52. This appears to be a genuine example of synergy with absence of both anti-B and secretor substance being necessary to give an increased risk of urinary tract infection. The ratios of observed to expected prevalence in the table show this finding clearly, with only those for non-secretors in blood groups B and AB differing substantially from unity.

The larger series of controls (in whom only ABO group and not secretor state was known) did not differ significantly from the smaller series in their blood group frequencies, but showed a highly significant difference ($\chi^2 = 16{\cdot}34$, 3 df, $p < 0{\cdot}001$) from the patients with urinary tract infections (table) (14% in blood groups B and AB *v* 21%). This confirms previous findings and is consistent with the suggested model in which non-secretors in blood groups B and AB have an increased likelihood of recurrent urinary tract infection. The proportion of anti-B negative patients in the study group is inflated by the excess prevalence of those who are also non-secretors, even though anti-B negative secretors are not at a higher risk. Thus, absence of anti-B when considered alone shows a significant risk relative to presence of anti-B (95% confidence limits of 1·24 and 21·7 (table)). This risk is lower than the threefold increase in risk found for non-secretors in blood groups B and AB, because the anti-B negative group is "diluted" with anti-B negative secretors.

Discussion

The highly significant excess of non-secretors in blood groups B or AB in the study group strongly suggests a synergistic link between the absence of anti-B isohaemagglutinin and secretor substances and an increased risk of recurrent urinary tract infection in women. In the controls the prevalence of non-secretors of 26·6% reflects an increased incidence of non-secretors found in Scottish and Irish ethnic groups compared with that reported for other regions in the United Kingdom.[13] This highlights the need to use local control groups in surveys of this nature.

The association of infections with ABO blood group and secretor state[1-9] has led to investigation of the mechanisms concerned.[6 14 15] Many bacteria cross-react with ABO blood groups.[14-20] Drach *et al*[18] found A and B blood group activity in 47% of 34 urinary tract pathogens. Isohaemagglutinins may have an important protective role against these cross-reacting bacteria.[15 19] Our finding that individuals of blood groups B and

AB with no anti-B isohaemagglutinins are more susceptible to urinary tract infection is interesting in that Drach[18 20] found antigens like those of blood groups A and B on urinary tract pathogens, often with both group A and group B activity on the same bacterium. These workers noted that anti-B titres were sometimes raised in the disease but anti-A ones were not, suggesting that the antigen to blood group A was less immunogenic on the bacteria or that there were deficiencies in recognition of antigens of blood group A. Springer *et al*[16] found *E coli* 086 B:7 to be highly interactive with the blood group: thus growth was retarded in the presence of anti-B sera; if complement was added a bactericidal reaction ensued. Isohaemagglutinins may also interact with blood group like antigens on bacterial cell walls to inhibit attachment to uroepithelial or periurethral cells. Thus persons capable of producing anti-B isohaemagglutinins may have a greater degree of protection against urinary tract infection. In general, the extent of bacterial cross-reaction with ABO blood groups suggests that a selective advantage linked to ABO blood group might operate in combating infections.

Secretor state is associated with certain differences in immunoglobulin concentrations in that anti-B serum IgA and (in white people) serum IgG concentrations are lower in non-secretors than in secretors.[21 22] This suggests that non-secretors may have less effective immune protection than secretors and is consistent with our findings that non-secretors were more susceptible to recurrent urinary tract infection. This probably entails the participation of other factors, and the finding that excretion of blood group substances in urine is an active process in the kidney and not a simple filtration process[23] suggests that these substances may have a protective part to play in secretions. Boat *et al*[24] found that ABH blood group substances of saliva inhibited haemagglutination of influenza B virus and suggested that some of these substances may interfere with access of influenza virus to binding sites. One of the protective roles of these blood group substances may be their ability to occupy or in some way interfere with binding sites either on the bacterium or on the epithelial cell with possible effects on bacterial colonisation and subsequent invasion and infection; this appeared to be the case in our studies on diabetic mice.[25]

Determination of blood groups and secretor state may provide additional information in identifying individuals who are at risk. In children under the age of 5 the combination of infection and vesicoureteral reflux predisposes to renal scarring.[26] Few if any tests are available for providing prognostic information for adults with a normal urinary tract and a history of recurrent infection. Blood group and secretor state may be of relatively minor importance in relation to all other aspects of susceptibility.

Nevertheless, it may be worthwhile for the clinician to take account of ABO and secretor state in addition to already established factors in the assessment of patients with urinary tract infection when considering their long-term susceptibility and management. Meanwhile, there is a good case for research workers concerned with mechanisms of microbial pathogenicity to add this consideration to an extending list of host factors that bear on host-parasite interactions.

This work was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department and by grants G81/103811/SB and G979/74/S from the Medical Research Council.

We thank the Blood Transfusion Service, Royal Infirmary, Edinburgh for supplying reagents for blood grouping and determination of secretor state and for undertaking random verification checks on selected saliva (50) and plasma (48) samples; Dr J McMurdoch and Dr J A Gray for permission to study their patients; the doctors and staff of Ward 14, City Hospital, Edinburgh; and Professor J G Collee and Messrs A McGowan and G Neill for their help and advice.

References

[1] Barua D, Paguio AS. ABO blood group and cholera. *Ann Hum Biol* 1977; **4**:489-92.

[2] Socha W, Bilinska M, Kaczera Z, Padjak E, Stankiewicz D. *Escherichia coli* and ABO blood groups. *Folia Biol (Krakow)* 1969;**17**:259-69.

[3] Robinson MG, Tolchrin D, Halpern C. Enteric bacterial agents and the ABO blood groups. *Am J Hum Genet* 1971;**23**:135-45.

[4] Cruz-Coke R, Parades L, Montengro A. Blood groups and urinary microorganisms. *J Med Genet* 1965;**2**:185-8.

[5] Foster MT, Labrum AH. Relation of infection with *Neisseria gonorrhoeae* to blood groups. *J Infect Dis* 1976;**133**:329-30.

[6] Miler JJ, Novotny P, Walker PD, Harris JRW, MacLennan IPB. *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect Immun* 1977; **15**:713-9.

[7] Haverkorn MJ, Goslings WRO. Streptococci ABO blood groups and secretor status. *Am J Hum Genet* 1969;**21**:360-75.

[8] Pradhan AC, Chawla TN, Samuel KC, Pradhan S. The relationship between periodontal disease and blood groups and secretor status. *J Periodont Res* 1971;**6**:194-300.

[9] Chaudhuri A, Das Adchickary CR. Possible role of blood group secretory substances in the aetiology of cholera. *Trans R Soc Trop Med Hyg* 1978;**72**:664-5.

[10] Kopeć AC. *The distribution of the blood groups in the United Kingdom.* Oxford: Oxford University Press, 1970:26.

[11] Mollinson PL. *Blood transfusion in clinical medicine.* 6th ed. Oxford: Blackwell, 1979:414-82.

[12] Armitage P. *Statistical methods in medical research.* Oxford: Blackwell, 1971:375.

[13] Lincoln PJ, Dodd BE. Variation in secretor and Lewis type frequencies within the British Isles. *J Med Genet* 1972;**9**:43-5.

[14] Springer GF, Williamson P, Brandes WC. Blood group activity of Gram-negative bacteria. *J Exp Med* 1961;**113**:1177-93.

[15] Muschel L, Osawa E. Human blood group substances B and *Escherichia coli* 086. *Proc Soc Exp Biol Med* 1959;**101**:614-7.

[16] Springer GF. Importance of blood group substances in interactions between man and microbes. *Ann NY Acad Sci* 1970;**169**:134-52.

[17] Springer GF. Blood group and Forssman antigenic determinants shared between microbes and mammalian cells. *Prog Allergy* 1971;**15**:9-77.

[18] Drach GW, Reed WP, Williams RC. Antigens common to human and bacterial cells: urinary tract pathogens. *J Lab Clin Med* 1971;**78**:725-35.

[19] Check JH, O'Neill EA, O'Neill KE, Fuscaldo KE. Effect of anti-B antiserum on the phagocytosis of *Escherichia coli*. *Infect Immun* 1972;**6**: 95-6.

[20] Drach GW. Antigens common to human and bacterial cells: tolerance or resistance to urinary and surgical infections. *Surg Forum* 1971;**22**:67-8.

[21] Grundbacher FJ, Shreffler DC. Effects of secretor, blood and serum groups on isoantibody and immunoglobulin levels. *Am J Hum Genet* 1970;**22**:194-202.

[22] Grundbacher FJ. Immunoglobulins, secretor status and the incidence of rheumatic fever and rheumatic heart diseases. *Hum Hered* 1972;**25**: 399-404.

[23] Kalinowski M. Urinary elimination of group substances ABO(H) in different renal diseases. *Folia Haematol (Liepz)* 1972;**100**:144-63.

[24] Boat TF, Davis J, Stern RC, Cheng PW. Effect of blood group determinants on binding of human salivary mucous glycoproteins to influenza virus. *Biochim Biophys Acta* 1978;**540**:127-33.

[25] Weir DM, Blackwell CC, McLean CA. Impaired bacterial binding to peritoneal exudate cells from mice with alloxan induced diabetes. *Journal of Clinical and Laboratory Immunology* 1981;**5**:37-40.

[26] Rolleston GL, Shannon FT, Utley JF. Relationship of infantile cystoureteric reflux to renal damage. *Br Med J* 1970;i:460-3.

(*Accepted 19 April 1982*)

Printed in Great Britain by Bourne Offset (Iver) Ltd., Iver, Bucks.

Clin. Lab. Immunol. (1982), 9, 169–172

PHAGOCYTE RECOGNITION OF NEISSERIA GONORRHOEAE

D. F. KINANE, C. C. BLACKWELL, D. M. WEIR and F. P. WINSTANLEY
Department of Bacteriology, University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland, UK
(Received 15 February 1982)

SUMMARY Human monocytes show an increased ability to bind pilate rather than non-pilate gonococci. Polymorphonuclear leucocytes (PMN) do not discriminate between pilate and non-pilate variants. PMN, however, bind appreciably more gonococci (either pilate or non-pilate) than monocytes. The results help to explain the role of pili on gonococci as virulence factors and in the immune response.

ITRODUCTION

TTACHMENT of *Neisseria gonorrhoeae* to mucosal cells nd their subsequent invasion are the initial steps in e establishment of gonococcal infection. When the rganisms have penetrated the mucosal barriers, the itcome then largely depends on their interaction with nagocytic cells. Attachment and uptake by polymorpho-uclear leucocytes (PMN) may lead to destruction of the rganism. Attachment to macrophages is likely to lead to itiation of an immune response by presentation of icrobial antigen to the lymphoid cells. The interaction etween phagocytes and bacteria is clearly an important eterminant in the pathogenesis of the disease. Binding the phagocyte membrane is dependent on the presence appropriate adhesin receptor interactions between acteria and phagocytes (1).

The virulence of *N. gonorrhoeae* has been linked with rticular colonial types, pilate types T_1 and T_2, but not ith non-pilate types T_3 and T_4 (2). Pilate gonococci ave been found to adhere more efficiently to certain uman tissues than non-pilate organisms, e.g. epithelial lls (3), sperm cells (4), buccal cells (5), erythrocytes (3) nd tissue culture cells (6, 7). No difference in binding to MN has been observed with pilate and non-pilate onococci (7, 8); attachment of gonococci to PMN ppears to be mediated by another surface component, e leukocyte association factor (9).

In view of the different roles PMN and macrophages ay in host defence, we sought differences in the ability these cells to bind pilate and non-pilate gonococci cently isolated from an infected patient.

rrespondence: Dr. D. M. Weir, Reader, Immunology Laboratory, epartment of Bacteriology, University Medical School, Teviot Place, dinburgh EH8 9AG, Scotland, UK.

MATERIALS AND METHODS

Bacterial Strain

Neisseria gonorrhoeae strain E757, recently isolated from a patient attending the Department of Genitourinary Medicine was used throughout these experiments. Opaque colony types 2 (T_2) and 3 (T_3) were differentiated with a Zeiss stereoscopic microscope with a double system of substage lighting and selectively subcultured and maintained on Difco GC base supplemented as described by Young (10) (GC agar). T_2 and T_3 growths, with more than 95% of the colonies stable, were employed. Bacteria were harvested from GC agar plates at 14–16 hr, suspended in Dulbecco's phosphate buffered saline (D.PBS) by gentle pipetting, washed and finally re-suspended gently in D.PBS. The pili were sheared by vortexing a suspension of gonococci in D.PBS with a Rotamixer Deluxe (Hook and Tucker Ltd., England) at maximum setting for 5 min.

Grids negatively stained with phosphotungstic acid were examined by electron microscopy (Hitachi HU12A). This confirmed the presence of pili on type 2 gonococci and their absence on type 3 and vortexed type 2 and 3 gonococci (3, 11).

Bacterial concentrations were determined at E650 nm on a CE292 spectrophotometer (Cecil Instruments, Cambridge, England) by reference to standards enumerated by microscope counts with a counting chamber. Dose response experiments indicated that the optimal concentration of gonococci for these assays was 10^8 organisms/ml and this concentration was used throughout.

Phagocytes

Blood from healthy donors with no history of gonorrhoea was collected in plastic tubes containing 10 units of heparin ml^{-1}. Monocytes and PMN were separated on Ficoll-Hypaque cushions (Ficoll, Pharmacia, London)—specific gravity 1·078—according to the method of Böyum (12). After the interface cells (monocytes and lymphocytes) were collected, the pellet containing granulocytes and red blood cells was re-suspended in 0·82% ammonium chloride to lyse the erythrocytes (13). The separated cells were finally washed twice with D.PBS.

Preparation of Monolayers

PMN and monocytes were re-suspended in Eagle's minimum essential medium (MEM) without serum, buffered at pH 7·3 with HEPES (final concentration, 30 mM; Wellcome Research Laboratories, Beckenham), and supplemented with penicillin/streptomycin (final concentration, 100 $u.ml^{-1}$ of each) and glutamine (final concentration, 2 mM) to give

41–2760/82/1200–0169 $03.50

a total cell count of 2×10^5/ml. Aliquots (1 ml) of the above cell preparations were layered onto 13 mm diameter (No. 1) glass coverslips in tissue culture plates 16 mm well diameter; (Costar, 295 Broadway, Cambridge, Mass.) and incubated for 1 hr at 37°C to allow adhesion. Non-adherent cells were removed by repeated washing with D.PBS.

Binding Assay

Coverslips were overlaid with 1 ml of gonococci in D.PBS containing Ca^{++} and Mg^{++} ions (0·9 mM and 0·5 mM, respectively) and incubated for: (1) 30 min at 37°C, and (2) 2 hr at 4°C. Non-attached organisms were removed by repeated washing with D.PBS. Coverslips were air dried, fixed in methanol and stained with May Grunwald/Giemsa. Bacterial binding was estimated by counting leucocytes with bacteria attached at two or more discrete points. Duplicate coverslips were used; for each coverslip, 200 leucocytes were counted and the results expressed as the percentage of cells binding organisms.

Analysis of Results

Statistical analysis of the results was by paired sample *t* tests.

Results

Attachment of Pilate and Non-pilate Variants

Figure 1 summarises the results of three experiments comparing the attachment of pilate (T_2) and non-pilate (T_3) gonococci to PMN and macrophages. There was no significant difference in association of the two types to PMN at either 4°C or 37°C ($P > 0·5$). The association of T_2 cells with monocytes was significantly greater than that observed with T_3 cells at both 4°C ($P < 0·02$) and 37°C ($P < 0·01$). A significantly greater number of PMN than monocytes bound both T_2 and T_3 gonococci ($P < 0·01$) (table 1).

Effect of Removal of Pili

Figure 2 shows that the difference in binding of T_2 and T_3 cells to monocytes illustrated in Figure 1 was abolished by removal of pili from T_2 cells by mechanical shearing. While there was a slight overall reduction in binding of gonococci to the phagocytes following shearing treatment (table 2), there was still a significantly greater number of PMN than monocytes binding gonococci ($P < 0·05$).

Comparison of 4°C and 37°C Assays

The results for the assays done at 4°C represent attachment only as the monocytes and PMN do not phagocytose at this temperature. The 37°C assays are for attachment and phagocytosis, i.e. association. Differentiation between phagocytosis and attachment was not done, but the general trends in phagocyte/gonococcal association at 4°C and 37°C were consistent and suggest that the type of attachment seen at 4°C also occurs at physiological temperatures at which phagocytosis also occurs.

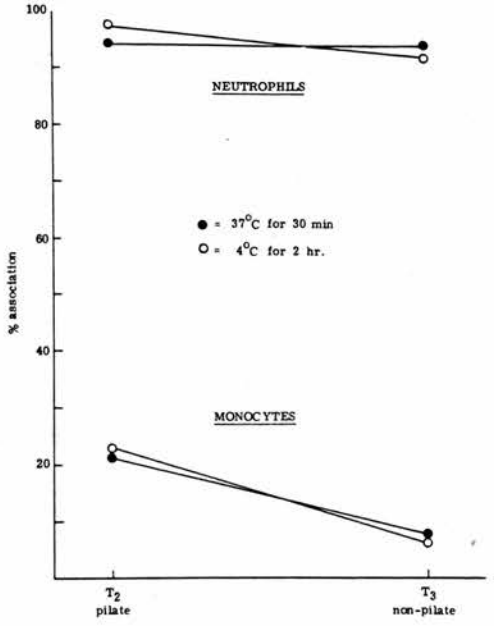


FIG. 1. Association of pilate (T_2) and non-pilate (T_3) gonococci with PMN and monocytes at 37°C and 4°C. Each point represents the mean of three experiments.

Discussion

These results indicate that the recognition of *N. gonorrhoeae* by PMN and monocytes differs. Our findings agree with the observation of Swanson (7) that PMN do not appear to discriminate in their ability to bind pilate and non-pilate variants of the strain used. Monocytes, in contrast, showed an increased binding of pilate organisms similar to that observed for epithelial cells (3), sperm (4), buccal cells (5), erythrocytes (3) and tissue culture cells (6, 7).

Two roles have been suggested for pili in the establishment of gonococcal infection: (1) pili assist the organism in attaching to the mucosa (6,. 14); and (2) pili increase

Table 1 Association of pilate (T_2) and non-pilate (T_3) gonococci with PMN and monocytes at 37°C and 4°C

N. gonorrhoeae E757	*Temperature*	*Percentage binding to* PMN	Monocytes	*% difference*	*P value*
T_2	37°C	94·17	21·17	73	<0·01
	4°C	97·33	23·67	64	<0·001
T_3	37°C	93·17	7·00	86	<0·01
	4°C	92·17	6.50	86	<0·01

Table 2 Effect of loss of pili on association of gonococci with PMN and monocytes at 37°C and 4°C

N. gonorrhoeae E757	*Temperature*	*Percentage binding to*		*% difference*	*P value*
		PMN	*Monocytes*		
T_2 (vortexed)	37°C	75·67	10·25	65	<0·02
	4°C	61·67	6·33	55	<0·05
T_3 (vortexed)	37°C	70·25	8·33	62	<0·05
	4°C	58·58	9·33	49	<0·05

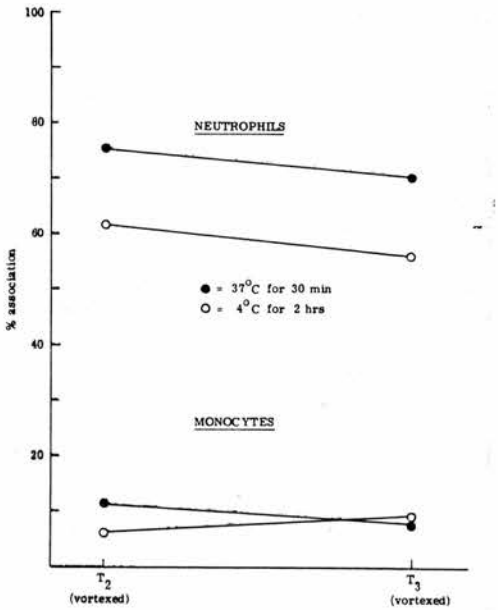


FIG. 2. The effect on binding of both T_2 and T_3 gonococci to PMN and monocytes following mechanical shearing treatment of the gonococci. Each point represents the mean of three experiments.

gonococcal resistance to phagocytosis by PMN (3, 15, 16, 17). Our findings suggest a third role in conjunction with the hypothesis of Novotny and his colleagues (1977) that "gonorrhoea appears to be a specific disease of human macrophages".

Novotny and his colleagues observed "infectious units" of gonococci in electron micrographs of pus from more than 70 infected individuals. These are clusters of organisms that appear to be undamaged and in an exponential growth state. Surrounding the gonococci is a coat made up of granules and remnants of mitochondria that are thought to be derived from macrophages. There is evidence, from labelled antibody studies, to suggest that the dense accumulation of granules protects the gonococci from humoral defences. These granular coats also seem to allow the gonococci to go unrecognised by phagocytic cells as long as the coat remains dense.

According to Novotny's hypothesis, a host is infected with both free non-multiplying gonococci and multiplying gonococci in the protective infectious units. The multiplying organisms infiltrate the mucosa and submucosa of the genitourinary tract. Free gonococci phagocytosed by PMN are destroyed, but those ingested by monocytes are believed to interfere with regulation of the phagocytic cell and multiply within it. This results in the destruction of the monocyte, the remnants of which surround the gonococci to form a new infectious unit. The results of the present study encourage us to postulate that the presence of pilar antigen on the gonococcal surface is a virulence factor that increases the probability of the bacteria becoming attached to and subsequently phagocytosed by monocytes.

The large difference we have shown in binding of gonococci (whether pilate, non-pilate or vortexed) between PMN and monocytes is most striking and contrasts with work done on *Staph. albus* in our department; in the staphylococcal studies, binding to PMN and monocytes showed no differences (19). Our results with gonococci may also provide an explanation for gonorrhoeal recurrence additional to the theory of broad heterogeneity of the gonococcal antigens (20, 21, 22). An ineffective immune response to gonococcal infection may be explained by the much greater ability of PMN to take up the organism in comparison with the smaller numbers that are recognised and taken up by monocytes. If these are largely incorporated into "infectious units" and survive, the presentation of gonococcal antigen by the monocytes to the lymphocytes will be reduced in comparison with other types of antigens. Pili are not readily demonstrated on gonococci in human tissues (23, 24). If proteolytic enzymes in pus and tissues remove these from the surface of the organism, the probability of attachment to monocytes would be further reduced. As pili seem to enhance attachment to epithelial cells and macrophages, loss of pili would be likely to limit colonisation and to reduce the possibility of the development of infectious units in macrophages with a consequent reduction in virulence.

ACKNOWLEDGEMENTS

We are grateful to Mrs. Marilyn Cole for help in typing the manuscript and to Professor J. G. Collee for his advice in the preparation of the manuscript. This investigation was supported by Grant No. K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department and by Grants G81/103811/SB and G979/74/S from the Medical Research Council.

REFERENCES

1. Weir, D. M. (1980). Surface carbohydrates and lectins in cellular recognition. *Immunology Today*, **1**, 45.
2. Kellog, D. S. Jr., Peacock, W. L. Jr., Deacon, W. E., Brown, L. and Pirkel, C. I. (1963). *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. *J. Bacteriol.*, **85**, 1274.
3. Punsalang, A. P. Jr. and Sawyer, W. D. (1973). Role of pili in the virulence of *Neisseria gonorrhoeae*. *Infect. Immun.*, **8**, 255.
4. James-Holmquest, A. N., Swanson, J., Buchanan, T. M., Wende, R. D. and Williams, R. P. (1974). Differential attachment by pilated and non-pilated *Neisseria gonorrhoeae* to human sperm. *Infect. Immun.*, **9**, 897.
5. Tramont, E. M. and Wilson, C. (1977). Variations in buccal cell adhesion of *Neisseria gonorrhoeae*. *Infect. Immun.*, **16**, 709.
6. Swanson, J. (1973). Studies on gonococcus infection. IV. Pili: their role in attachment of gonococci to tissue culture cells. *J. Exp. Med.*, **137**, 571.
7. Swanson, J., King, G. and Zeligs, B. (1975). Studies on gonococcus infection. VIII. ^{125}Iodine labelling of gonococci and studies on their *in vitro* interactions with eukaryotic cells. *Infect. Immun.*, **11**, 453.
8. Swanson, J., Sparks, E., Zeligs, B., Siam, M. and Parrott, C. (1974). Studies on gonococcus infection. V. Observations on *in vitro* interactions of gonococci and human neutrophils. *Infect. Immun.*, **10**, 633.
9. Swanson, J., Sparks, E., Young, O. and King, G. (1975). Studies on gonococcus infection. X. Pili and leukocyte association factor as mediators in interactions between gonococci and eukaryotic cells *in vitro*. *Infect. Immun.*, **11**, 1352.
10. Young, H., Patterson, I. C. and McDonald, D. R. (1976). Rapid carbohydrate utilisation test for the identification of *Neisseria gonorrhoeae*. *Brit. J. Vener. Dis.*, **52**, 172.
11. Rosenthal, R. S., Fulbright, R. S., Eads, M. E. and Sawyer, W. D. (1977). Ethylenediaminetetracetic acid-sensitive antiphagocytic activity of *Neisseria gonorrhoeae*. *Infect. Immun.*, **15**, 817.
12. Böyum, A. (1968). Isolation of leucocytes from human blood. Further observation. Methyl cellulose dextran and Ficoll as erythrocyte aggregating agents. *J. Clin. Lab. Invest.*, **21**, Suppl. 97, 31.
13. Boyle, W. (1968). An extension of the ^{51}Cr release assay for the estimation of mouse cytotoxins. *Transplantation*, **67**, 761.
14. Ward, M. E., Watt, P. J. and Robertson, J. N. (1974). The human fallopian tube: a laboratory model for gonococcal infection. *J. Infect. Dis.*, **129**, 650.
15. 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.
16. Thongthai, C. and Sawyer, W. D. (1973). Studies on the virulence of *Neisseria gonorrhoeae*. 1. Relation of colonial morphology and resistance to phagocytosis by polymorphonuclear leukocytes. *Infect. Immun.*, **7**, 373.
17. Ofek, I., Beachey, E. H. and Bisno, A. L. (1974). Resistance of *Neisseria gonorrhoeae* to phagocytosis: relationship to colonial morphology and surface pili. *J. Infect. Dis.*, **129**, 310.
18. Novotny, P., Short, J. A., Hughes, M., Miler, J. J., Syrett, C., Turner, W. H., Harris, J. R. W. and Maclennan, I. P. B. (1977). Studies on the mechanisms of pathogenicity of *Neisseria gonorrhoeae*. *J. Med. Microbiol.*, **10**, 347.
19. Glass, E. J., Stewart, J. and Weir, D. M. (1981). Presence of bacterial binding "lectin-like" receptors on phagocytes. *Immunology*, **44**, 529.
20. Kasper, D. L., Rice, P. A. and McCormack, W. M. (1977). Bactericidal antibody in genital infection due to *Neisseria gonorrhoeae*. *J. Infect. Dis.*, **135**, 243.
21. McMillan, A., McNeillage, G., Young, H. and Bain, S. S. R. (1979). Secretory antibody response of the cervix to infection with *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.*, **55**, 265.
22. McMillan, A., McNeillage, G., Young, H. and Bain, S. S. R. (1979). Serum immunoglobulin response in uncomplicated gonorrhoea. *Br. J. Vener. Dis.*, **55**, 5.
23. Novotny, P., Short, J. A. and Walker, P. D. (1975). An electron-microscope study of naturally occurring and cultured cells of *Neisseria gonorrhoeae*. *J. Med. Microbiol.*, **8**, 413.
24. Evans, B. A. (1977). Ultrastructural study of cervical gonorrhoea. *J. Infect. Dis.*, **136**, 248.

Teviot Scientific Publications Ltd
31 Montpelier Park
Edinburgh EH10 4LX, UK

Br J Vener Dis 1983;**59**:44-6

Blood group, secretor status, and susceptibility to infection by Neisseria gonorrhoeae

D F KINANE, C C BLACKWELL, F P WINSTANLEY, AND D M WEIR
From the Department of Bacteriology, University of Edinburgh Medical School, Edinburgh

SUMMARY To determine whether the presence or absence of anti-B isohaemagglutinin in individuals of blood group B increases their susceptibility to gonococcal infections 567 new patients attending a sexually transmitted disease clinic were screened for blood group and secretor status. Of the patients with blood group B, 20·1% had gonorrhoea and 12% had not. A higher percentage (20·9%) of patients with no anti-B isohaemagglutinin had gonorrhoea compared with those without (12·1%). There was, however, no synergy between the absence of anti-B isohaemagglutin and non-secretion of water-soluble blood group B antigen. Further research is needed to determine the underlying host-parasite interactions responsible for the increased susceptibility to gonorrhoea in these individuals.

Introduction

Over the past six years there have been reports of increased susceptibility of individuals of blood group B to infection with *Neisseria gonorrhoeae*.[1,2] Foster and Labrum[1] suggested that the presence or absence of anti-B isohaemagglutinin may be the causal factor for the reported increase in susceptibility to gonorrhoea of individuals with blood group B. In a recent study we found that individuals of blood groups B and AB — that is, those with no anti-B isohaemagglutinin — who were also non-secretors of blood group antigen were significantly more susceptible to urinary tract infection.[3] To determine whether there is a synergistic effect between those two host factors and susceptibility to gonococcal infection we screened patients attending a department of genitourinary medicine for blood group and secretor status, and we related the data to the occurrence of gonorrhoea in these patients.

Patients and methods

During a three-month period all new patients attending the department of genitourinary medicine at the Royal Infirmary, Edinburgh, were asked to provide a specimen of blood and saliva for determination of blood group and secretor status.

Address for reprints: Dr D F Kinane, Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

Accepted for publication 29 August 1982

Control data for blood group frequency were available for 6662 donors from the same geographical area.[4] Secretor status control data were produced by testing 334 plasma specimens from blood donors in the survey area.

SCREENING PROCEDURES

Blood from each patient was collected at the clinic and stored in heparinised tubes. The saliva from each patient was boiled for 20 minutes, centrifuged at $500 \times g$ for 10 minutes and the supernate stored briefly at 4°C until tested for the presence of blood group antigen.

Blood group was determined by agglutination tests in plastic wells (WHO plates). Secretor status was determined as described by Mollinson[5] with saliva or plasma samples. Samples of saliva or plasma from secretors and non-secretors representative of the four blood groups were used as controls for each experiment. Agglutinins used were anti-A and anti-B sera and *Ulex europaeus* lectin. The red blood cells used were of groups A_2, B, and O. The Blood Transfusion Service, Royal Infirmary, Edinburgh, kindly supplied the reagents for blood grouping and determination of secretor status and also performed random verification tests on 50 selected saliva samples and 46 plasma samples.

Results

The distribution of blood groups of patients with and without gonorrhoea and of the controls is given in

TABLE I *Distribution of ABO blood groups in 567 patients with and without gonorrhoea and controls*

	Culture results for N gonorrhoeae			
Blood group	*No (%) negative*	*No (%) positive*	*Total No (%) of patients*	*No (%) of controls*[4]
O	247(50·3)	35(46·1)	282(49·7)	3323(49·9)
A	176(35·8)	23(30·3)	199(35·1)	2410(36·2)
B	59(12·0)	16(21·1)	75(13·2)	715(10·7)
AB	9(1·8)	2(2·6)	11(1·9)	214(3·2)
Total	491(99·9)	76(100·1)	567(99·9)	6662(100·0)

able I. The blood group frequencies of the controls nd the total number of patients attending the clinic howed no significant differences ($\chi^2 = 5 \cdot 864$, $>0 \cdot 1$). The frequency of blood group B in these atients with gonorrhoea was 21·1% compared with 2% in those without. This difference was not, owever, significant ($\chi^2 = 5 \cdot 103$, $p > 0 \cdot 1$). When the requency of blood group B in the patients with gonorrhoea was compared (21·1%) with that of the ontrols (10·7%) the difference ($\chi^2 = 8 \cdot 404$, $<0 \cdot 05$) was significant.

The effect of the presence or absence of anti-B on usceptibility to gonorrhoea is shown in table II. A igher percentage (20·9%) of patients with no anti-B sohaemagglutinin had gonorrhoea compared with hose with anti-B isohaemagglutinin (12·1%) ($\chi^2 = 4 \cdot 947$, $p < 0 \cdot 05$). Using the relative risk method f Woolf[6] we found that the relative risk of gonorrhoea as 1·93 for individuals without anti-B isohaem-gglutinin. This means that they are 93% more usceptible to gonorrhoea than persons with anti-B sohaemagglutinin.

The distribution of secretor status for patients with nd without gonorrhoea and controls is given in table II. No significant differences were noted between the otal patients and the controls ($\chi^2 = 0 \cdot 917$, $p > 0 \cdot 1$) nor etween those patients with and without gonorrhoea ($\chi^2 = 0 \cdot 168$, $p > 0 \cdot 5$).

ABLE II *Analysis of data from table I by presence or bsence of anti-B isohaemagglutinin*

	Culture results for N gonorrhoeae		
nti-B	*No (%) negative*	*No (%) positive*	*Total*
resent (O and A)	423(87·9)	58(12·1)	481(100)
bsent (B and AB)	68(79·1)	18(20·9)	86(100)
otal	491	76	567

Discussion

Our findings confirm those of others[1,2] that individuals of blood group B are more susceptible to gonococcal infection and further suggest that the absence of anti-B isohaemagglutinin is important.

An increase in the number of non-secretors among patients with gonococcal infections had been predicted, but this was not observed. Although there was a significant increase in the number of blood group B individuals among the infected patients, there was no synergy between the absence of anti-B isohaemagglutinin and non-secretion of water soluble blood group B antigen comparable to that found in patients with urinary tract infections.[3]

The underlying host-parasite interactions responsible for the increased susceptibility of individuals with no anti-B isohaemagglutinin to gonococcal infection are not yet known; they appear, however, to differ from those involved with other Gram-negative, urinary tract pathogens. Accordingly, we are currently investigating the role of anti-B isohaemagglutinin in normal human serum acting as an opsonin or as bactericidal antibody, gonococcal interactions with human phagocytic cells of the different blood groups, and differences in the attachment of gonococcal strains to epithelial cells from individuals with different ABO blood groups.

We are most grateful to Dr D H H Robertson and his colleagues in the department of genitourinary medicine, Royal Infirmary, Edinburgh, for their assistance in the collection of blood and saliva specimens, to Dr H Young for the results of bacteriological investigations, and to Dr R A Elton for assistance with the statistical analysis of our data. We also thank Professor J G Collee for his advice in

ABLE III *Distribution of secretor status for patients with and without gonorrhoea and controls*

	Culture results for N gonorrhoeae			
ecretor status	*No (%) negative*	*No (%) positive*	*Total No (%) of patients*	*No (%) of controls*
ecretor	344(70·1)	55(72·4)	399(70·4)	245(73·4)
on-secretor	147(29·9)	21(27·6)	168(29·6)	89(26·6)
otal	491(100)	76(100)	567(100)	334(100)

preparation of the manuscript and Mrs M Cole for its typing. This investigation was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department, and by grants G81/103811/SB and G979/64/S from the Medical Research Council.

References

1. Foster MT, Labrum AH. Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. *J Infect Dis* 1976;**133**:329.
2. Miler JJ, Novotny P, Walker PHD, Harris JRW, MacLennan IPB. *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect Immun* 1977;**15**:713.
3. Kinane DF, Blackwell CC, Brettle RP, Weir DM, Winstanley FP, Elton RA. ABO blood groups, secretory state, and susceptibility to recurrent urinary tract infection in women. *Br Med J* 1982;**285**:7.
4. Kopec AC. *The Distribution of the Blood Groups in the United Kingdom*. London, New York, Toronto: Oxford University Press, 1970;26.
5. Mollinson PL. *Blood Transfusion in Clinical Medicine*. 6th ed. Oxford: Blackwell, 1979;414.
6. Woolf B. On estimating the relation between blood group and disease. *Ann Hum Genet* 1954/55;**19**:251.

. Clin. Lab. Immunol. (1983), 4, 173–178

ABO BLOOD GROUP AND SUSCEPTIBILITY TO GONOCOCCAL INFECTION. I. FACTORS AFFECTING PHAGOCYTOSIS OF *NEISSERIA GONORRHOEAE*

C. C. BLACKWELL, M. KOWOLIK*, F. P. WINSTANLEY, D. F. KINANE, D. M. WEIR, J. A. LAW and Y. L. HO WANG CHOK

*Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh, and *Department of Oral Medicine, University of Edinburgh, U.K.*

(Received 24 June 1982)

SUMMARY The effect of opsonization of *Neisseria gonorrhoeae* by isohaemagglutinins from normal serum on attachment to human polymorphonuclear leukocytes (PMN) was investigated. No significant differences between sera from blood groups O, A, B or AB were found. Differences in attachment of gonococci were related to differences in lipopolysaccharide detected by sensitivity to R-type pyocines of *Pseudomonas aeruginosa*. Non-pilate variants of each test strain markedly stimulated nitroblue tetrazolium (NBT) reduction in PMN, but their pilate variants were essentially inactive in the NBT test.

NTRODUCTION

THE establishment of an infection by *Neisseria onorrhoeae* depends on a variety of factors including the bility of the bacteria to attach to the appropriate target ells, their uptake and destruction within phagocytic ells, and the effects of serum components on their via- bility and opsonization. A higher incidence of gonor- hoea among individuals of blood group B has been eported (1, 2), and a theoretical explanation is that the nti-A isohaemagglutinin characteristic of B serum may e less effective as an opsonin than the anti-B isohaemag- lutinin which occurs in the sera of persons of groups A nd O (1).

In this study we have investigated the effects of isohae- naggluti nins in the sera of individuals of groups A, B, AB nd O on the attachment to polymorphonuclear leuko- ytes (PMN) of gonococcal strains with different cell nvelope characteristics. The effects of differences in acterial cell envelope structure and composition as re- lected by variation in complexity of pyocine receptors of he lipopolysaccharide (LPS) (3) have been examined as actors that may affect attachment of non-opsonized acteria to the PMN membrane or the subsequent hagocytosis and killing of the bacteria.

In an earlier study with one of the strains used here we ave shown that human PMN do not discriminate in heir binding ability between pilate and non-pilate ariants of the strain of *N. gonorrhoeae* tested (4), hough these variants differ in their infectivity. Either (1) he binding mechanism studied does not reflect a more significant surface event *in vivo*, or (2) intracellular events within the PMN significantly determine the outcome of the challenge. To assess the effects of variation in the cell surface of the organism on the metabolism of the PMN with implications for the survival of the gonococci, we have tested the NBT response induced by pilate and non-pilate variants of various pyocine types.

Correspondence: Dr. C. C. Blackwell, Department of Bacteriology, University Medical School, Teviot Place, Edinburgh.

MATERIALS AND METHODS

Bacteria

The isolates of *N. gonorrhoeae* used are listed in Table 1. Strains 9 and F62 are laboratory cultures; and strains 7422 and 7502 from disseminated gonococcal infection were obtained from Dr. Joan S. Knapp, Neisseria Reference Laboratory, United States Public Health Service, Seattle, Washington. Eight others are clinical isolates from patients attending the Department of Genito-Urinary Medicine, Royal Infirmary, Edinburgh. The pyocin types of these isolates were determined by the method previously described (3). Pilate colony type T1 and non-pilate colony type T4 variants of these strains were differentiated with a Zeiss stereoscopic microscope with a double system of substage lighting and selectively subcultured and maintained on Difco GC agar base supplemented as described by Young *et al.* (5). Bacteria were harvested from these plates at 14–16 hr, suspended in Hanks' balanced salt solution (HBSS) by gentle pipetting, washed and finally resuspended in HBSS.

Sera

Serum from individuals of groups A, B, O and AB with no history of gonorrhoea was stored at −20°C in 0·5 ml aliquots and thawed immediately before use. For some experiments sera inactivated by heating at 56°C for 30 min were used.

Phagocytosis Assay

Blood was collected from a healthy group O donor with no history of gonorrhoea. Volumes of blood (7 ml) were collected in heparinized (10 iu/ml) plastic tubes to which 2 ml of 5% dextran (Sigma Chemicals) in phosphate buffered saline (PBS) pH 7·2 was added.

141–2760/83/0400–0173 $04.50

Table 1 Pyocin types of *N. gonorrhoeae* strains

N. gonorrhoeae strain	*R1 strain*					*Unclassified strain*		*R2 strain*			*R3 strain*	*R5 strain*	
	ISD	*2285*	*IS4*	*IS6*	*IS8*	*R205*	*9579*	*ISB*	*R21*	*430*	*ISE*	*ISA*	*ISC*
Group I													
E757	−	−	−	−	−	−	+	−	−	−	−	+	±
M9131	−	−	−	−	−	−	+	−	−	−	−	+	+
7502	−	−	−	−	−	−	−	−	−	−	−	±	−
Group II													
F62	+	±	+	+	+	+	+	−	−	−	−	+	+
9	+	−	+	+	+	+	+	−	−	−	−	+	+
M8865	+	±	+	+	+	+	+	−	−	−	−	+	+
E760	+	−	+	+	+	+	+	−	−	−	−	+	+
P152	+	−	+	+	+	+	+	−	−	−	−	+	+
M6967	+	−	+	+	+	+	+	−	−	−	−	+	+
7422	+	−	+	+	+	+	+	−	−	−	−	+	+

+ inhibition.
± inhibition with some growth.
− no inhibition.

Erythrocytes were sedimented at 37°C and the leukocyte rich portion was centrifuged at 380 g for 10 min. The leukocytes were washed twice with heparinized saline and suspended in HBSS. The cell suspension was counted in a haemocytometer and the concentration adjusted to approximately 10^7 PMN/ml.

Bacterial suspensions (approximately 10^8/ml) were prepared in HBSS. The numbers of viable organisms in suitable dilutions in HBSS were determined by colony counts on GC agar.

The phagocytosis assay was essentially as described by van Furth and co-workers (6). Leukocytes (10^7/ml) and bacteria (10^8/ml) were mixed in equal volumes in plastic tubes with 0·2 ml (10%) of the appropriate serum and incubated at 37°C with rotation (16 rev/min). Aliquots of 0·4 ml were removed at 0, 10 and 30 min and added to 10 ml ice cold HBSS to prevent further phagocytosis. The leukocytes were sedimented by centrifugation at 380 g for 10 min.

Bacterial suspensions were opsonized for 30 min with serum 10% (v/v) from a healthy individual of blood group A, B or O with no history of gonorrhoea. For the control, the serum was replaced by HBSS.

For tests at each time interval, 0·5 ml samples of the pelleted leukocytes were spun onto glass slides in a cytocentrifuge (Shandon Elliot Cytospin) at 500 rpm. The slides were then studied by light microscopy. The number of leukocytes containing bacteria in vacuoles or adherent to the cell surface were expressed as a percentage of the 200 leukocytes counted. The number of bacteria per cell were calculated as a mean of 10 counts.

Nitroblue Tetrazolium Reduction (NBT)

The NBT reduction was assayed as previously described (7) with PMN and bacteria from the phagocytosis experiments. For the stimulation test, 0·5 ml of the saline dextrose solution was replaced by an equal volume of a suspension of the *N. gonorrhoeae* strain being tested. Percentage positive counts (i.e. cells containing formazan deposits) were made of the preparations and the mean of 10 counts compared statistically by a paired *t*-test with the unstimulated control cells performed at the same time.

Attachment Experiments

Blood from healthy donors with no history of gonorrhoea was collected in plastic tubes containing 10 units of heparin ml^{-1}. Monocytes and PMN were separated on Ficoll-Hypaque cushions (Ficoll, Pharmacia, London; specific gravity 1·078) by the method of Böyum (8). After the interface cells (monocytes and lymphocytes) were collected, the pellet containing granulocytes and red blood cells was resuspended in 0·8% ammonium chloride to lyse the erythrocytes (9). The separated cells were finally washed twice with Dulbecco's phosphate buffered saline (DPBS) pH 7·2. PMN were resuspended in Eagles' minimal essential medium (MEM) without serum, buffered with HEPES at pH 7·3 (final concentration, 30 mM; Wellcome Research Laboratories, Beckenham), and supplemented with penicillin/streptomycin (final concentration 100 μml^{-1} of each) and glutamine (final concentration, 2 mM) to give a total cell count of 2×10^5 (3 ml). Aliquots (1 ml) of the above cell preparations were layered onto glass coverslips 13 mm diameter in tissue culture plates with wells of 16 mm diameter (Costar, 295 Broadway, Cambridge, Ma.) and incubated for 1 hr at 37°C to allow adhesion. Non-adherent cells were removed by repeated washing in DPBS.

Coverslips were overlaid with 1 ml of gonococci (5×10^8/ml) in DPBS containing Ca^{++} 0·9 mM and Mg^{++} 0·5 mM and incubated for (a) 3 hr at 4°C, or (b) 30 min at 37°C. Non-attached organisms were removed by repeated washing with DPBS. Coverslips were air-dried, fixed in methanol and stained with May Grünwald/Giemsa. Bacterial binding was estimated by counting leukocytes with bacteria attached at two or more discrete points. Duplicate coverslips were used; for each coverslip, 200 leukocytes were counted and the results expressed as the percentage of cells binding organisms. The percentages of leukocytes with 1, 2–3, 4–5, 6 or 6 bacteria per cell were also determined.

Fresh normal sera were obtained from the same healthy donors of blood groups A, B, O and AB from whom the leukocytes were isolated. A portion of each serum was inactivated at 56°C for 30 min. The effect of opsonization on attachment was assayed as above by preincubating the bacterial suspensions with 0·1 ml of the homologous heat-inactivated serum with 0·9 ml of bacteria in HBSS before they were added to the phagocytic cells.

Results

Isohaemagglutinins as Opsonins

Sera from individuals of blood groups A, B or O were used as a source of isohaemagglutinins; the control contained HBSS instead of serum. The titres of isohaemagglutinin were 256 for anti-A and anti-B in the B and A individuals respectively and 64 for anti-A and 32 for anti-

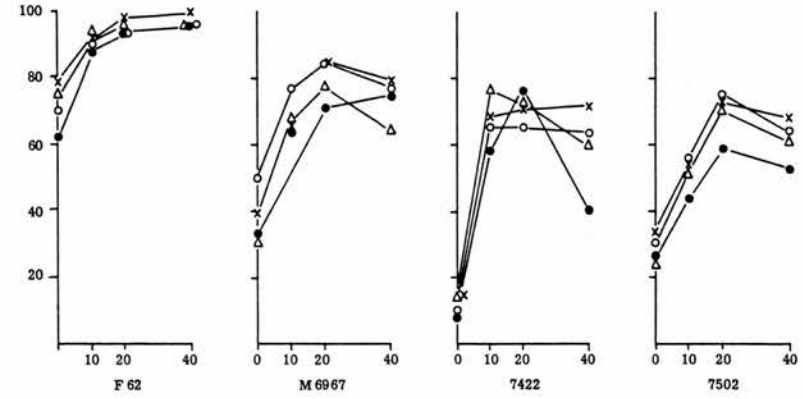


FIG. 1. Phagocytosis of *N. gonorrhoeae* strains opsonized with A, B, or O serum. ●: no serum, ○: A serum, △: B serum, X: O serum.

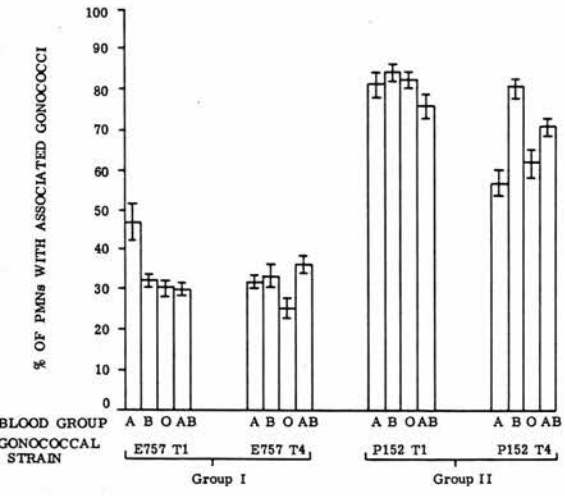
in the O individual. Figure 1 shows the effect of these arious sera on attachment and phagocytosis (i.e. ssociation) of various strains of gonococci at 37°C.

The figure shows that association was independent of e presence of the isohaemagglutinins; the variations bserved were dependent on strain differences. The rains included a laboratory strain (F62) subcultured for number of years that showed the highest degree of sociation; a freshly isolated clinical strain (M6967); nd two strains isolated from patients with disseminated onococcal infection (7422 and 7502).

ttachment to PMN of Strains with Different Lipopoly-ccharide Composition Determined by Pyocin Type

The strains used can be subdivided according to the omplexity of their LPS as defined by sensitivity to R-pe pyocins. Group I strains can be simply regarded as nsitive to the pyocins that attach to LPS structures earest the cytoplasmic membrane, whereas group II strains are sensitive to the same pyocins as group I and also to additional ones that attach to LPS components in positions more distal from the cytoplasmic membrane. A clinical isolate of group I (E757) and one of group II (P152) were examined for their ability to attach to PMN from individuals of blood groups A, B, O and AB. At 4°C and 37°C (data not shown) in the absence of homologous serum, the group II organisms with the more complex LPS associated with larger numbers of PMN than the group I organism (fig. 2). The same differences in association were apparent between the two groups when the bacteria were preincubated with homologous heat-inactivated serum (data not shown).

The numbers of gonococci associating with individual PMN were determined for organisms of both groups (figs. 3 and 4). More bacteria of group II than group I were



G. 2. Association of pilate and non-pilate variants with PMN from ifferent blood groups at 4°C in the absence of serum.

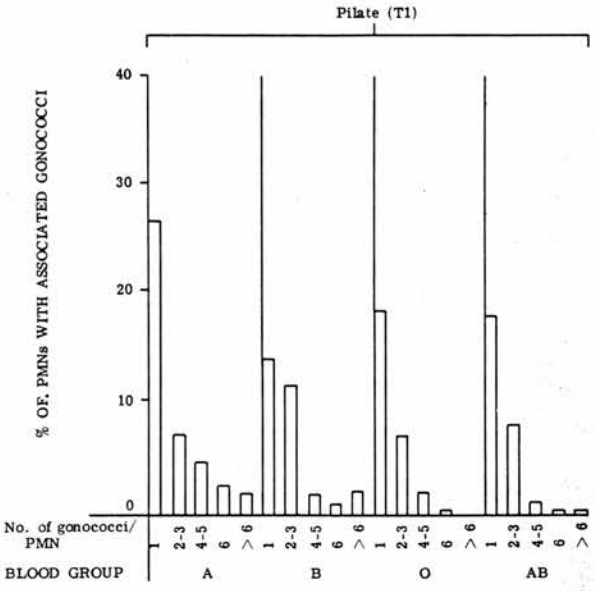


FIG. 3. Numbers of pilate gonococci of group I associated with individual PMN of different blood groups at 4°C in the absence of serum.

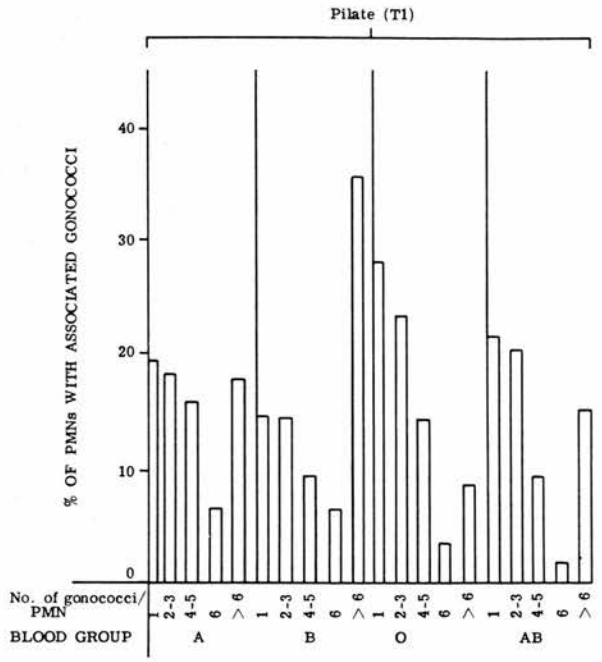


FIG. 4. Numbers of pilate gonococci of group II associated with individual PMN of different blood groups at 4°C in the absence of serum.

associated with individual PMN (fig. 3). Similar patterns were observed in the presence of homologous heat-inactivated serum at 4°C and 37°C, although fewer organisms of either group attached at 4°C.

Factors Affecting NBT Reduction

Pilate and non-pilate variants of group I and group II strains were assessed for their potential to stimulate NBT reductase in PMN. Two strains of group I (M9131 and E757) and 5 strains of group II (F62, M8865, M6967, E760 and 9) were used. No differences in the number of formazan-containing PMN were found in NBT tests with non-pilate variants of the two groups (table 2). In contrast, for each strain tested, the presence of pili resulted in a significantly lower number of PMN-containing formazan deposits compared to results obtained with its non-pilate variant (table 3).

Table 2 Stimulation of NBT reduction by non-pilate (T4) variants of gonococci of groups I and II (percentage PMN containing formazan)

Strain	*Number of experiments*	*Mean percentage PMN containing formazan (SE)*	
		Unstimulated (control)	*Stimulated (test)*
Group I			
M9131	3	3·1 (0·62)	8·87 (2·8)
E757	2	3·8 (0·55)	24·2 (4)
Group II			
F62	3	5·9 (1·1)	24·03 (2·11)
M6967	2	8·4 (2·9)	22·4 (0·5)
9	2	3·9 (1·5)	13·9 (2·1)
M8865	3	3·1 (0·62)	19·3 (2·1)
E760	5	6·0 (2·2)	27·6 (6·6)

Table 3 The effect of pilation on NBT reduction with gonococci of group I and group II (percentage PMN containing formazan)

Strain	*Number of experiments*	*Mean percentage PMN containing formazan (SE)*	
		Unstimulated (control)	*Stimulated (test)*
Group I			
E757 (T4) non-pilate	2	3·8 (0·55)	24·2 (4)*
E757 (T1) pilate	2	3·8 (0·55)	7 (4·5)†
Group II			
F62 (T4) non-pilate	1	10·6	25·9*
F62 (T1) pilate	1	10·6	10·6†
E760 (T4) non-pilate	5	6 (2·2)	27·6 (6·6)*
E760 (T1) pilate	5	6 (2·2)	11·3 (3)†

* $p < 0.001$.
† N.S.

DISCUSSION

We have tested the hypothesis proposed by Foster and Labrum (1) that the observed excess of blood group B individuals among patients with gonococcal infection may indicate that the anti-B isohaemagglutinin is a more effective opsonin than the anti-A isohaemagglutinin. We were unable to demonstrate any significant difference in the association of individual strains of *N. gonorrhoeae* opsonized with fresh sera from healthy A, B, O or AB individuals to PMN of the respective blood groups.

Our results indicate that differences in association of gonococcal strains with PMN from A, B, O or AB individuals was independent of the serum and paralleled differences in LPS composition as detected by the sensitivity of the strain to R-type pyocins of *P. aeruginosa* (10, 3, 11). The strain of group II gonococci with the more complex LPS was found to attach to a greater percentage of PMN than the strain of group I. The same pattern emerged when the numbers of gonococci per individual PMN were determined.

Differences in attachment of gonococci to PMN from donors of all four blood groups appears to depend on the LPS composition as determined by pyocin type, rather than the presence of pili or opsonization by anti-B antiserum.

In contrast to our observations on association with MN, we found no differences in the NBT test with MN when non-pilate strains of either group I or group were tested. Both groups were found to show an crease in number of formazan-containing PMN com-ared with the control to which no bacteria were added. esults for pilate variants of both groups were not signifi-antly different from the unstimulated controls.

Densen and Mandell (12) reported that in the presence f serum, pilate and non-pilate gonococci of their test rain F62 stimulated specific granule release measured y the appearance of lactoferrin in the medium, and that oth stimulated increases in oxidative metabolism. In ontrast, Kreiger, Schiller and Roberts (13) reported a inimal increase in glucose oxidation and oxygen con-umption of PMN to which pilate gonococci were ttached compared with t[illegible] marked increase found with on-pilate gonococci. It must be noted that the latter orkers were using a pilate strain and a non-pilate strain btained from two different patients with disseminated fections. Our results in studies with pilate and non-ilate variants of strain F62 and with isolates from calized genital infections support the findings of reiger *et al.* and not those of Densen and Mandell.

We conclude that in host parasite interactions between MN of the four different blood groups and *N. gonor-oeae*, the surface characteristics of the bacteria detected y the pyocin typing system are the major determinants f recognition. These surface characteristics appear to ave no effect on subsequent intracellular events that are nked with the NBT assay. Our findings support pre-ious reports (13, 14) that gonococcal cells with pili, cell rface components associated with virulence, provoke ss stimulation of intracellular bactericidal mechanisms human PMN than do non-pilate organisms. Although ntibodies against pilar proteins are produced during onococcal infection (15), electron microscopic studies of fected material have prompted debate about their hysical presence on the bacteria *in vivo* (16, 17). It is ossible, however, that proteases present in the tissue uids and released by phagocytes may remove these ructures.

The greater ability of group II gonococci with more omplex LPS to adhere to PMN would be expected to ct to the disadvantage of the organism in establishing fection. This would, however, be offset by any dvantage such organisms would have in attaching to roepithelial cells. Group II organisms are frequently ecovered from patients (3); thus, it will be necessary to etermine if the differences in attachment to PMN bserved for groups I and II are found also with roepithelial cells. Pili are already known to play a part mediating such attachment (18, 19) but, the relative ontributions of pili and LPS are not yet established.

The present report has concentrated on gonococcal-MN interactions. Novotny and colleagues (20) have proposed that gonorrhoea is a disease of human monocytes. Whether or not the presence of pili or differences in LPS have any significance as determinants of susceptibility in individuals of different blood groups has not yet been elucidated. As a result of the present study and our previous findings (4) that pili are an important determinant for attachment to monocytes, investigations are underway to examine various factors, of both host and parasite, affecting recognition of gonococcal strains by monocytes of different ABO blood groups.

ACKNOWLEDGEMENTS

We are grateful to Professor J. G. Collee for help in the preparation of the manuscript and to Mrs. Marilyn Cole for its typing. The work was supported by Grant No. K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department and by Grants G81/103811 SB and G979/64/S from the Medical Research Council.

REFERENCES

1. Foster, M. T. and Labrum, A. H. (1976). Relation of infection with *Neisseria gonorrhoeae* to blood groups. *J. Infect. Dis.*, **133**, 329.
2. Miler, J. J., Novotny, P., Walker, P. D., Harris, J. R. W. and MacLennan, I. P. B. (1977). *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect and Immun.*, **15**, 713.
3. Blackwell, C. C., Young, H. and Anderson, I. (1979). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing. *J. Med. Microbiol.*, **12**, 321.
4. Kinane, D. F., Blackwell, C. C., Weir, D. M. and Winstanley, F. P. (1982). Phagocyte recognition of *Neisseria gonorrhoeae*. *J. Clin. Lab. Immunol.*, **9**, 169.
5. Young, H., Patterson, I. C. and McDonald, D. R. (1976). Rapid carbohydrate utilization test for the identification of *Neissera gonorrhoeae*. *Brit. J. vener. Dis.*, **52**, 172.
6. Van Furth, R., Van Zwet, T. L. and Leijh, P. C. J. (1978). *In vitro* determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes. In *Handbook of Experimental Immunology*, (edited by D. M. Weir). Blackwell Scientific Publications, Oxford.
7. Kowolik, M. J. and Moody, G. H. (1979). A simplified technique for the NBT test. *IRCS Medical Science Cell and Molecular Biology*, **7**, 100.
8. Böyum, A. (1968). Isolation of leucocytes from human blood. Further observation. Methyl cellulose dextran and Ficoll as erythrocyte aggregating agents. *J. Clin. Lab. Invest.*, **21**, Suppl. 97, 31.
9. Boyle, W. (1968). An extension of the ^{51}Cr release assay for the estimation of mouse cytotoxins. *Transplantation*, **67**, 761.
10. Sidberry, H. D. and Sadoff, J. C. (1977). Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infect and Immun.*, **15**, 628.
11. Sadoff, J. C., Zollinger, W. D. and Sidberry, H. D. (1978). Cell surface structures of *Neisseria gonorrhoeae*. In *Immunobiology of* Neisseria gonorrhoeae, pp. 93–100. American Society for Microbiology, Washington D.C.
12. Densen, P. and Mandell, G. (1977). The role of microbial attachment and ingestion in polymorphoneutrophil function. *Clin. Res.*, **25**, 375A.
13. Krieger, A. G., Schiller, N. L. and Roberts, R. B. (1980). Gonococci—human polymorphonuclear leucocyte interactions: metabolic studies associated with attachment and ingestion. *Infect. and Immun.*, **8**, 991.

14. Densen, P. and Mandell, G. L. (1978). Gonococcal interaction with polymorphonuclear neutrophils—importance of phagosome for bactericidal activity. *J. Clin. Invest.*, **62**, 1161.
15. Tramont, E. C., Ciak, J., Boslego, J., McChesney, D. G., Brinton, C. C. and Zollinger, W. D. (1980). Antigenic specificity of a genital antibody response to the infecting strain of *Neisseria gonorrhoeae*. *J. Infect. Dis.*, **142**, 23.
16. Evans, B. A. (1977). Ultrastructural study of cervical gonorrhoea. *J. Infect. Dis.*, **136**, 248.
17. James, J. F. and Swanson, J. (1978). Piliation of gonococci *in vivo*. *J. Infect. Dis.*, **137**, 94.
18. Swanson, J., King, G. and Zeligs, B. (1975). Studies on gonococcus infection VIII. ^{125}Iodine labelling of gonococci and studies on their *in vitro* interactions with eukaryotic cells. *Infect. and Immun.*, **11**, 453.
19. Tramont, E. M. amd Wilson, C. (1977). Variations in buccal cell adhesion of *Neisseria gonorrhoeae*. *Infect. and Immun.*, **16**, 709.
20. Novotny, P., Short, J. A., Hughes, M., Miller, J. J., Syrett, C., Turner, W. H., Harris, J. R. W. and MacLennan, I. P. B. (1977). Studies on the mechanisms of pathogenicity of *Neisseria gonorrhoeae*. *J. Med. Microbiol.*, **10**, 347.

Teviot Scientific Publications Ltd
31 Montpelier Park
Edinburgh EH10 4LX, UK

Clin. Lab. Immunol. (1983), 11, 27–32

BLOOD GROUPS AND SUSCEPTIBILITY TO GONOCOCCAL INFECTION.
II. THE RELATIONSHIP OF LIPOPOLYSACCHARIDE TYPE TO GONOCOCCAL SENSITIVITY TO THE BACTERICIDAL ACTIVITY OF NORMAL HUMAN SERUM

F. P. WINSTANLEY, C. C. BLACKWELL, D. M. WEIR and D. F. KINANE
Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, UK
(Received 2 August 1982)

SUMMARY This study examines the bactericidal activity of normal human sera from individuals of blood groups A and B for gonococcal strains with simple and complex lipopolysaccharides (defined by pyocin-sensitivity) isolated from localised and disseminated infection. The bactericidal activity did not depend on A or B isohaemagglutinins. Resistance to normal human serum exhibited by strains from localised infections appeared to be due to lack of part of the lipopolysaccharide antigen, whereas resistance of strains from disseminated infection appeared to depend on a separate mechanism yet to be defined.

TRODUCTION

DIVIDUALS of blood group B are especially susceptible genito-urinary infections with *Escherichia coli* (1, 2) d *Neisseria gonorrhoeae* (3, 4, 5). There is evidence that e anti-B isohaemagglutinin found in individuals of ood groups A and O may play a role in host-protection ainst infection by gram-negative bacteria (6).

In this study we investigated the sensitivity of a number strains of *N. gonorrhoeae* with different lipopolysaccaride (LPS) to "normal human serum" (NHS) from dividuals of blood groups A and B to determine nether anti-B isohaemagglutinin is involved in a nonecific host defence mechanism against gonorrhoea.

Previous work from this laboratory has indicated that tachment of gonococcal strains to polymorphonuclear ukocytes (PMN) varies according to differences in the ructure of the LPS (7) as detected by differences in nsitivity to partially purified R-type pyocins of *Pseudonas aeruginosa* (8). Since LPS is one of the major antins involved in bactericidal killing (9, 10), strains of both the pyocin-defined groups (7) were tested to determine nether differences in LPS structure could be correlated th differences in sensitivity to the bactericidal effects of HS and conventional immune sera prepared in mice.

rrespondence: Dr. D. M. Weir, Department of Bacteriology iiversity of Edinburgh Medical School, Teviot Place, Edinburgh, I8 9AG.

MATERIALS AND METHODS

Bacterial Strains

Ten gonococcal strains from localised infections were supplied by the Department of Genito-urinary Medicine, Royal Infirmary of Edinburgh (R.I.E.). Three β-lactamase-producing strains were kindly supplied by Dr. K. Shannon and Professor I. Phillips, St. Thomas's Hospital Medical School, London, and two strains from disseminated infections were obtained from Dr. Joan S. Knapp, Neisseria Reference Laboratory, United States Public Health Service, Seattle, Washington. All strains, together with the grouping based on pyocin sensitivity (7) are listed in Table 1. They all conformed to the morphological and biochemical characteristics of *N. gonorrhoeae* and were of colony type 4 (11).

Culture

Strains were cultured on Modified New York City (MNYC) medium (12) at 37°C in a humidified incubator with 10% CO_2 in air.

Isolation of Pyocin-resistant Mutant

Colonies growing within a zone of inhibition produced by pyocin IS8 were isolated and re-typed. One mutant strain (P280μ), sensitive to Kageyama group R5 pyocins only, was used in the study.

Preparation of Immunising Antigens

An 18-hr growth of gonococci was harvested from MNYC plates with sterile cotton swabs and suspended in 0·01M phosphate-buffered saline (PBS) pH 7·2. An equal volume of PBS containing 2·4% formaldehyde was added to the suspension which was stored overnight at 4°C. The formaldehyde-treated organisms were centrifuged at 900 × g for 1 hr and resuspended in PBS containing 0·01% formaldehyde to the opacity of Brown's Tube No. 3 as judged by eye (13). This is equivalent to $1·1 \times 10^9$ gonococci/ml (14).

1–2760/83/0500–0027 $04.50

Table 1 Pyocin type of gonococcal strains

		Pyocins with Kageyama group												
		R1					*Unclassified*		*R3*	*R2*			*R5*	
	Gonococcal strain	*ISD*	*2285*	*IS4*	*IS6*	*IS8*	*R205*	*9579*	*ISE*	*ISB*	*R21*	*430*	*ISA*	*ISC*
Group I	M9131													
	E757													
	*849209													
	*845650													
	*934936	−	−	−	−	−	−	+	−	−	−	−	+	+
	P280μ													
	P3309													
	†7425													
Group II	M8865	+	±	+	+	+	+	+	−	−	−	−	+	+
	P280													
	E2590													
	E728													
	E759	+	+	+	+	+	+	+	−	−	−	−	+	+
	M5287													
	P3290													
	†1560													

\+ clear zone of inhibition.
± zone of partial inhibition.
− no inhibition.
* β-lactamase producing strains.
† strains from disseminated infection.

Immunisation Schedule

Female CF1 mice, 5–6 weeks old, caged in groups of 10, were given 0·25 ml of the standard suspension of formaldehyde-treated gonococci (gonococcal antigen) intravenously, via the lateral tail veins. The mice were immunised at day 0 and again on days 7 and 10. On day 14 a small quantity of blood was taken from the retro-orbital plexus of each mouse of the group and pooled; the serum obtained was inactivated by heating to 56°C for 30 min and then distributed into small tubes and stored at −20°C until required. The mice thereafter received booster doses (0·25 ml) of the gonococcal antigen at 14-day intervals and blood was obtained 5 days after each immunisation.

Complement

Human AB serum from a donor with no history of gonococcal infection was adsorbed for 24 hr at 4°C with a live suspension of the gonococci used in the study, distributed in 200 μl aliquots and stored at −70°C before use. The minimum haemolytic titre of the serum was 32–64.

Normal Human Sera from Individuals of Blood Groups A and B

Kindly supplied by Drs. S. Urbaniak and P. L. Yap of the Blood Transfusion Service, R.I.E.

Screening Test for Bactericidal Activity of Normal Human Serum

An 18-hr culture of gonococci was suspended in Dulbecco's Phosphate Buffered Saline supplemented with Mg^{++} and Ca^{++} ions (0·5 mM and 0·9 mM respectively) (DPBS + B) to give approximately 10^4 cfu/ml.

A series of two-fold dilutions, from 15 to 480, were made in a microtitre plate for each serum to be screened to give a final volume of 50 μl per dilution, 40 μl of gonococcal suspension and 10 μl of a two-fold dilution of the complement source were added to each well and incubated at 37°C for 30 min. Two drops of 20 μl from each well were plated onto MNYC medium and incubated for 24 hr. The highest serum dilution to give a reduction in viable count of ⩾ 80% was considered to be the bactericidal titre of the serum.

Bactericidal Assay with Immune Mouse Serum

These were performed in round-bottomed glass tubes (15 mm × 75 mm). An 18-hr culture of gonococci was suspended in D.PBS + B to give approximately 10^6 cfu/ml. The assay mixture contained 160 μl of bacterial suspension, 20 μl of a two-fold dilution of heat-inactivated mouse serum and 20 μl of freshly thawed AB serum as the complement source. Controls lacking serum and/or complement were included for each experiment. After static incubation at 37°C for 30 min in a humidified atmosphere with 10% CO_2 in air, 25 μl volumes were removed from each tube and viable counts were performed (15). A reduction in viable count of 80% or greater when compared with controls was considered to be significant.

Results

The gonococcal strains were all typed by pyocin sensitivity and classified into two broad groups: Group I strains were sensitive to Kageyama group R5 pyocine; Group II strains were sensitive to Kageyama groups R5 and R1 pyocins (table 1).

The AB serum used as the exogenous complement source for the bactericidal reactions was found to have slight microbicidal activity against strains of Group II. This activity was removed by absorption at 4°C with the sensitive strains and the procedure had no effect on the complement titres. Absorbed AB serum was used in all the bactericidal tests.

able 2 Bactericidal titre of normal human serum from Group A individuals

	Test strain of N. gonorrhoeae	Sera A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Group I	M9131, E757, 849209, P3309, 934936, P280μ, *7425	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<
Group II	M8865	15	240	60	60	60	30	120	120	30	120	120	60	30	30	15	60
	P280	>	>	>	>	>	>	240	240	240	60	120	30	120	120	30	120
	E2590	<	<	60	30	<	30	<	<	30	30	120	60	60	60	15	60
	P3290	15	15	30	30	30	30	15	60	120	120	15	15	15	60	30	120
	*1560	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<

< less than 15.
> greater than 480.
* strains from disseminated infection.

Normal human sera from individuals of blood groups
and B were found to be bactericidal to gonococcal
rains of Group II to a titre of ⩾ 15, but no activity
owards Group I strains from localised infections or a
isseminated infection could be demonstrated at the
owest serum dilution of 15. The titre of bactericidal anti-
ody against strains of Group II was slightly greater in
ndividuals of blood group A (tables 2 and 3). No bacteri-
idal activity could be demonstrated towards strain 1560
Group II) from a disseminated infection (tables 2
nd 3).

This bactericidal activity of NHS towards strains of
Group II is inhibited by 10 mM Mg-EGTA which
equesters Ca^{++} ions necessary for the classical pathway
f complement activation.

There was no discrimination between strains of Group I and Group II by the microbicidal mechanism in immune mouse serum. Serum raised against a strain from Group I (M9131) killed other strains in Group I and also strains in Group II. This same lack of discrimination was shown by antiserum raised against a strain from Group II (M8865) (table 4). Similar experiments with two gonococcal strains from disseminated infections (1560 and 7425) showed no detectable microbicidal activity.

One serum from each of the blood groups A and B was absorbed twice for 24 hr at 4°C with a heavy suspension of erythrocytes expressing A or B antigens to remove the iso-haemagglutinins. This absorption did not remove any bactericidal activity from the sera. Absorption with a heat-killed suspension of *P. aeruginosa* strain ZD8 slightly reduced the bactericidal activity of the sera (table 5).

able 3 Bactericidal titre of normal human serum from Group B individuals

	Test strain of N. gonorrhoeae	Sera i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii	xiv	xv	xvi
Group I	M9131, E757, 849209, P3309, 934936, P280μ, *7245	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<
Group II	M8865	15	30	30	30	>	15	120	30	15	15	<	30	<	15	30	15
	P280	<	15	<	15	240	15	15	30	120	60	60	60	240	60	120	60
	E2590	<	<	<	15	60	<	<	<	30	30	30	60	30	60	30	30
	P3290	<	<	<	30	60	<	120	60	120	30	30	15	15	120	30	60
	*1560	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<	<

< titre less than 15.
> titre greater than 480.
* strains from disseminated infection.

Table 4 Microbicidal action of immune mouse serum. Number of tests out of 4 giving ⩾ 80% kill

	Gonococcal strains	Antiserum	
		M9131 (group I)	*M8865 (group II)*
Group I	M9131	3	2
	E757	1	2
	849209	3	4
	845650	4	4
	934936	2	3
	P280μ	0	3
Group II	M8865	4	4
	M5287	4	4
	E728	3	2
	E759	2	2
	P280	1	4

Discussion

Our findings suggest that the increased susceptibility of individuals of blood group B to infection by *N. gonorrhoeae* is not related to differences in susceptibility of the organism to isohaemagglutinins. Although *N. gonorrhoeae* isolates have been shown to absorb anti-A and anti-B isohaemagglutinins (4) the bactericidal activity of normal human serum (NHS) could not be removed by absorbtion with red blood cells expressing A and B antigens.

Variations in the susceptibility of gonococci to the bactericidal effects of NHS have been reported (9, 16, 17). Although the LPS has been found to be the target antigen for "natural" antibody (16, 18), the particular components involved in the reaction had not been identified. Classification of gonococcal isolates by the sensitivity pattern to partially-purified R-type pyocins of *P. aeruginosa* has provided a method for detecting differences in LPS antigens. Clinical isolates of Group I which appear to have a simpler LPS structure are resistant to NHS while those with more complex LPS (Group II) are sensitive. The importance of LPS components in determining the reaction with NHS is illustrated by the change from sensitivity to resistance found in the mutant P280μ (Group I) derived from a NHS-sensitive clinical isolate, P280 (Group II). Alterations in the LPS associated with this mutation was shown by gel-electrophoresis and gas-liquid chromatography (unpublished data). These findings are similar to those of Morse and Apicella (19) for their gonococcal LPS mutant selected for resistance to pyocin 611–131.

Table 6 Relative positions of pyocin receptor sites on lipopolysaccharide (LPS) of *Pseudomonas aeruginosa*

*R3**	*R4**	*R2**	*R1**	*R5**
I	H	D	K	A
		F	L	B
		J	M	C
		U	N	E
			O	G
			P	
			Q	
(Sidberry and Sadoff 1977)			R	
			S	
			T	
ISE	—	ISB	ISD	ISC
		R21	IS4	ISA
		430	IS6	
			IS8	
(This study)			2285	

* Arranged in order of the receptor sites on the lipopolysaccharide (LPS) fraction of *P. aeruginosa* suggested by Kageyama (1975).
The receptor for R5 is nearest to, and that for R3 furthest from the cytoplasm.

A schematic representation of the relative positions of pyocin receptors on the LPS of *P. aeruginosa* proposed by (20) is shown for our range of pyocins and those used by Sidberry and Sadoff (21) in Table 6. The "natural" antibody of NHS appears to be directed against either: (1) the LPS structure(s) that form the receptor site(s) for pyocins of Kageyama's group R1; or (2) to one or more structures distal, but linked, to the receptor site for the R1 pyocins. Evidence for this second proposal comes from the work of Sadoff and co-workers (22). Gonococcal isolates were typed with pyocins from their collection and they found that the growth of serum-sensitive strains was inhibited by pyocins D, H and I. These three pyocins belong to Kageyama groups R2, R4 and R3 respectively which attach to LPS receptors distal to those for R1 (table 6). In contrast, our pyocins of these three groups

Table 5 Titres of bactericidal antibody before and after absorption

	N. gonorrhoeae strain	*Before absorbtion*	*Absorbed with erythrocytes*	*Absorbed with* P. aeruginosa
Group A serum (K)	P280	120	120	60
	M8865	120	120	60
	E2590	30	30–60	15
Group B serum (xi)	P280	120	120	15
	M8865	15	<	15
	E2590	15	15	<

activity against any of the gonococcal isolates (8).

putative "natural" antibody responsible for the icidal activity of NHS differs in specificity com- with conventional immune sera raised in mice. the "natural" antibody recognises determinants only on organisms expressing the more complex he immune sera had a wider specificity and were cidal for strains of both Group I and Group II.

ious work has suggested that resistance of occi to NHS is not due to the presence of masked ns, blocking IgA antibodies, or deficiencies in com- t function, but to the resistant organism's lack of S determinant that binds the "natural" antibody ur findings support this view. The bactericidal acti- f NHS towards strains of Group II could be ed in part by a heat-killed suspension of *P. aosa* strain ZD8, suggesting that the activity may e to "natural" antibody induced by commensal

implication of our findings is that the more NHS- ve Group II organisms should be restricted to ed infections and that Group I should predominate eminated infections. In an earlier study on pyocin f gonococci from a number of geographic sources (86·4%) of 110 isolates from localised infections f Group II compared with only 15 (13·6%) of I. In contrast, of the 24 isolates from disseminated ons, a higher percentage of Group I organisms %) were found. The proportion of isolates of Group 58·3%. Since organisms of Group II have been d from disseminated infections, simplicity of LPS ot appear to be the sole factor in determining the teristics of a "disseminated" strain.

roup II isolate from a disseminated infection was nt to immune mouse serum in addition to NHS is finding is the subject of further studies. This ts that loss or masking of antigens can occur t interfering with the receptor sites for the R-type s. This resistance to bactericidal antibody of a from disseminated infections was not found to be ated with anti-complementary activity of the sm.

conclude that the bactericidal effect of NHS on of *N. gonorrhoeae* is mediated by antibodies that a LPS portion associated with the binding site of ocins present in gonococci of Group II. Strains g this portion of LPS—(Group I)—are resistant to Thus "natural immunity" to gonorrhoea appears lirected at organisms of Group II.

basis for the increased susceptibility of blood B individuals to gonorrhoea is still not established ems not to be due to isohaemagglutinins. There is ce in *E. coli* urinary tract infections that the anti-B are raised during infection with organisms sing B-like antigens. This rise did not occur with anti-A isohaemagglutinins in response to A-like organisms (24). If this difference is reflected in other "natural antibody" responses of B individuals a possible basis for increased susceptibility to infection arises.

The sharing of antigens (galactose determinants) between the LPS of gonococci and blood group B antigen could also account for a diminished response in individuals of blood group B.

ACKNOWLEDGEMENTS

We are most grateful to Professor J. G. Collee for his advice in preparation of the manuscript and to Mrs. M. Cole and Mrs. N. Marr for its typing. This investigation was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department, and by grants G81/103811/SB and G979/63/S from The Medical Research Council.

REFERENCES

1. Cruz-Coke, R., Paredes, L. and Montenegro, A. (1965). Blood groups and urinary microorganisms. *J. Med. Genet.*, **2**, 185.
2. Kinane, D. F., Blackwell, C. C., Brettle, R. P., Weir, D. M., Winstanley, F. P. and Elton, R. H. (1982). ABO blood group, secretor state and susceptibility to recurrent urinary tract infection in women. *Br. Med. J.*, **285**, 3.
3. Foster, M. T. and Labrum, A. H. (1976). Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. *J. Inf. Dis.*, **133**, 329.
4. Miler, J. J., Novotny, P., Walker, P. D., Harris, J. R. W. and MacLennan, I. P. B. (1977). *Neisseria gonorrhoeae* and ABO isohaemagglutinins. *Infect. Immun.*, **15**, 713.
5. Kinane, D. F., Blackwell, C. C., Winstanley, F. P. and Weir, D. M. Blood group, secretor status and susceptibility to infection by *Neisseria gonorrhoeae*. *Br. J. Vener. Dis.* (in press).
6. Springer, G. F., Williamson, P. and Brindes, W. C. (1961). Blood group activity of gram-negative bacteria. *J. exp. Med.*, **113**, 1077.
7. Blackwell, C. C., Kowolik, M., Winstanley, F. P., Kinane, D. F., Weir, D. M., Law, J. A. and Ho Wang Chok, Y.-L. (1982). Blood group and susceptibility to gonococcal infection. I. Factors affecting phagocytosis of *Neisseria gonorrhoeae*, *J. Clin. Lab. Immunol.* (in press).
8. Blackwell, C. C., Young, H. and Anderson, I. (1978). Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocins and a possible approach to epidemiological typing. *J. Med. Microbiol.*, **12**, 321.
9. Glynn, A. A. and Ward, M. E. (1970). Nature and heterogeneity of the antigens of *Neisseria gonorrhoeae* involved in the serum bactericidal reaction. *Infect. Immun.*, **2**, 162.
10. Ward, M. E., Lambden, P. R., Heckels, J. E. and Watt, P. J. (1978). The surface properties of *Neisseria gonorrhoeae*: Determinants of susceptibility to antibody-complement killing. *J. Gen. Microbiol.*, **108**, 205.
11. Kellog, D. S. Jr., Peacock, W. L. Jr., Deacon, W. E., Brown, L. and Pirkle, C. I. (1963). *Neisseria gonorrhoeae* I. Virulence genetically linked to clonal variation. *J. Bacteriol.*, **85**, 1274.
12. Young, H. (1978). Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br. J. Ven. Dis.*, **54**, 36.
13. Brown, H. C. (1919–1920). Further observations on the standardisation of bacterial suspensions. *Ind. J. Med. Res.*, **7**, 238.
14. Cunningham, J. and Timothy, B. (1924). A comparison between the

numerical content of certain bacterial suspensions obtained by the haemocytometer method and Brown's opacity tubes. *Ind. J. Med. Res.*, **11**, 1253.
15. Miles, A. A., Misra, S. S. and Irwin, J. O. (1938). The estimation of the bactericidal power of the blood. *J. Hyg. Camb.*, **38**, 732.
16. Tramont, E. C., Sadoff, J. C. and Wilson, C. (1977). Variability of the lytic susceptibility of *Neisseria gonorrhoeae* to human sera. *J. Immunol.*, **118**, 1843.
17. Rice, P. A., McCormack, W. M. and Kasper, D. L. (1980). Natural serum bactericidal activity against *Neisseria gonorrhoeae* isolates from disseminated, locally invasive and uncomplicated disease. *J. Immunol.*, **124**, 2105.
18. Ward, M. E. and Glynn, A. A. (1971). Antibodies to gonococcal lipopolysaccharides in patients with gonorrhoea. *J. Med. Microbiol.*, **4**, p. ii.
19. Morse, S. A. and Apicella, M. (1982). Isolation of a lipopolysaccharide mutant of *Neisseria gonorrhoeae*—an analysis of the antigenic and biologic differences. *J. Inf. Dis.*, **145**, 206.
20. Kageyama, M. (1975). Bacteriocins and Bacteriophages in *Pseudomonas aeruginosa*. In *Microbial Drug Resistance: Report of the International Symposium on Bacterial Resistance* (edited by S. Mitsuhasi and H. Hashimoto), p. 291. University of Tokyo Press.
21. Sidberry, H. D. and Sadoff, J. C. (1977). Pyocin sensitivity of *Neisseria gonorrhoeae* and its feasibility as an epidemiological tool. *Infect. Immun.*, **15**, 628.
22. Sadoff, J. C., Zollinger, W. D. and Sidberry, H. (1978). Cell surface structures of *Neisseria gonorrhoeae*. In *Immunobiology of* Neisseria gonorrhoeae (edited by G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer and F. E. Young), p. 93. American Society for Microbiology, Washington D.C.
23. Schneider, H., Griffiss, J. McC., Williams, G. D. and Pier, G. B. (1982). Immunological basis of serum resistance of *Neisseria gonorrhoeae*. *J. Gen. Microbiol.*, **128**, 13.
24. Drach, G. W., Reed, W. P. and Williams, R. C. (1971). Antigens common to human and bacterial cells: urinary tract pathogens. *J. Lab. Clin. Med.*, **78**, 725.

Teviot Scientific Publications Ltd
31 Montpelier Park
Edinburgh EH10 4LX, UK