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The elusive determinants of bacterial interference with non-specific host defences

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The identification of the determinants of bacterial interference with non-specific host defences during the early stages of infection is approached rather than attained. Recognizing a relevant biological property (e.g. resistance to phagocytosis) by an *in vitro* test and associating bacterial surface components with it are relatively easy. Proving causation, however, is usually not completed because the biological test is complex and the surface component(s) act only *in situ*. Nevertheless, evidence in addition to mere association can be sought to show that a putative determinant is strongly implicated in biological activity. Even then, proving that the biological activity concerned is relevant to infection *in vivo*, and that the putative determinant is produced there, is often not accomplished. Again, however, distinction can be made between those cases probably relevant *in vivo* and those only possibly so. Finally, bacteria grown *in vitro* can be deficient in some of the determinants of pathogenicity expressed during infection, and this situation requires the study of organisms grown *in vivo*. These points are discussed and then illustrated in a brief survey of the activities of many pathogenic bacteria and a description of recent work on the resistance of gonococci to killing by human serum and phagocytes.

Introduction

Bacterial interference with non-specific host defences has received much attention (Smith 1976; Schlessinger 1979; Densen & Mandell 1980; O'Grady & Smith 1981; Falconi *et al.* 1983; Easmon *et al.* 1983). I cannot add to this up-to-date information but only summarize it and emphasize important points. These publications should be consulted for detail and original references; most of the latter I cannot quote because of lack of space and I apologize to their authors. I confine my attention to interference with bactericidins and phagocytes that bacteria meet in the early stages of infection, to some extent on mucous surfaces but more intensely when they enter the tissues and spread in the lymph and blood. Breaching the skin and counteracting non-specific mucosal defences, such as moving mucus or lumen contents and the activity of commensal flora, have been covered elsewhere (Freter 1980; Smith 1982). Similarly, overcoming nutritional or environmental restriction of metabolism, such as iron limitation for most bacterial pathogens and high oxygen tension for anaerobes, has received sufficient attention (Braude 1981; Griffiths, this symposium). I have divided the paper into three parts. First, I shall describe the difficulties that beset attempts to identify the determinants of bacterial interference with host defence. Second, I shall survey briefly what we know in this area for all pathogenic bacteria. Finally, I shall describe recent work on the resistance of gonococci to killing by human serum and human phagocytes.

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Difficulties in attempts to identify the determinants of bacterial interference with non-specific host defences

The first step of research in this area should be to observe the early stages of infection in animals (see later) and identify an aspect of host defence, for example ingestion by phagocytes, that appears to be counteracted by the chosen bacterial species *in vivo*. Then, by using a relevant biological test *in vitro*, virulent strains can be examined, preferably in comparison with avirulent strains, for their ability to resist the selected aspect of host defence. Sometimes the crucial preliminary observations on behaviour *in vivo* are made but often a biological test is chosen, for example resistance to killing by serum, whose relevance *in vivo* is possible but has not been investigated.

It is usually easy to demonstrate *in vitro* an ability of virulent strains to interfere with a selected aspect of host defence, for example, resistance to ingestion by phagocytes. Also, the first step in identifying the determinant, proving an association between one or more surface components and biological activity, does not usually present problems. Active and less active strains can be examined for differences in capsulation (by morphological and chemical means), outer membrane proteins, lipopolysaccharides (LPS) and other surface components. Removal or destruction of selected components by chemicals or enzymes can sometimes be related to loss of biological activity. Both biological activity and certain surface components might be transferred from one strain to another by plasmids or other genetic manipulations.

The difficulty, often insurmountable, comes with trying to prove causation. Ideally, the putative determinant(s) should be extracted, purified and used to confer biological activity on to either the organism for which it was removed or an avirulent strain lacking it. Sometimes activity is conferred by treating the test bacteria with the purified component or by including it in the biological test. Capsular poly-D-glutamic acid extracted from resistant, virulent *Bacillus anthracis* prevented killing of susceptible organisms by serum and their ingestion by phagocytes (Keppie *et al.* 1963). Similarly, complete (i.e. with a full polysaccharide side chain) LPS extracted from serum resistant *Escherichia coli* attached to the surface and prevented killing of otherwise sensitive, LPS-deficient, homologous organisms harvested from early exponential growth (Allen & Scott 1981). Often, however, this method fails, probably because reattachment to the bacterial surface, essential for biological activity, is not achieved under the test conditions. An association between a surface antigen of *Brucella abortus* and resistance to killing by bovine phagocytes was established by three findings: virulent strains resisted intracellular killing more than attenuated strains; phagocytosed cell walls from virulent organisms inhibited intracellular killing of attenuated strains; and removal of the antigen from the surface of virulent organisms decreased their resistance to killing by phagocytes. However, attempts to promote the intracellular survival of an attenuated strain by treatment with solutions of the antigen failed (Frost *et al.* 1972). Similarly, capsular carbohydrates from pneumococci, which have been strongly associated with resistance to phagocytosis (Densen & Mandell 1980; Quie *et al.* 1981), were unable to confer resistance on susceptible organisms (Dhingra *et al.* 1979). The requirement for reattachment to the bacterial surface to restore biological activity is not required by pathogens that are dependent on extracellular products, for example toxins; consequently these determinants of biological activity are more easily identified.

Even if resistance to a defence system is conferred by treatment with the isolated putative determinant, the result should be interpreted with caution (Wilton 1981). For example,

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protein A is a surface component of staphylococci that *in situ* appears to confer resistance to phagocytosis when 'natural' antibody is present by combining with the latter's Fc regions, thereby preventing opsonization (Quie *et al.* 1981). When isolated, it conferred resistance to phagocytosis on susceptible strains in tests *in vitro* but not by combining with antibody; it appeared to activate complement extracellularly, thereby using the complement that would otherwise have opsonized the susceptible strains (Densen & Mandell 1980; Quie *et al.* 1981). A similar explanation could have accounted for resistance to killing by human serum by otherwise susceptible *E. coli* after treatment with isolated complete LPS from resistant organisms (see above). The lipid A and core of the latter could have activated complement extracellularly (Penn 1983), leaving insufficient for activation by the target site on the susceptible strain. However, control experiments showed that the added LPS attached to the surface of the susceptible organisms and complement was not activated (Allan & Scott 1981). Furthermore, the evidence for the complete LPS being the specific resistance determinant was strengthened by the fact that LPS from three different virulent enterobacteria did not protect the susceptible organisms from serum killing (Allan & Scott 1981).

Modern genetic manipulations provide a powerful new approach to proving causation for a putative determinant of interference with host defence. Isogenic strains that differ only in the gene that codes for this determinant can be compared in the appropriate biological test. For example, a comparison of genetically manipulated strains of *Salmonella typhimurium*, in tests for resistance to phagocytosis and activation of complement, provided evidence that the resistance determinant was a complete LPS-containing abequose and other sugars in its side chain (Liang-Takasaki *et al.* 1982). The two strains must differ only by the DNA segment in question and, to be certain of this, critical genetic analysis is required (Sparling 1979) of both plasmid and genomic DNA. Even if this analysis is forthcoming, pleiotropy may occur; the gene product itself may not be the determinant but may influence the production of other cell constituents, one or more of which is the true determinant. Hence the genetic approach must be applied correctly to be effective and is not completely certain.

Other ways of strengthening the evidence for causation are as follows. Antibody specific to a purified determinant may neutralize the biological activity of intact virulent organisms, for example the opsonization and promotion of phagocytosis of otherwise resistant encapsulated organisms by antibodies to capsular materials (Quie *et al.* 1981). The specificity of the antibody is crucial and, unfortunately, antisera raised by conventional means against purified bacterial components often contain antibodies to minor contaminants. Monoclonal antibodies have an obvious role in future work. Neutralization by specific antibody is not foolproof; for example, antibody to a surface component different from the real determinant of resistance to phagocytosis might opsonize the bacteria sufficiently for ingestion to take place.

If an identified surface component can be measured either chemically or serologically, then examination of many strains, or the same strain grown under different conditions, may show a good correlation between the amount of the component and degree of biological activity, for example between the amount of K polysaccharide antigens of *E. coli* and resistance to complement-mediated killing by serum (Glynn 1972; Taylor 1978; Penn 1983). Such evidence is still that of association but the quantitative aspect strengthens the possibility of causation.

The case for a surface component as the determinant of biological activity is strengthened if the activity is abolished by specific chemical change of the component *in situ*. For example, the inability of group B streptococci to inactivate complement, and their corresponding

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resistance to phagocytosis, were abolished by changing the tertiary structure of the capsular polysaccharide through reduction of the carboxyl group of the terminal sialic acid and by treatment with neuraminidase (Edwards *et al.* 1982). Formalin-killed organisms were used in this work, but similar experiments might be conducted on living bacteria if sufficiently mild aqueous chemical reactants were available.

In addition to the uncertainty of causation in a particular test *in vitro*, sooner or later the question of relevance *in vivo* must be faced. Ideally, the significance to infection in animals of resistance to the particular aspect of host defence should have been assessed at the beginning of the research (see above), but often this is not so. In addition to filling the possible gap, the production *in vivo* of the putative determinant of the biological activity *in vitro* should be checked. The relevance of the selected biological property *in vivo* can be indicated by studying the behaviour of virulent and attenuated strains in the early stages of infection of the skin, peritoneal cavity, mucous surfaces and tissue chambers by methods described elsewhere (Smith 1964). Are differences seen between virulent and avirulent organisms? For instance, are large numbers of virulent bacteria seen extracellularly, indicating resistance to humoral bactericidins and ingestion by phagocytes? Are bacteria seen within phagocytes, suggesting possible resistance to intracellular killing? Are few phagocytes present, pointing to a lack of stimulation or suppression of the inflammatory response? Are phagocytes being destroyed, suggesting the production of a toxin? The presence of the putative determinant *in vivo* can be established by biochemical and antigenic analysis of bacteria isolated directly from experimental animals (Smith 1964) and by serological (especially by fluorescent antibody methods) examination of tissue samples and sera for the determinant or its antibody. If vaccination with the putative determinant protects against infection, for example with pneumococcal or meningococcal capsular polysaccharides, this indicates that the determinant is produced during infection and is important in the primary invasive stage of the disease when non-specific defences are overcome unless strengthened by prior immunization. However, it does not necessarily mean that the determinant promotes (or its antibody neutralizes) resistance to the particular aspect of host defence studied *in vitro* because some surface components can inhibit more than one aspect of host defences that operate *in vivo*. Also lack of protection after inoculating the putative determinant does not mean that it is irrelevant to infection because some virulence determinants are not antigenic (Smith 1983).

The final uncertainty as regards relevance *in vivo* is that the important determinant of interference with a particular aspect of host defence may be expressed by bacteria only under the conditions of growth *in vivo*; owing to selection and phenotypic change, organisms grown *in vitro* may be deficient in this and other determinants of interference with host defence (Smith 1964, 1980). This problem should be countered by examining organisms grown *in vivo* without subculture in the appropriate biological tests; two examples of this approach are included in the last section of this paper.

The theme of the above discussion applies to all experimental biology, but emphasizing it in relation to pathogenicity, which involves complex interactions between different organisms, seems important to me. Even for interference with a single facet of host defence tested *in vitro*, complete evidence that a particular surface component is the determinant concerned is hardly ever obtained. In most cases the best that can be done is to improve on mere association and provide evidence for *strong implication* by one or more of the methods described above, i.e. isolation and biological characterization of the putative determinant; comparison of genetically

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manipulated and analysed isogenic strains; observation of the effect of specific antibody; quantitatively relating the putative determinant with biological activity; and modification of the biological effect by specific chemical changes. Similarly, proof of relevance *in vivo* is often difficult; however, we can recognize those cases that are *possibly relevant* but insufficiently examined and those that are *probably relevant* from experiments conducted so far. In the survey below I shall judge for each determinant whether the evidence indicates association with, or strong implication in, a particular facet of resistance to host defence. Also, I shall consider possible and probable relevance *in vivo*. This categorization will be subjective and sometimes wrong because of borderline cases and ignorance of pertinent facts. The objective, however, is not to decide each case correctly but to emphasize the fact that proving causation *in vivo* is difficult and we should be aware of the level of evidence obtained.

A survey of bacterial interference with non-specific host defences

(*a*) *Interference with humoral bactericidins*

For Gram-negative organisms the major humoral bactericidins are the later components of the complement cascade (see later) and, for Gram-positive organisms, lysozyme, β lysins and basic peptides (Smith 1976; Penn 1983).

In tests with sera of various animals where the actual bactericidin antagonized is often not clear (although in many cases heat lability indicates complement involvement), virulent strains of many bacterial species resist killing more than avirulent strains: *B. anthracis*, *Staphylococcus aureus*, *E. coli*, *Salmonella* spp., *Leptospira* spp., *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Br. abortus* (Smith 1976; Braude 1981; Penn 1983).

In some cases surface components have been associated with resistance to killing and are possibly relevant *in vivo*: the outer membrane proteins coded by the *tra T* gene of a drug-resistance plasmid in genetically manipulated rough laboratory strains of *E. coli*; complete LPS of *Salmonella* spp. and *Shigella sonnei*; capsular materials of type b *Haemophilus influenzae*; and a surface protein and LPS of *Aeromonas salmonicida* (Braude 1981; Madonna & Allen 1981; Easmon 1983; Penn 1983).

In other cases putative determinants have been strongly implicated in resistance to killing and are probably relevant *in vivo*: the capsular poly-D-glutamic acid of *B. anthracis*; the K polysaccharides of encapsulated *E. coli* and the complete LPS of other smooth virulent *E. coli*; a complex (lipid, protein, carbohydrate) surface antigen of *Br. abortus*; and capsular materials of meningococci (Keppie *et al.* 1963; Glynn 1972; Tee & Scott 1980; Allan & Scott 1981; Smith 1977; Penn 1983).

(*b*) *Interference with the phagocytic defences*

Inhibitors of all four stages of the phagocytic defence system, i.e. mobilization by inflammation, chemotaxis, attachment and ingestion, and intracellular killing will be considered first, followed by discussion of toxins that either kill phagocytes or, at lower concentrations, inhibit their activities.

Staphylococci produce an anti-inflammatory, cell-wall peptidoglycan that is relevant to infection in mice (Glynn 1972; Easmon 1983). *Treponema pallidum* may have an envelope associated with lack of stimulation of the inflammatory response that is possibly relevant to infection in rabbits and man (Penn 1983).

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Some virulent bacteria (*Salm. typhi*, meningococci, gonococci from disseminated infection, *Pseudomonas aeruginosa*, *Serratia* spp. and *Capnocytophaga* spp.) inhibit chemotaxis of phagocytes *in vitro* (Densen & Mandell 1980; Wilton 1981). Also, while many bacterial products stimulate chemotaxis *in vitro*, others are inhibitory, for example cord factor of *Mycobacterium tuberculosis*, a second peptidoglycan from staphylococci and various toxins (see later) (Densen & Mandell 1980; Wilton 1981). This inhibition of chemotaxis has yet to be proved to be relevant *in vivo*.

Many putative determinants have been strongly implicated in the resistance of bacteria to attachment and ingestion by phagocytes and are probably relevant *in vivo*: capsular polysaccharides of some pneumococci, the M protein of *Streptococcus pyogenes*, the Vi antigen of *Salm. typhi*, the capsular poly-D-glutamic acid of *B. anthracis*; fraction I of *Yersina pestis*; protein A of staphylococci; K polysaccharide antigens of encapsulated *E. coli* including K1 types and the complete LPS of other smooth virulent unencapsulated *E. coli*; complete LPS of *Salm. typhimurium*; acid polysaccharides of encapsulated staphylococci; a glycolipoprotein from the surface slime of *Ps. aeruginosa*; the capsular polysaccharide of *H. influenzae* type b; and the capsular material of *Bacteroides fragilis* (Smith 1976, 1977; Denson & Mandell 1980; Bartell & Krikszens 1980; Bortolussi & Ferrieri 1980; Wilton 1981; Quie *et al.* 1981; Hunter *et al.* 1982; Easmon 1983; Penn 1983; Stendahl 1983). As yet the capsular materials of the following organisms have only been associated with resistance to attachment and ingestion *in vitro*, although more investigation would show them strongly implicated and probably relevant to infection *in vivo*: meningococci, type B streptococci, *Klebsiella pneumoniae*, *Pasteurella multocida* and *Campylobacter fetus* (Densen & Mandell 1980; Robbins *et al.* 1980; Wilton 1981; Quie *et al.* 1981; Easmon 1983; Penn 1983). In some cases inhibition of ingestion has been distinguished from inhibition of attachment; the pili of gonococci are strongly implicated in attachment to phagocytes and resistance to ingestion (Densen & Mandell 1980; Jones *et al.* 1980; Siegel *et al.* 1982) and pilated *E. coli* with complete LPS or K capsular antigens (or both) attach to phagocytes but are not ingested (Stendahl 1983). As regards mechanisms of inhibition of attachment and ingestion, masking of specific interactions between opsonins and bacterial cell-wall components appear most important (see next section), but non-specific increase of surface hydrophilicity also appears to play a role, for example in the action of the complete LPS of *Salm. typhimurium* and *E. coli* (Stendahl 1983).

Several bacterial species resist intracellular killing by phagocytes and this resistance is probably relevant *in vivo*, especially to bacterial survival in chronic disease (Draper 1981; Smith 1983). In some cases, the bacteria prevent phago-lysosome fusion (*Myc. tuberculosis*, *Mycobacterium microti*, *Nocardia asteroides*); in others they resist intraphagosome killing (*Myc. tuberculosis* after treatment with antibody: *Mycobacterium lepraemurium*, *Listeria monocytogenes*, *Salm. typhimurium*, *Legionella pneumophila* and some staphylococci); in others the ingested organisms escape from the phagosome (*Mycobacterium leprae*); and in others the mechanism is not yet known (*Br. abortus*) (Densen & Mandell 1980; Draper 1981; Smith 1983). Putative determinants have been associated with these mechanisms in certain pathogens: (*a*) prevention of phago-lysosome fusion by *Myc. tuberculosis* has been associated with surface sulphatides (glycolipid sulphates), cell-wall polyglutamic acid and ammonia production; (*b*) resistance to intra-phagosome killing of *Myc. lepraemurium*, *Myc. tuberculosis*, staphylococci and *Salm. typhimurium* has been associated respectively with mycoside C (a peptidoglycolipid), catalase and possibly a compound like mycoside C, catalase and complete LPS; (*c*) the unknown resistance mechanism of *Br. abortus* has been attributed to a surface antigen (Smith 1976, 1983;

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Densen & Mandell 1980; Draper 1981). Some of these putative determinants are possibly relevant *in vivo*.

The following extracellular toxins of bacteria kill phagocytes *in vitro*: staphylococcal α toxin, β toxin and leucocidin, the streptolysins, the α and θ toxins of *Clostridium perfringens* and leucocidins of *Ps. aeruginosa*, *Actinobacillus actinomycetemcomitans*, *Fusiformis necrophorum* and *Pasteurella haemolytica* (Densen & Mandell 1980; Wilton 1981; Arbuthnott 1981; Scarlan *et al.* 1982; Hemmel *et al.* 1982). In small quantities several of these toxins and the enterotoxins of *E. coli* and *Vibrio cholerae* inhibit chemotaxis *in vitro* and some prevent ingestion by phagocytes (Densen & Mandell 1980; Wilton 1981; Arbuthnott 1981). Their relevance to infection *in vivo* is possible, even probable for some (the staphylococcal toxins), but has not yet been investigated (Arbuthnott 1981).

(*c*) *Interference with the action of complement and 'natural' antibody*

Resistance to one humoral bactericidin and to some aspects of the phagocyte defences can have the following humoral origin. The complement cascade is activated either by the classical pathway after reaction of 'natural' antibody (evoked by normal exposure to microbes and part of 'non-specific' defence (Penn 1983)) with bacterial surface components or through the alternative pathway by direct reaction with cell-wall materials such as peptidoglycan of Gram-positive organisms or lipid A and the core of Gram-negative organisms' LPS (Braude 1981; Quie *et al.* 1981; Wilton 1981; Penn 1983). The terminal components of this cascade, acting alone or in consort with lysozyme, form the most important humoral bactericidin against Gram-negative organisms (Braude 1981; Penn 1983; Easmon 1983). Earlier components of the cascade promote inflammation and chemotaxis of the mobilized phagocytes towards bacteria (Wilton 1981; Penn 1983). Also, they opsonize bacteria for attachment to and ingestion by the phagocytes (Wilton 1981; Quie *et al.* 1981; Penn 1983). Thus any bacterial product that interferes with the initial activation of the complement cascade by either pathway, or destroys or prevents the action of its early components, particularly the crucial C3b (Penn 1983), will impair both humoral and phagocytic defences. Furthermore, the demonstration that one of these mechanisms operates in one relevant test, for example inhibition of killing by serum, means that other aspects of host defence affected by complement, for example phagocytosis, will also be impaired.

In tests related to resistance to killing by serum or to prevention of opsonization, or to both (and occasionally to inhibition of chemotaxis), some surface or capsular materials have been strongly implicated in masking cell-wall components that would otherwise activate complement directly or after reaction with 'natural' antibody. These are: the K polysaccharide antigens of *E. coli* (including K1 types), the complete LPS of other virulent smooth unencapsulated *E. coli*, the complete LPS of *Salm. typhimurium*, and the sialic acid-containing capsular material or group B streptococci (Glynn 1972; Densen & Mandell 1980; Robbins *et al.* 1980; Wilton 1981; Quie *et al.* 1981; Edwards *et al.* 1982; Penn 1983; Easmon 1983). In addition, the following have been associated with masking, the M protein of streptococci, the capsular polysaccharides of pneumococci, meningococci and type b *H. influenzae*, and the surface layers of fresh clinical isolates of *Salm. typhi*, *K. pneumoniae*, *Proteus mirabilis* and gonococci from cases of disseminated gonorrhoea (Glynn 1972; Densen & Mandell 1980; Robbins *et al.* 1980; Wilton 1981; Quie *et al.* 1981; Penn 1983; Easmon 1983). These cases of masking should be classed as possibly relevant *in vivo* on present evidence but if investigated further might well prove to

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be probably relevant. Staphylococcal capsules appear to allow activation of complement by underlying cell-wall components but then prevent, by steric hindrance, the opsonic components from forming ligands with their receptors on the phagocytes (Densen & Mandell 1980; Quie *et al.* 1981; Verbrugh *et al.* 1982). In some cases that are probably relevant *in vivo* the masking and steric hindrance associated with capsular and surface components seems to prevent 'natural' antibody from opsonizing bacteria such as pneumococci (Densen & Mandell 1980; Quie *et al.* 1981).

Two examples that involve the likely destruction of complement components by bacterial action are known. The elastase of *Ps. aeruginosa* destroys C_1, C_3, C_5, C_8 and C_9 of the complement cascade (Densen & Mandell 1980; Quie *et al.* 1981) and is probably relevant *in vivo*. Also, ammonia produced by *Proteus* and *Klebsiella* spp. in the kidney could inactivate complement *in vivo* (Braude 1981).

This section underlines the uncertainty of knowing precisely what happens *in vivo*. The demonstration that a pathogenic bacterium interferes with one aspect of host defence in a relevant test *in vitro* that depends on the action of complement does not mean that this aspect is crucial *in vivo*; inhibition of other defence mechanisms depending on complement but not tested *in vitro* may be equally or more important (Penn 1983).

Studies on the determinants of the resistance of gonococci to killing by human serum and by human phagocytes

These studies illustrate many of the points made in the first section.

(*a*) *Complexity of the resistance of gonococci to complement-mediated killing by human serum: importance of phenotypic changes* in vivo *and* in vitro

The resistance of gonococci to complement-mediated killing appears important in the pathogenesis of both disseminated gonorrhoea and urogenital infections because complement components are in the blood and during inflammation will escape to the mucous surfaces and underlying tissues (Brooks *et al.* 1978; Braude 1982). Indeed, gonococci obtained from both disseminated infections and from urethral exudate are resistant to complement-mediated killing by human serum (Brooks *et al.* 1978; Ward *et al.* 1970). The serum resistance is of two types. Strains from disseminated gonorrhoea and a few strains from urethral exudates exhibit resistance that is retained on subculture (Ward *et al.* 1970; Schoolnik *et al.* 1976; Brooks *et al.* 1978). The second type of resistance is lost on subculture *in vitro* and is shown by most strains from urethral exudates (Ward *et al.* 1970; Brooks *et al.* 1978).

The determinants of the stable type of resistance have received much attention because adequate amounts of resistant strains could be obtained *in vitro* for comparisons with susceptible strains. No single determinant has been strongly implicated in resistance by the type of evidence outlined in the first part of this paper. However, the three main outer-membrane components, protein I (the principle outer-membrane protein, molecular mass 32–40 kDa), the proteins II (several heat-modifiable proteins of lower molecular mass, 27–29 kDa) and LPS have been associated with resistance. More than one gene locus is involved. The complex situation is summarized as follows. In opacity variants of the P9 strain a protein IIa (molecular mass 28.5 kDa) was usually but no exclusively associated with serum resistance (Lambden *et al.* 1979). The resistance to male and female sera of 43 clinical isolates was most closely but not

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entirely associated with proteins I of low molecular mass (35 kDa); proteins II were associated with resistance to male serum only, with no particular protein II prominent (James *et al.* 1982). Transformation studies have indicated that two genetic sites, *Sac-1*$^+$ and *Sac-3*$^+$, are associated with serum resistance as well as the site governing production of protein I (Shafer *et al.* 1982); the products of *Sac-1*$^+$ and *Sac-3*$^+$ are unknown. Mutation and other studies have indicated that LPS structure affects serum resistance (Brooks *et al.* 1978; Guymon *et al.* 1982). Finally, some gonococci form capsules under particular growth conditions and these have been associated with resistance to serum killing (Brooks *et al.* 1978; Ward *et al.* 1978). Clearly, the determinants of the stable type of resistance to killing by serum are multiple, complex and may vary from strain to strain (James *et al.* 1982; Shafer *et al.* 1982; Guymon *et al.* 1982). It has been suggested that they may act by providing receptors for incomplete antibody which then blocks attachment of complete antibody and hence complement activation (Braude, 1982; Rice & Kasper 1982). However, it appears that complement is activated by resistant gonococci and the membrane attack complex attaches to them (Harrisman *et al.* 1982).

The determinants of the unstable type of resistance have not been investigated because, as yet, sufficient resistant gonococci have not been produced *in vitro*. Nevertheless, the nature of the resistance and the host factors that induce it have received attention.

First, gonococci grown *in vivo*, which are impossible to obtain in sufficient quantities from urethral pus, were produced by infecting plastic chambers implanted subcutaneously into guinea-pigs (Penn *et al.* 1976). Like organisms from urethral pus, gonococci from the chambers were resistant to killing by human serum, and most strains lost their resistance on subculture (Penn *et al.* 1976, 1977). The change from resistance to susceptibility in laboratory media, and vice versa in the chambers, was phenotypically determined because it occurred in one or two generations (Rittenberg *et al.* 1977). Susceptible gonococci were rendered resistant *in vitro* by short-term (3 h) incubation at 37 °C, first in fluid from guinea-pig chambers, then in guinea-pig serum and finally in a mixture of diffusate from guinea-pig serum, bovine serum albumin (0.1 %) and a defined medium (Goldner *et al.* 1979; Veale *et al.* 1981). The resistance was not induced by treatment at 8 °C for 3 h or 37 °C for $\frac{1}{2}$ h, indicating that simple adsorption of guinea-pig material was not effective: a metabolic event was required. Ultrafiltration showed that the molecular mass of the heat-labile and acid-labile inducing factor was 500–1000 Da (Veale *et al.* 1980; P. V. Patel, P. M. V. Martin, N. J. Parsons & H. Smith, unpublished observations).

Turning to human material, about 25 % of 147 sera examined had resistance-inducing activity with the same characteristics as that of guinea-pig serum, though at a lower level (Martin *et al.* 1981, 1982*a*); the activity depended on fractions of high and low molecular masses, and the latter was similar to that from guinea-pig serum (Martin *et al.* 1981). Resistance-inducing activity was demonstrated in cervical secretions and seminal plasma from uninfected, clinically normal persons and vaginal exudates from patients with infections other than gonorrhoea (Martin *et al.* 1982*b*). All the above work was conducted with one serum-sensitive strain, but recently all of 30 gonococcal isolates from urethritis that were serum-susceptible when subcultured were induced to resistance by the low molecular mass inducer of guinea-pig serum (Martin *et al.* 1983). This work explains why gonococci from urethritis are resistant to serum killing *in vivo* but most of them are sensitive *in vitro*. It also underlines the fact that dissemination of the disease, which occurs only occasionally, must be due to factors in addition to the serum resistance possessed by strains *in vivo*.

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Another type of resistance to killing by human serum should be mentioned. McCutchan *et al.* (1976) selected a resistant strain by eight serial 1 h passages in bactericidal human serum. Resistance was lost on subculture after two serial overnight cultures on agar; this contrasted with the 2–3 generations required for the phenotypically acquired resistance described above (Rittenberg *et al.* 1977).

In summary, the resistance of gonococci to killing by human serum is complex. The stable type of resistance has been associated with more than one outer-membrane component (proteins I, proteins II and LPS), which may vary from strain to strain; none of these components can yet be considered to have been strongly implicated in resistance by the necessary evidence. The unstable type of resistance is mostly phenotypic and induced by materials present in guinea-pig and human serum and human genital secretions, one of them of low molecular mass. Identification of the inducer will allow an investigation of the determinants. This situation, after much work by many groups, epitomizes the difficulty of identifying the determinants of even one facet of interference with host defence.

(b) Studies on the determinant of the resistance of some gonococci to killing by human phagocytes: the effect of selection in vivo

In addition to resistance to killing by humoral factors, virulent gonococci resist ingestion by phagocytes to some extent, and pili have been strongly implicated in this resistance to ingestion (Densen & Mandell 1980; Jones *et al.* 1980; Siegel *et al.* 1982). Nevertheless, microscopy of urethral exudates shows that some gonococci are ingested by phagocytes, and the following evidence indicates that intracellular survival and growth contribute significantly to the pathogenesis of gonorrhoea. About 50% of all gonococci present in urethral pus are associated with, and are probably within, the phagocytes (King *et al.* 1978; Veale *et al.* 1979). Electron microscopy by several groups showed that although some gonococci disintegrate within the phagocytes others appear to be intact and, occasionally dividing (for references see Veale *et al.* 1979). These visual indications of intracellular survival and growth were confirmed by phagocytosis tests *in vitro* with gonococci grown *in vitro* and *in vivo* and phagocytes (predominantly polymorphonuclear with some mononuclear leucocytes) from human peripheral blood or urethral exudates (Veale *et al.* 1979; Casey *et al.* 1979, 1980). Finally, in tests *in vitro*, large numbers of gonococci lysed polymorphonuclear phagocytes and this may explain the clumps of extracellular gonococci that are seen in granule-like debris that reacted with antibody against polymorphonuclear phagocytes (Veale *et al.* 1979).

Investigations of the determinants of resistance to killing by human phagocytes began with examination of gonococci grown in guinea-pig chambers. When examined without subculture these gonococci were more resistant to killing by human phagocytes than the parent laboratory strain and, unlike their resistance to killing by human serum, the resistance to killing by phagocytes was retained on minimal subculture (Penn *et al.* 1977). The conditions *in vivo* had *selected* phagocyte-resistant organisms from the original population as shown by the following. Resistant gonococci from guinea-pig chambers produced colonies with a characteristic double-highlight morphology in a system of transmitted and reflected light. A few organisms in the unpassaged parent laboratory strain produced such double-highlight colonies and were resistant to killing by human phagocytes, whereas the majority of organisms were sensitive to phagocytic killing and formed colonies with a single highlight. They were also less pilated than

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double-highlight organisms (Penn *et al.* 1977), thus associating pilation with resistance to killing by phagocytes.

In seeking to identify the determinants of resistance to intracellular killing, the first requirement was to develop a test for them. The simplest test, direct promotion of resistance to intracellular killing in susceptible single-highlight organisms by addition of extracts of the resistant double-highlight organisms was attempted. The results, although sometimes positive, were inconsistent (D. R. Veale, N. J. Parsons & H. Smith, unpublished observations); probably for the reason expressed previously, the determinant did not become attached to the surface of the susceptible organisms. Two indirect tests became available when the resistance of double-highlight organisms to phagocytic killing was shown to be neutralized by antiserum against intact homologous organisms but not by the antiserum after adsorption with either homologous organisms or extracts of them (Veale *et al.* 1978). First, fractions of surface extracts could be examined for their ability to prevent the reduction in resistance to phagocytic killing produced by antisera to whole organisms. Second, antisera raised to purifed surface components of the resistant organisms could be examined for ability to promote intracellular killing. Both methods were used, as follows (Parsons *et al.* 1981, 1982). A surface wash of the phagocyte-resistant pilated double-highlight strain neutralized the activity of the whole organism antiserum, but a similar wash from the phagocyte-susceptible single-highlight strain had little neutralizing activity. Pili separated from the wash of the double-highlight strain did not neutralize the activity of the whole organism antiserum, and antiserum against pili failed to reduce the intracellular survival of resistant gonococci. On the other hand, when outer-membrane vesicles were removed from the surface wash by precipitating them with wheat-germ agglutinin, the antiserum-neutralizing activity of the wash was removed, and antiserum raised against outer-membrane vesicles reduced the intracellular survival of resistant gonococci. Furthermore, outer-membrane vesicles purified from lithum chloride extracts of the resistant double-highlight strain neutralized the activity of the antiserum to the whole organism, whereas vesicles similarly purified from the susceptible single-highlight strain had poor neutralizing activity. Finally, comparison of the proteins of the outer-membrane vesicles from the resistant double-highlight strain with those of the susceptible single-highlight strain showed that three proteins were associated with resistance to phagocyte killing.

Thus, in summary, resistance to intracellular killing appears to play a part in the pathogenesis of gonorrhoea; conditions *in vivo* select for gonococcal types with this resistance; the search for the determinants has to rely on an indirect test involving neutralization of their activity by antisera; and the determinants are not pili and appear to be present in outer-membrane vesicles, and three proteins of the latter have been associated with activity.

Conclusion

All sections of the paper emphasize the difficulty of obtaining unequivocal evidence that defined components of bacteria are the determinants of interference with certain defence mechanisms of known importance to infection *in vivo*. Often the most we can do is to strengthen evidence of association between a putative determinant and a particular interference into that which strongly implicates it, and then go on to devise experiments that show that it probably acts *in vivo*.

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References

Allan, R. J. & Scott, G. K. 1981 Comparisons of the effects of different lipopolysaccharides on the serum bactericidal reactions of two strains of *Escherichia coli*. *Infect. Immun.* **31**, 831–832.

Arbuthnott, J. P. 1981 Membrane-damaging toxins in relation to interference with host defence mechanisms. In *Microbial perturbation of host defences* (ed. F. O'Grady & H. Smith), pp. 97–120. London: Academic Press.

Bartell, P. F. & Krikszens, A. 1980 Influence of anti-slime glycolipoprotein serum on the interaction between *Pseudomonas aeruginosa* and macrophages. *Infect. Immun.* **27**, 777–783.

Bortolussi, R. & Ferrieri, P. 1980 Protection against *Escherichia coli* K_1 infection in newborn rats by antibody to K_1 capsular polysaccharide antigen. *Infect. Immun.* **28**, 111–117.

Braude, A. I. 1981 Bacterial interference with non-specific non-phagocytic defences. In *Microbial perturbation of host defences* (ed. F. O'Grady & H. Smith), pp. 31–48. London: Academic Press.

Braude, A. I. 1982 Resistance to infection with the gonococcus. *J. infect. Dis.* **145**, 623–634.

Brooks, G. F., Gotschlich, E. C., Holmes, K. K., Sawyer, W. D. & Young, F. E. 1978 *Immunobiology of Neisseria gonorrhoeae*. Washington, D.C.: American Society for Microbiology.

Casey, S. G., Veale, D. R. & Smith, H. 1979 Demonstration of intracellular growth of gonococci in human phagocytes using spectinomycin to kill extracellular organisms. *J. gen. Microbiol.* **113**, 395–398.

Casey, S. G., Veale, D. R. & Smith, H. 1980 Intracellular survival of *Neisseria gonorrhoeae* in human urethral exudate. *FEMS Microbiol. Lett.* **8**, 97–100.

Densen, P. & Mandell, G. I. 1980 Phagocyte strategy vs. microbial tactics. *Rev. infect. Dis.* **2**, 817–838.

Dhingra, R. K., Williams, R. C. Jr & Reed, W. P. 1977 Effects of pneumococcal mucopeptide and capsular polysaccharide on phagocytosis. *Infect. Immun.* **15**, 169–174.

Draper, P. 1981 Microbial inhibition of intracellular killing. In *Microbial perturbation of host defences* (ed. F. O'Grady & H. Smith), pp. 143–164. London: Academic Press.

Easmon, C. S. F. 1983 Inhibition of non-specific humoral and phagocytic defence mechanisms by bacteria. In *Bacterial and viral inhibition and immuno-modulation of host defences* (ed. G. Falconi, A. Campa, H. Smith & G. M. Scott). London: Academic Press. (In the press.)

Easmon, C. S. F., Jeljaszcwicz, J., Brown, M. R. W. & Lambert, P. A. (eds) 1983 *Medical microbiology*, vol. 3. (*The role of the envelope in survival of bacteria in infection*). London: Academic Press.

Edwards, M. S., Kasper, D. L., Jennings, H. J., Baker, C. J. & Nicholson-Weller, A. 1982 Capsular sialic acid prevents activation of the alternate complement pathway by type III group B streptococci. *J. Immunol.* **128**, 1278–1283.

Falconi, G., Campa, A., Smith, H. & Scott, G. M. (eds) 1983 *Bacterial and viral inhibition and immuno-modulation of host defences* London: Academic Press.

Freter, R. 1980 Prospects of preventing association of harmful bacteria with host mucosal surfaces. In *Bacterial adherence*, pp. 441–458. London: Chapman Hall.

Frost, A. J., Smith, H., Witt, K. & Keppie, J. 1972 The chemical basis of the virulence of *Brucella abortus*. X. A surface virulence factor which facilitates intracellular growth of *Brucella abortus* in bovine phagocytes. *Br. J. exp. Path.* **53**, 587–596.

Glynn, A. A. 1972 Bacterial factors inhibiting host defence mechanisms. *Symp. Soc. gen. Microbiol.* **22**, 75–112.

Goldner, M., Penn, C. W., Sanyal, S. C., Veale, D. R. & Smith, H. 1979 Phenotypically determined resistance of *Neisseria gonorrhoeae* to normal human serum: environmental factors in subcutaneous chambers in guinea pigs. *J. gen. Microbiol.* **114**, 169–177.

Guymon, L. F., Esser, M. & Shaffer, W. M. 1982 Pyocin-resistant lipopolysaccharide mutants of *Neisseria gonorrhoeae*: alterations in sensitivity to normal human serum and polymyxin B. *Infect. Immun.* **36**, 541–547.

Harrisman, G. R., Podack, E. R., Braude, A. I., Corbeil, L. C., Esser, A. F. & Curd, J. G. 1982 Activation of complement by serum resistant *Neisseria gonorrhoeae*: assembly of the membrane attack complex without subsequent cell death. *J. exp. Med.* **156**, 1235–1249.

Hemmel, M. E., Yates, M. D., Lauerman, L. H. & Squire, P. G. 1982 Purification and partial characterisation of a macrophage cytotoxin from *Pasteurella haemolytica*. *Am. J. vet. Res.* **43**, 764–767.

Hunter, K. W., Hemming, V. G., Fischer, G. W., Wilson, S. R., Hartzmann, R. J. & Woody, J. N. 1982 Anti-bacterial activity of a human monoclonal antibody to *Haemophilus influenzae* type B capsular polysaccharide. *Lancet* ii, 798–799.

James, J. F., Zurlinden, E., Lammel, C. J. & Brooks, C. F. 1982 Relation of protein I and colony opacity to serum killing of *Neisseria gonorrhoeae*. *J. infect. Dis.* **145**, 37–44.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Jones, R. B., Newland, J. C., Olsen, D. A. & Buchanan, T. M. 1980 Immune-enhanced phagocytosis of *Neisseria gonorrhoeae* by macrophages: characterization of the major antigens to which opsonins are directed. *J. gen. Microbiol.* **121**, 365–372.

Keppie, J., Harris-Smith, P. W. & Smith, H. 1963 The chemical basis of the virulence of *Bacillus anthracis*. IX. Its aggressins and mode of action. *Br. J. exp. Path.* **44**, 446–453.

King, G., James, J. F. & Swanson, J. 1978 Studies on gonococcus infection. XI. Comparison of *in vivo* and *in vitro* association of *Neisseria gonorrhoeae* with human neutrophils. *J. infect. Dis.* **137**, 38–43.

Lambden, P. R., Heckels, J. E., James, L. T. & 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.

Liang-Takasaki, C., Makela, P. H. & Leive, L. 1982 Phagocytosis of bacteria by macrophages: changing the carbohydrate of lipopolysaccharides alters interaction with complement and macrophages. *J. Immunol.* **128**, 1229–1235.

McCutchan, J. A., Levine, S. & Braude, A. I. 1976 Influence of colony type on susceptibility of gonococci to killing by human serum. *J. Immunol.* **116**, 1652–1655.

Madonna, G. S. & Allen, R. C. 1981 *Shigella sonnei* Phase I and Phase II: susceptibility to direct serum lysis and opsonic requirements necessary for stimulation of leukocyte redox metabolism and killing. *Infect. Immun.* **32**, 153–159.

Martin, P. M. V., Patel, P. V., Parsons, N. J. & Smith, H. 1981 Induction of phenotypically determined resistance of *Neisseria gonorrhoeae* to human serum by factors in human serum. *J. gen. Microbiol.* **127**, 213–217.

Martin, P. M. V., Patel, P. V., Parsons, N. J. & Smith, H. 1982*a* Induction of phenotypically determined resistance of *Neisseria gonorrhoeae* to human sera by sera from patients with gonorrhoea. *Br. J. vener. Dis.* **58**, 302–304.

Martin, P. M. V., Patel, P. V., Parsons, N. J. & Smith, H. 1982*b* Induction in gonococci of phenotypic resistance to killing by human serum by human genital secretions. *Br. J. vener. Dis.* **58**, 363–365.

Martin, P. M. V., Patel, P. V., Parsons, N. J. & Smith, H. 1983 Induction of serum-resistance to recent isolates of *Neisseria gonorrhoeae* by a low molecular weight fraction of guinea pig serum. *J. infect. Dis.* (In the press.)

O'Grady, F. & Smith, H. (eds) 1981 *Microbial perturbation of host defences*. London: Academic Press.

Parsons, N. J., Kwaasi, A. A. A., Perera, V. Y., Patel, P. V., Martin, P. M. V. & Smith, H. 1982 Outer membrane proteins of *Neisseria gonorrhoeae* associated with survival within human polymorphonuclear phagocytes. *J. gen. Microbiol.* **128**, 3077–3081.

Parsons, N. J., Kwaasi, A. A. A., Turner, J. A., Veale, D. R., Perera, V. Y., Penn, C. W. & Smith, H. 1981 Investigation of the determinants of the survival of *Neisseria gonorrhoeae* within human polymorphonuclear phagocytes. *J. gen. Microbiol.* **127**, 103–112.

Penn, C. W. 1983 Envelope and humoral defences. In *Medical microbiology*, vol. 3. (*Role of the envelope in the survival of bacterial infection*) (ed. C. S. F. Easmon, J. Jeljaszewicz, M. R. W. Brown & P. A. Lambert). London: Academic Press. (In the press.)

Penn, C. W., Sen, D., Veale, D. R., Parsons, N. J., Smith, H. & Witt, K. 1976 Morphological, biological and antigenic properties of *Neisseria gonorrhoeae* adapted to growth in guinea pig subcutaneous chambers. *J. gen. Microbiol.* **97**, 35–43.

Penn, C. W., Veale, D. R. & Smith, H. 1977 Selection from gonococci grown *in vitro* of a colony type with some virulence properties of organisms adapted *in vivo*. *J. gen. Microbiol.* **100**, 147–158.

Quie, P. G., Gielbrink, G. S. & Peterson, P. K. 1981 Bacterial mechanisms for inhibition of ingestion by phagocytic cells. In *Microbial perturbation of host defences*. (ed. F. O'Grady & H. Smith), pp. 121–142. London: Academic Press.

Rice, P. A. & Kasper, D. L. 1982 Characterisation of serum resistance of *Neisseria gonorrhoeae* that disseminate. Role of blocking antibody and gonococcal outer membrane proteins. *J. clin. Invest.* **70**, 157–167.

Rittenberg, S. C., Penn, C. W., Parsons, N. J., Veale, D. R. & Smith, H. 1977 Phenotypic changes in the resistance of *Neisseria gonorrhoeae* to killing by normal human serum. *J. gen. Microbiol.* **103**, 69–75.

Robbins, J. B., Schneerson, R., Egan, W. B., Vann, W. & Lui, D. T. 1980 In *The molecular basis of microbial pathogenicity* (ed. H. Smith, J. J. Skehel & M. J. Turner), pp. 115–132. Weinheim: Verlag Chemie.

Scarlan, C. M., Berg, J. H. & Fales, W. H. 1982 Comparative *in vitro* leukotoxin production of three strains of *Fusobacterium necrophorum*. *Am. J. vet. Res.* **43**, 1329–1333.

Schlessinger, D. (ed.) 1979 *Microbiology – 1979*. Washington, D.C.: American Society for Microbiology.

Schoolnik, G. K., Buchanan, T. M. & Holmes, K. K. 1976 Gonococci causing disseminated gonococcal infection are more resistant to the bactericidal action of normal human sera. *J. clin. Invest.* **58**, 1163–1173.

Shafer, W. M., Guymon, L. F. & Sparling, P. F. 1982 Identification of new genetic site (*sac-3*$^{+}$) in *Neisseria gonorrhoeae* that affects sensitivity to normal human serum. *Infect. Immun.* **35**, 764–769.

Siegel, M., Olsen, O., Critchlow, C. & Buchanan, T. M. 1982 Gonococcal pili: safety and immunogenicity in humans and antibody function *in vitro*. *J. infect. Dis.* **145**, 300–310.

Smith, H. 1964 Microbial behaviour in natural and artificial environments. *Symp. Soc. gen. Microbiol.* **14**, 1–29.

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. *Bact. Rev.* **41**, 475–500.

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Smith, H. 1980 The effect of environmental conditions *in vivo* and *in vitro* on the determinants of microbial pathogenicity. In *The molecular basis of microbial pathogenicity*, (ed. H. Smith, J. J. Skehel & M. J. Turner), pp. 159–174. Weinheim: Verlag Chemie.

Smith, H. 1982 The role of microbial interactions in infectious disease. *Phil. Trans. R. Soc. Lond.* B **297**, 551–561.

Smith, H. 1983 Bacterial subversion rather than suppression of immune defences. In *Bacterial and viral inhibition and immuno-modulation of host defences* (ed. G. Falconi, A. Campa, H. Smith & G. M. Scott). London: Academic Press. (In the press.)

Sparling, P. F. 1979 Use of microbial genetics in the study of pathogenicity: differentiation between correlation and causation. In *Microbiology – 1979* (ed. D. Schlessinger), pp. 249–253. Washington, D.C.: American Society for Microbiology.

Stendahl, O. 1983 The physicochemical basis of surface interaction between bacteria and phagocytic cells. In *Medical microbiology*, vol. 3 (*Role of the envelope in the survival of bacteria in infection*) (ed. C. S. F. Easmon, J. Jeljaszewicz, M. R. W. Brown & P. A. Lambert). London: Academic Press. (In the press.)

Taylor, P. W. 1978 The effect of growth environment on serum sensitivity of some urinary *Escherichia coli* strains. *FEMS Microbiol. Lett.* **3**, 119–122.

Tee, G. L. & Scott, G. K. 1980 Analysis of outer membrane components of *Escherichia coli* ML 308 225 and of a serum-resistant mutant. *Infect. Immun.* **28**, 387–392.

Veale, D. R., Goldner, M., Penn, C. W., Ward, J. & Smith, H. 1979 The intracellular survival and growth of gonococci in human phagocytes. *J. gen. Microbiol.* **113**, 383–393.

Veale, D. R., Penn, C. W., Parsons, N. J. & Smith, H. 1980 Preliminary studies of a factor in guinea pig serum which induces serum resistance in *Neisseria gonorrhoeae*. *Soc. gen. Microbiol. Q.* **7**, 188.

Veale, D. R., Penn, C. W. & Smith, H. 1981 Factors affecting the induction of phenotypically determined serum resistance of *Neisseria gonorrhoeae* grown in media containing serum or its diffusible components. *J. gen. Microbiol.* **122**, 235–245.

Veale, D. R., Penn, C. W., Sutton, S. & Smith, H. 1978 The effects of specific antiserum on the resistance of *Neisseria gonorrhoeae* to intracellular killing by phagocytes of human blood. *J. gen. Microbiol.* **106**, 129–136.

Verbrugh, H. A., Peterson, P. K., Nguyen, B. T., Sisson, S. P. & Kim, Y. 1982 Opsonization of encapsulated *Staphylococcus aureus*: the role of specific antibody and complement. *J. Immunol.* **129**, 1681–1687.

Ward, M. E., Lambden, P. R., Heckels, E. J. & Watt, P. J. 1978 The surface properties of *Neisseria gonorrhoeae*: determinants of susceptibility to antibody complement killing. *J. gen. Microbiol.* **108**, 205–212.

Ward, M. E., Watt, P. J. & Glynn, A. A. 1970 Gonococci in urethral exudates possess a virulence factor lost on subculture. *Nature, Lond.* **227**, 382–384.

Wilton, J. M. A. 1981 Microbial interference with inflammation and phagocyte function. In *Microbial perturbation of host defences*, (ed. F. O'Grady & H. Smith), pp. 67–96. London: Academic Press.

Discussion

P. H. Cleat (*Division of Comparative Medicine, M.R.C. Clinical Research Centre, Harrow, U.K.*). We have a strain of group B streptococcus that survives inside neutrophils. If, however, we coat the organism with specific antiserum, it activates the neutrophil to kill the ingested organisms like the gonogocci that Professor Smith has been describing. Does Professor Smith think that, like toxoplasma and mycobacteria in macrophages, coating the organisms with specific antibody overcomes their inhibition of lysosome–phagosome fusion, or does he have any other ideas?

H. Smith. I know the story with toxoplasma and mycobacteria, but for gonococci we don't know how the coating with antiserum overcomes its resistance to killing. Does Mr Cleat know for group B streptococcus?

P. H. Cleat. Not as yet, but we do know that $F(ab^1)_2$ fragments of specific antibody and not whole antiserum will initiate killing by neutrophils. Also, if we add specific $F(ab^1)_2$ fragments after phagocytosis has occurred, we can also get killing. How the antibody comes into contact with the bacterium, whether by pinocytosis or whatever, we do not yet know.

D. R. Veale (*Safety Unit, University of Birmingham, U.K.*). I should like to confirm Professor Smith's remarks on the cytotoxicity of *Neisseria gonorrhoeae* for human peripheral blood

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phagocytes. It is a common observation that some polymorphonuclear phagocytes in urethral exudates of patients with gonorrhoea contain large numbers of gonococci (50 or more). If the ingestion of large numbers of gonococci by human peripheral blood phagocytes (chiefly polymorphonuclear phagocytes) is induced *in vitro* by incubation at high gonococci:phagocyte ratios, a substantial proportion of the phagocytes show visual evidence of cell damage or lysis with the release of cellular contents, including ingested gonococci.

A. A. GLYNN (*Central Public Health Laboratory, London, U.K.*). The suggestion by a previous questioner that gonococci in urethral exudate might resist serum killing because they were passively coated by some factor was tested by Ward, Watt & Glynn in their original paper (*Nature, Lond.* **227**, 382–384 (1970), describing loss of serum resistance by gonococci on culture. A serum-sensitive strain of gonococcus, G11, bearing a streptomycin-resistant marker, was mixed with exudates but retained its sensitivity to complement. I agree entirely that genetic manipulation can be extraordinarily useful in pathogenesis research experiments, but genetic, like other experiments, can be misleading. If molecular geneticists say that they have transferred a gene, they usually have, but what others besides? A few years ago we received from Helena Makela some transductants of *Salmonella typhimurium* in which only one O antigen was said to have been altered. Yet Bryon Wilson in my laboratory found significant differences in the outer-membrane proteins as well. Newer genetic techniques may be more precise, but still need watching.

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