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Physiology and Metabolism of *Neisseria gonorrhoeae* and *Neisseria meningitidis*: Implications for Pathogenesis

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Gonococci and meningococci are obligate human pathogens that can infect diverse sites within the human host. Each of these sites represents a unique niche with respect to nutrients, environmental factors, and competing microorganisms. The growth environment has a marked effect on the metabolism and cellular composition of *Neisseria gonorrhoeae* and *N. meningitidis*. Altered cellular composition is often reflected by changes in the cell surface that can ultimately affect the interaction of these microorganisms with the human host. The physiology and metabolism of the pathogenic *Neisseria* spp. have not been reviewed since 1979 (41). This review will discuss selected areas that have implications for the pathogenesis of these important microorganisms.

IRON METABOLISM

After entry into the human host, *N. gonorrhoeae* and *N. meningitidis* must multiply to colonize mucosal surfaces and to establish an infection. Cell growth and multiplication require essential nutrients such as iron. To obtain iron, the pathogenic *Neisseria* spp. must acquire it from the host. Despite the relative abundance of iron in the host, there is little free iron because of its sequestration by the iron-binding proteins transferrin (TF) and lactoferrin (LF) (19). In serum and interstitial fluid, iron is associated with TF; in breast milk, semen, and mucosal surfaces, it is associated primarily with LF. Consequently, gonococci and meningococci must possess mechanisms for utilizing the iron associated with the host iron-binding proteins as well as other potential in vivo iron sources such as heme and hemoglobin.

Several studies have implicated iron in the virulence of the pathogenic *Neisseria* spp. Calver et al. (7) demonstrated that injection of ferrous sulfate prior to or injection of either iron sorbitol citrate or iron-dextran concomitantly with injection of *N. meningitidis* increased the lethality of several different meningococcal serogroups for mice by up to a 10^6-fold. The effect of the added iron was partially abrogated by the prior incubation of *N. meningitidis* with Desferal (CIBA-GEIGY Corp.), an iron chelator from which meningococci and gonococci are unable to remove iron (36). Holbein et al. (24) showed that the 50% lethal dose of *N. meningitidis* strains in a mouse model was decreased 10^9-fold by the concomitant administration of iron-dextran with the inoculum. Payne and Finkelstein (50) found that the intravenous inoculation of iron-containing compounds together with avirulent (nonpiliated) gonococci increased the lethality of these avirulent organisms for chicken embryos.

Hafiz et al. (20) observed that high concentrations of ferric citrate appeared to stimulate the reversion of nonpiliated gonococci to piliated gonococci during batch culture in a liquid medium. Odugbemi and Hafiz (49) further demonstrated that the apparent rate of reversion from the nonpiliated state to the piliated state was influenced by both iron and iron chelators. These results are somewhat at odds with recent findings (26) that iron-limited gonococci remained piliated. Nevertheless, these studies provide evidence that iron has an important role in the virulence of the pathogenic *Neisseria* spp. Iron probably influences cellular function(s) since ferric chloride, ferric nitrate, and ferric ammonium citrate did not increase the attachment of gonococci to human spermatozoa (25).

All strains of *N. gonorrhoeae* and *N. meningitidis* are able to grow with 25% iron-saturated TF as their sole source of iron (37). Archibald and DeVoe (1, 2) found that meningococci were capable of obtaining iron from a variety of iron-containing compounds including gastric mucin, ferric citrate, hemoglobin, myoglobin, and human TF. Iron complexed with a number of metabolic organic acids, polyphosphates, and several synthetic polycarboxylic acids was also readily utilized by all meningococcal strains examined. However, *N. meningitidis* was unable to use iron bound to some common hydroxymate- and catechol-type siderophores.

All strains of *N. meningitidis* used LF as an iron source, whereas approximately 60% of the gonococcal strains examined in one study (36) were able to utilize this iron source. The percentage of gonococcal strains that were capable of utilizing LF-bound iron was related to the auxotype of the strain; 86% of prototrophic gonococci were able to utilize LF-bound iron, whereas only 14% of Arg^- Hyx^- Ura^- strains were able to utilize this iron source. Their inability to utilize LF-bound iron has been offered as an explanation for the association of these strains with asymptomatic infection (5). The actual LF concentration in human vaginal mucus varies during the menstrual cycle and is lowest just before menses (13). The implication of this observation in the pathogenesis of pelvic inflammatory disease or disseminated gonococcal infection remains to be determined.

N. meningitidis was shown to acquire TF-bound iron by an iron-repressible, energy-dependent mechanism that required a functional respiratory chain (60). The uptake of iron from TF required direct contact of the TF with the meningococcal surface; during this process, the TF remained extracellular (61). Approximately 70% of the ^{55}Fe taken up by cyanide-treated meningococci was located in the outer membrane in association with a major outer membrane protein with an apparent molecular mass of 36,500 daltons. McKenna et al. (35) have shown that, like meningococci, gonococci remove iron from TF and LF by an iron-repressible, energy-dependent mechanism. The acquisition of TF- and LF-bound iron required direct contact of the protein with the gonococcal cell surface. Gonococci were extremely efficient at utilizing the TF- or LF-bound iron as the sole source of iron for growth; a level of 5% iron saturation was sufficient to support normal growth in vitro, provided that

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enough protein was available to supply the required amount of iron (35).

The mechanism by which gonococci release the iron from TF and LF is unclear. West et al. (63) postulated that the TF and LF bound nonspecifically to the gonococcal cell surface and that the iron was subsequently released by a mechanism involving polyphosphate. The Fe^{3+} bound to TF or LF may also be released by reduction to Fe^{2+}; both cytoplasmic and membrane-bound ferric reductase activities have been detected in gonococci and meningococci (A. E. LeFaou and S. A. Morse, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, D20, p. 75). Meningococcal and gonococcal iron-regulated proteins may play a role in the binding of TF or LF, the release of iron, and its transport across the outer and inner membranes. A putative meningococcal TF receptor was recently detected in an assay in which human TF conjugated to horseradish peroxidase was used (54). Further studies involving sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western immunoblot analysis showed that it was a 71,000-dalton, iron-regulated outer membrane protein (54). Competitive-binding experiments indicated that the receptor exhibited a specificity for human TF and was distinct from the LF receptor. An LF-binding protein (105,000 daltons) was subsequently identified in three different meningococcal strains by using biotinylated human LF and streptavidin-agarose (55). The LF-binding protein exhibited a specificity for human LF, and its synthesis was regulated by the level of iron in the medium. The specificity exhibited for human TF and LF may be an important factor in the host specificity of these pathogens.

Free hemoglobin or hemoglobin bound to haptoglobin can be used as an iron source by most gonococcal and meningococcal strains (17). *N. gonorrhoeae* and *N. meningitidis* can also utilize free heme as an iron source (37, 66), but not when it is complexed to hemopexin or albumin (17).

Yancey and Finkelstein (67) first reported the production of a siderophore by disseminating strains of *N. gonorrhoeae*. Subsequently, they isolated a dihydroxamate-type siderophore from concentrated culture supernatants of *N. gonorrhoeae* and *N. meningitidis* (66). However, Archibald and DeVoe (2) and Norrod and Williams (48) were unable to detect any siderophore activity in spent culture supernatants from these organisms. These conflicting reports were recently clarified by West and Sparling (64), who were also unable to detect siderophore activity in *N. gonorrhoeae*. They further showed that the quantity of siderophore detected by bioassay of culture supernatants from *N. gonorrhoeae* was never greater than the amount already present in the uninoculated medium.

Gonococci are capable of utilizing siderophores produced by other microorganisms. Yancey and Finkelstein (66) have reported that gonococci can utilize the dihydroxamate siderophores aerobactin, arthrobactin, and schizokinen. West and Sparling (65) confirmed that gonococci could utilize ferri-aerobactin as a sole iron source. In addition, they probed gonococcal genomic deoxyribonucleic acid with the cloned *Escherichia coli* aerobactin-biosynthetic genes (*iucABCD*) as well as the aerobactin receptor (*iutA*) and hydroxamate utilization (*fhuCDB*) genes; hybridization was detected with *fhuB* sequences but not with the other genes. West and Sparling (65) identified and cloned the region of the gonococcal genome that exhibited homology with *fhuB* and demonstrated its ability to complement *fhuB* mutations in *E. coli*.

Norqvist et al. (46) were the first to report that gonococci expressed several iron-regulated proteins when grown under iron-limited conditions. These iron-regulated proteins were heterogeneous with respect to both their relative migration during sodium dodecyl sulfate-polyacrylamide gel electrophoresis and their presence in different strains. Only one protein, with an apparent molecular mass of 97,000 daltons, appeared to be conserved among all the gonococcal strains examined. Mietzner et al. (40) confirmed the previous observation and identified a previously unrecognized iron-regulated protein with an apparent molecular mass of 37,000 daltons. This protein comigrated with the protein I of some strains and was resolved in sodium dodecyl sulfate-polyacrylamide gel electrophoresis only under conditions of increased ionic strength, which permitted protein I and the 37,000-dalton protein to migrate as distinct bands. Although the expression of most of the other iron-regulated proteins varied among strains, the 37,000-dalton protein was common to all the gonococcal strains examined. Two-dimensional peptide maps of this protein obtained from two unrelated gonococcal strains were identical, suggesting that it was highly conserved.

West and Sparling (64) examined the expression of gonococcal iron-regulated proteins during growth in medium containing different iron sources. They found that, with the exception of the 37,000-dalton protein that was expressed under all conditions of iron limitation, most of the iron-regulated proteins were not coordinately regulated. The expression of the 37,000-dalton protein under all conditions of iron limitation and by all of the strains examined prompted speculation that this protein played a key role in the acquisition of iron by *N. gonorrhoeae*.

The gonococcal 37,000-dalton protein was purified by a combination of selective extraction with cetyltrimethylammonium bromide and column chromatography and used to produce both rabbit monospecific antiserum and murine monoclonal antibodies (39). Using these reagents, Mietzner et al. (38) found that this protein was antigenically conserved among strains of *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, and *N. cinerea*. Two-dimensional peptide maps and N-terminal amino acid sequences from the 37,000-dalton proteins isolated from these species confirmed that the primary structure of the protein was highly conserved (C.-Y. Chen, Ph.D. thesis, Oregon Health Sciences University, Portland, 1988). The antigenic and structural conservation of these 37,000-dalton proteins, particularly among the pathogenic members of the genus *Neisseria*, suggested that they may serve a common function in pathogenesis. Acute-phase serum specimens, as well as vaginal wash fluids from patients with disseminated gonococcal infection and uncomplicated gonococcal infections (18, 44) and with meningococcal infection (18), contained immunoglobulins that reacted with the 37,000-dalton protein, indicating that this protein is both expressed and antigenic in vivo.

The 37,000-dalton protein, purified to homogeneity from both *N. gonorrhoeae* and *N. meningitidis*, contains approximately 1 mol of Fe^{3+} per mol of protein (39, 42, 43). Furthermore, when isolated from gonococci grown in a liquid medium containing [^{55}Fe]TF as the only source of iron, the protein was labeled with ^{55}Fe (42). Whether the ^{55}Fe was directly transferred from TF to the 37,000-dalton protein or whether one or more intermediate steps were involved in this process is not known. The susceptibility of the 37,000-dalton protein to proteolytic cleavage following treatment of intact gonococci with the serine protease lysosomal cathepsin G suggested that certain portions of this protein were surface exposed (56). This surface exposure, subsequently confirmed by surface peptide mapping (C.-Y.

Chen and S. A. Morse, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, D-185, p. 102), indicated that a direct interaction between TF and the 37,000-dalton protein was possible.

The gene encoding the gonococcal 37,000-dalton protein has been cloned in three overlapping fragments and sequenced (S. Berish, T. A. Mietzner, and S. A. Morse, unpublished data). The consensus amino acid sequence predicted a mature protein containing 308 amino acids, and the molecular weight based on this sequence was 33,571. Hybridization analysis suggested that there was a single copy of this gene in the gonococcal chromosome.

Tarkka and Sarvas (62) recently reported cloning a 37,000-dalton protein from *N. meningitidis*. However, this cloned gene did not hybridize to several different oligonucleotide probes specific for the 37,000-dalton gonococcal and meningococcal iron-regulated proteins and hence does not appear to be the same protein (C. Genco, unpublished results).

The availability of mutants that are altered in their ability to utilize specific iron sources has enabled researchers to more closely examine the relationship between iron and virulence and to study the mechanism of Fe uptake by *N. gonorrhoeae* and *N. meningitidis*. Streptonigrin, an aminoquinone, causes deoxyribonucleic acid degradation and requires iron for its bactericidal effect (68). It has been used to selectively enrich for mutants altered in their ability to utilize specific iron sources. Dyer et al. (15) described a mutant of *N. meningitidis* that was impaired in its ability to use TF-bound iron. This mutant did not produce the 85,000- and 95,000-dalton iron-repressible outer membrane proteins. However, genetic transformation experiments indicated that these outer membrane proteins were probably not responsible for the TF-deficient phenotype. Recently, Dyer et al. (16) isolated a pleiotropic iron uptake mutant of *N. meningitidis* that lacks a 70,000-dalton iron-regulated protein. This mutant was markedly deficient in the uptake of iron from TF, LF, citrate, and aerobactin. These authors suggested that the 70,000-dalton protein may be involved in the uptake or utilization of these iron sources; however, the data supporting this hypothesis were not conclusive.

We have isolated several mutants of *N. gonorrhoeae* 340 that grew normally with heme as a sole source of iron but were deficient in the uptake of TF-bound iron (our unpublished data). The parental strain was virulent in the mouse subcutaneous chamber model, whereas the mutants were avirulent. Results from these studies indicated that gonococcal strains defective in iron utilization were not capable of sustained growth in vivo and suggested that iron utilization was required to initiate an active infection.

Despite these studies on iron-regulated proteins and iron uptake systems, our knowledge of the biochemistry and mechanism of iron transport by the pathogenic *Neisseria* species is still rudimentary.

SULFUR METABOLISM

In contrast to other bacteria, gonococci and meningococci are restricted in the way they can obtain sulfur for growth. All strains of gonococci and some strains of meningococci require cystine (or cysteine) for growth (8).

DeVoe et al. (14) identified thiosulfate reductase activity in *N. meningitidis*. Other forms of sulfur that supported meningococcal growth included sulfate, sulfite, bisulfite, thiosulfate, dithionite, hydrosulfide, thiocyanate, L-cysteine, L-cystine, reduced glutathione, methionine, mercaptosuccinate, and lathionine (52).

LeFaou (31) demonstrated the presence of thiosulfate sulfur transferase, trithionate reductase, and tetrathionate reductase activities in *N. gonorrhoeae*. Despite a report that gonococci lacked sulfite reductase (32), Norrod (47) observed that the addition of sulfite to medium already containing cysteine and cystine resulted in an alteration in the structure of gonococcal lipooligosaccharide.

AMINO ACID METABOLISM

Auxotyping of gonococci (and to a lesser extent meningococci) has been used to assist epidemiologic investigations. Gonococcal amino acid metabolism has been reviewed previously (41); more recent investigations are described below.

Arginine biosynthesis and utilization are the most extensively studied, no doubt as a result of the predominance of the Arg$^-$ Hyx$^-$ Ura$^-$ auxotype among gonococci isolated from disseminated infections. A high proportion of Arg$^-$ strains utilized ornithine in place of arginine (9), suggesting that there were defects in the conversion of α-*N*-acetylornithine to ornithine or in the carbamylation of ornithine to citrulline. Ornithine transcarbamylase activity was found (9) and subsequently characterized (57) in strains that were incapable of growing on ornithine, suggesting that the inability to utilize ornithine was due to other defects. Powers and Pierson (53) purified and characterized ornithine transcarbamylase from *N. gonorrhoeae* and found that the enzyme was similar to that described in other bacteria.

Shinners and Catlin (58) examined Arg$^-$ Ura$^-$gonococcal strains for glutamate acetyltransferase, aspartate transcarbamylase, orotate phosphoribosyltransferase, and carbamyl phosphate synthetase. They discovered that strains incapable of growth on ornithine lacked carbamyl phosphate synthetase. The lack of this enzyme resulted in a concomitant requirement for pyrimidines, since carbamyl phosphate is also a precursor in the biosynthesis of these compounds.

A large number of clinical isolates of *N. gonorrhoeae* have been examined and grouped according to their amino acid requirements (23). These six groups included nonrequiring, Pro$^-$, Orn$^-$, Pro$^-$ Cit$^-$ Ura$^-$, Orn$^-$ Ura$^-$ Hyx$^-$, and Cit$^-$ Ura$^-$ Hyx$^-$ isolates. Several nonrequiring and Pro$^-$ auxotrophs were further studied to determine their requirement for proline and the kinetics of growth on proline (21). Growth in defined medium was very sensitive to proline concentration and to whether starch or fatty-acid-free bovine serum albumin was added as a protective agent. Chen and Buchanan (10) purified and characterized a proline iminopeptidase that allowed Pro$^-$ auxotrophs to grow in a medium consisting in part of proline-containing polypeptides. Proline utilization by nonrequiring, Pro$^-$, and thiamine-requiring auxotrophs was recently studied by Pillon et al. (51). They determined that proline was utilized efficiently as an energy source via reactions involving the tricarboxylic acid cycle.

Aromatic amino acid metabolism has been recently investigated. Some strains of gonococci are sensitive to growth inhibition by phenylalanine (22). This phenylalanine sensitivity was found to be due to feedback inhibition of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate, an enzyme active early in the common pathway of phenylalanine and tyrosine biosynthesis (3). Addition of tyrosine to the medium negated the phenylalanine repression.

ANAEROBIC GROWTH

N. gonorrhoeae proliferates and grows in the presence of a milieu of strict anaerobic organisms. Its normal sites of

infection are the cervix, rectum, pharynx, and genitourinary tract. In addition, gonococcal pelvic inflammatory disease is often a mixed infection with obligate anaerobes (6).

Kellogg et al. (29) reported the growth of *N. gonorrhoeae* under low oxygen tension and its survival under anaerobic conditions. Short et al. (59) reported the survival of both clinical and laboratory isolates of *N. gonorrhoeae* under anaerobic conditions. Of the laboratory strains examined, all maintained viability better at 27°C than at 37°C, and the Arg^- Hyx^- Ura^- strains survived better than strains of other auxotypes. Of 21 clinical isolates examined, 3 were able to grow anaerobically on prereduced Martin-Lewis agar plates; attempts to subculture these isolates anaerobically were not successful. The addition of the electron acceptors nitrate or fumarate or both to the medium did not support the anaerobic growth of these strains.

Knapp and Clark (30) examined 204 strains of *N. gonorrhoeae* for their ability to grow anaerobically with nitrite as the terminal electron acceptor. All strains grew anaerobically with subtoxic concentrations of nitrite. The generation time of *N. gonorrhoeae* growing anaerobically in the presence of nitrite was almost as rapid as that of gonococci growing aerobically in the same medium lacking nitrite; the final turbidity that was achieved anaerobically was comparable to that obtained aerobically. Cytochrome oxidase and nitrite reductase were produced constitutively under both anaerobic and aerobic conditions. There was no growth of *N. gonorrhoeae* with sulfite as a terminal electron acceptor. The ability of gonococci to grow either aerobically or anaerobically may allow them to proliferate on any mucosal surface of the body to which they can attach and may explain how they can be isolated from mixed infections with obligate anaerobes.

Clark et al. (11) compared the outer membrane protein composition of aerobically and anaerobically grown *N. gonorrhoeae* strains by one- and two-dimensional polyacrylamide gel electrophoresis. Anaerobic growth of *N. gonorrhoeae* resulted in both the induction and the repression of outer membrane proteins. The expression of at least three proteins (Pan 1 to Pan 3) was increased during anaerobic growth. Pan 1 and Pan 2 were highly conserved among gonococcal strains in that they had identical apparent molecular masses. During continuous culture, a protein with a molecular mass similar to that of Pan 1 was observed only under anaerobic conditions, whereas other proteins that were expressed under these conditions were also observed under other conditions of nutrient limitation (26). Thus, Pan 1 may be specifically induced by anaerobiosis, and Pan 2 and Pan 3 may be synthesized in response to nutritional stress. In addition, Clark et al. (11) observed at least five other proteins (Pox 1 to Pox 5) that were expressed at higher levels in aerobically grown cells. Anaerobic growth did not significantly alter the expression of other major outer membrane proteins (proteins I, II, III, pilin, and H.8). No apparent differences in lipopolysaccharide composition were observed between aerobically and anaerobically grown gonococci.

The expression of new outer membrane proteins during anaerobic growth indicated that these proteins were under genetic regulation and thus may be involved in colonization or pathogenesis at anaerobic sites. Indeed, Clark et al. (12) have found that serum specimens from convalescent patients with pelvic inflammatory disease or uncomplicated gonococcal infection, but not normal human serum, contained immunoglobulins that strongly reacted with Pan 1 in Western blots.

CONTINUOUS CULTURE

Studies of the pathogenesis of *N. gonorrhoeae* and *N. meningitidis* have classically involved the use of bacteria grown either on solid medium or in liquid culture to log phase. Although they provide important data, these studies suffer from problems that are inherent with the use of plate- or batch-grown cells. Bacterial growing in a closed system (e.g., batch culture) are exposed to a continually changing environment. In contrast, the in vivo environment is relatively constant, as ensured by homeostatic mechanisms of the host. The constraints of using bacteria grown in closed systems can be circumvented by using continuous culture. The chemostat is ideal for strictly controlling growth conditions and providing a uniform, constant environment.

Results of continuous-culture studies have demonstrated that nutrient-limited growth can produce significant alterations in the cell envelope. Peptidoglycan obtained from chemostat-grown gonococci had significantly less O-acetylation than that obtained from batch-grown cells did, although no differences in cross-linking were observed (4). Leith and Morse (33) grew nonpiliated gonococci under glucose-limited conditions and observed decreasing quantities of several outer membrane proteins when the dissolved oxygen concentration was reduced from 54 to 4% of saturation. Subsequently, gonococci grown under glucose limitation at high dissolved-oxygen levels (80 to 100%) became completely serum resistant at specific growth rates greater than 0.3 (45). This decrease in serum sensitivity occurred concurrently with decreasing cell surface hydrophobicity and increasing amounts of lipopolysaccharide serotype antigen. Coincidently, total serum resistance occurred at the point at which glucose was no longer totally depleted.

Manchee et al. (34) grew piliated gonococci in continuous culture in a defined medium at a dissolved oxygen concentration of 20%. The bacteria remained piliated for longer than 21 days and were virulent when evaluated in the guinea pig subcutaneous chamber model. Keevil et al. (26, 27) found that iron-limited gonococci remained piliated and were highly virulent for guinea pig subcutaneous chambers. However, switching to glucose-limited growth resulted in loss of piliation but retention of virulence. In contrast, cystine-limited gonococci were piliated but avirulent. Keevil et al. (28) have recently studied plasmid maintenance in two gonococcal strains grown in a chemostat under glucose limitation. One strain retained the conjugative (24.5-megadalton), β-lactamase (3.2-megadalton) and cryptic (2.6-megadalton) plasmids during 96 generations in continuous culture. The other strain, containing the conjugative, cryptic, and 4.4-megadalton β-lactamase plasmids, lost the conjugative plasmid after 30 generations; the 4.4- and 2.6-megadalton plasmids were lost by 100 generations.

ADDITIONAL COMMENTS

Studies of the physiology and metabolism of the pathogenic *Neisseria* species are important in furthering our knowledge of the interaction of these microorganisms with the human host. Studies similar to those cited in this review will complement studies on the genetics, molecular biology, and pathogenesis of *N. gonorrhoeae* and *N. meningitidis*. A thoroughly integrated approach will enable investigators to solve problems associated with the prevention of infections caused by these microorganisms.

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LITERATURE CITED

1. **Archibald, F. S., and I. W. DeVoe.** 1979. Removal of iron from human transferrin by *Neisseria meningitidis*. FEMS Microbiol. Lett. **6:**159–162.
2. **Archibald, F. S., and I. W. DeVoe.** 1980. Iron acquisition by *Neisseria meningitidis* in vitro. Infect. Immun. **27:**322–334.
3. **Berry, A., R. A. Jensen, and A. T. Hendry.** 1987. Enzymatic arrangement and allosteric regulation of the aromatic amino acid pathway in *Neisseria gonorrhoeae*. Arch. Microbiol. **149:**87–94.
4. **Blundell, J. K., C. W. Keevil, and H. R. Perkins.** 1988. Peptidoglycan structure in *Neisseria gonorrhoeae* grown in continuous culture, p. 499–502. *In* J. T. Poolman, H. C. Zanen, T. F. Meyer, J. E. Heckels, P. R. H. Makela, H. Smith, and E. C. Beuvery (ed.), Gonococci and meningococci. Kluwer Academic Publishers, Dordrecht, The Netherlands.
5. **Britigan, B. E., M. S. Cohen, and P. F. Sparling.** 1985. Gonococcal infection: a model of molecular pathogenesis. N. Engl. J. Med. **312:**1683–1694.
6. **Burnakis, T. G., and N. B. Hilderbrandt.** 1986. Pelvic inflammatory disease: a review with emphasis on antimicrobial therapy. Rev. Infect. Dis. **8:**86–116.
7. **Calver, G. A., C. P. Kenny, and G. Lavergne.** 1976. Iron as a replacement for mucin in the establishment of meningococcal infection in mice. Can. J. Microbiol. **22:**832–838.
8. **Catlin, B. W.** 1973. Nutritional requirements of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. J. Infect. Dis. **128:**178–194.
9. **Catlin, B. W., and E. H. Nash.** 1978. Arginine biosynthesis in gonococci isolated from patients, p. 1–8. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
10. **Chen, K. C. S., and T. M. Buchanan.** 1980. Hydrolases from *Neisseria gonorrhoeae*. The study of gonocosin, an aminopeptidase-P, a proline iminopeptidase, and an asparaginase. J. Biol. Chem. **255:**1704–1710.
11. **Clark, V. L., L. A. Campbell, D. A. Palermo, T. M. Evans, and K. W. Klimpel.** 1987. Induction and repression of outer membrane proteins by anaerobic growth of *Neisseria gonorrhoeae*. Infect. Immun. **55:**1359–1364.
12. **Clark, V. L., K. W. Klimpel, S. Thompson, and J. Knapp.** 1988. Anaerobically expressed outer membrane proteins of *Neisseria gonorrhoeae* are recognized by antibodies present in the sera of PID patients, p. 727–729. *In* J. T. Poolman, H. C. Zanen, T. F. Meyer, J. E. Heckels, P. R. H. Makela, H. Smith, and E. C. Beuvery (ed.), Gonococci and meningococci. Kluwer Academic Publishers, Dordrecht, The Netherlands.
13. **Cohen, M. S., B. E. Britigan, M. French, K. Bean.** 1987. Preliminary observations on lactoferrin secretion in human vaginal mucus; variation during the menstrual cycle, evidence of hormonal regulation, and implications for infection with *Neisseria gonorrhoeae*. Am. J. Obstet. Gynecol. **157:**1122–1125.
14. **DeVoe, I. W., J. Port, B. E. Holbein, and J. M. Ingram.** 1982. Thiosulfate reductase activity in *Neisseria meningitidis*. FEMS Microbiol. Lett. **14:**267–270.
15. **Dyer, D. W., W. McKenna, J. P. Woods, and P. F. Sparling.** 1987. Isolation by streptonigrin enrichment and characterization of a transferrin-specific iron uptake mutant of *Neisseria meningitidis*. Microb. Pathogen. **3:**351–363.
16. **Dyer, D. W., E. P. West, W. McKenna, S. A. Thompson, and P. F. Sparling.** 1988. A pleiotropic iron-uptake mutant of *Neisseria meningitidis* lacks a 70-kilodalton iron-regulated protein. Infect. Immun. **56:**977–983.
17. **Dyer, D. W., E. P. West, and P. F. Sparling.** 1987. Effects of serum carrier proteins on the growth of pathogenic neisseriae with heme-bound iron. Infect. Immun. **55:**2171–2175.
18. **Fohn, M. J., T. A. Mietzner, T. W. Hubbard, S. A. Morse, and E. W. Hook III.** 1987. Human immunoglobulin G antibody response to the major gonococcal iron-regulated protein. Infect. Immun. **55:**3065–3069.
19. **Griffiths, E.** 1985. Candidate virulence markers, p. 193–226. *In* M. Sussman (ed.), Virulence of *Escherichia coli*. Academic Press, Inc. (London), Ltd., London.
20. **Hafiz, S., M. G. McEntegart, and A. E. Jephcott.** 1977. Reversion of Kellogg's colonial types of *Neisseria gonorrhoeae* in liquid medium. J. Med. Microbiol. **10:**377–380.
21. **Hendry, A. T.** 1983. Growth responses of *Neisseria gonorrhoeae* auxotypes to required amino acids and bases in liquid medium. Can. J. Microbiol. **29:**1309–1313.
22. **Hendry, A. T., and J. R. Dillon.** 1984. Growth inhibition of *Neisseria gonorrhoeae* isolates by L-phenylalanine and its analogues in defined media. Can. J. Microbiol. **30:**1319–1325.
23. **Hendry, A. T., and I. O. Stewart.** 1979. Auxanographic grouping and typing of *Neisseria gonorrhoeae*. Can. J. Microbiol. **25:** 512–421.
24. **Holbein, B. E.** 1981. Enhancement of *Neisseria meningitidis* infection in mice by addition of iron bound to transferrin. Infect. Immun. **34:**120–125.
25. **Johnson, A. P., and M. F. Osborn.** 1979. Failure of iron to promote attachment of gonococci to human spermatozoa under physiological conditions. Br. J. Vener. Dis. **55:**329–333.
26. **Keevil, C. W., D. B. Davies, B. J. Spillane, and E. Mahenthiralingam.** 1988. Influence of iron-limited and excess continuous culture on the virulence properties of *Neisseria gonorrhoeae*, p. 727–730. *In* J. T. Poolman, H. C. Zanen, T. F. Meyer, J. E. Heckels, P. R. H. Makela, H. Smith, and E. C. Beuvery (ed.), Gonococci and meningococci. Kluwer Academic Publishers, Dordrecht, The Netherlands.
27. **Keevil, C. W., N. C. Major, D. B. Davies, and A. Robinson.** 1986. Physiology and virulence determinants of *Neisseria gonorrhoeae* grown in glucose-, oxygen-, or cystine-limited continuous culture. J. Gen. Microbiol. **132:**3289–3302.
28. **Keevil, C. W., B. J. Spillane, and N. C. Major.** 1987. Plasmid stability and antibiotic resistance of *Neisseria gonorrhoeae* during glucose-limited continuous culture. J. Med. Microbiol. **24:**351–357.
29. **Kellogg, D. S., J. A. Crawford, and C. S. Callaway.** 1983. Cultivation of *Neisseria gonorrhoeae* under low-oxygen conditions. J. Clin. Microbiol. **18:**178–184.
30. **Knapp, J. S., and V. L. Clark.** 1984. Anaerobic growth of *Neisseria gonorrhoeae* coupled to nitrite reduction. Infect. Immun. **46:**176–181.
31. **LeFaou, A.** 1983. Evidence for thiosulfate sulfur transferase (rhodanese), trithionate reductase, and tetrathionate reductase activities in *Neisseria gonorrhoeae*. FEMS Microbiol. Lett. **20:**279–283.
32. **LeFaou, A.** 1984. Sulphur nutrition and metabolism in various species of *Neisseria*. Ann. Inst. Pasteur (Paris) **135:**3–11.
33. **Leith, D. K., and S. A. Morse.** 1980. Effect of dissolved oxygen on outer membrane protein composition of *Neisseria gonorrhoeae* grown in continuous culture. FEMS Microbiol. Lett. **7:**191–194.
34. **Manchee, R. J., C. N. Wiblin, and A. Robinson.** 1980. Growth of *Neisseria gonorrhoeae* in continuous culture. FEMS Microbiol. Lett. **7:**115–118.
35. **McKenna, W. R., P. A. Mickelsen, P. F. Sparling, and D. W. Dyer.** 1988. Iron uptake from lactoferrin and transferrin by *Neisseria gonorrhoeae*. Infect. Immun. **56:**785–791.
36. **Mickelsen, P. A., E. Blackman, and P. F. Sparling.** 1982. Ability of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and commensal *Neisseria* species to obtain iron from lactoferrin. Infect. Immun. **35:**915–920.
37. **Mickelsen, P. A., and P. F. Sparling.** 1981. Ability of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and commensal *Neisseria* species to obtain iron from transferrin and iron compounds. Infect. Immun. **33:**555–564.
38. **Mietzner, T. A., R. C. Barnes, Y. A. JeanLouis, W. M. Shafer, and S. A. Morse.** 1986. Distribution of an antigenically related

iron-regulated protein among the *Neisseria* spp. Infect. Immun. **51**:60–68.

39. **Mietzner, M. A., G. Bolan, G. K. Schoolnik, and S. A. Morse.** 1987. Purification and characterization of the major iron-regulated protein expressed by pathogenic *Neisseria*. J. Exp. Med. **165**:1041–1057.
40. **Mietzner, T. A., G. H. Luginbuhl, E. Sandstrom, and S. A. Morse.** 1984. Identification of an iron-regulated 37,000-dalton protein in the cell envelope of *Neisseria gonorrhoeae*. Infect. Immun. **45**:410–416.
41. **Morse, S. A., A. F. Cacciapuoti, and P. G. Lysko.** 1979. Physiology of *Neisseria gonorrhoeae*. Adv. Microb. Physiol. **20**:251–320.
42. **Morse, S. A., C.-Y., Chen, A. LeFaou, and T. A. Mietzner.** 1988. A potential role for the major iron-regulated protein expressed by pathogenic *Neisseria* species. Rev. Infect. Dis. **10**:S306–S310.
43. **Morse, S. A., T. A. Mietzner, G. Bolan, A. LeFaou, and G. Schoolnik.** 1987. Characterization of the major iron-regulated protein of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Antonie van Leewenhoek J. Microbiol. **53**:465–469.
44. **Morse, S. A., T. A. Mietzner, W. O. Schalla, C. J. Lammel, and G. F. Brooks.** 1988. Serum and vaginal fluid antibodies against the major iron-regulated protein in women with gonococcal pelvic inflammatory disease or uncomplicated infection, p. 761–765. *In* J. T. Poolman, H. C. Zanen, T. F. Meyer, J. E. Heckels, P. R. H. Makela, H. Smith, and E. C. Beuvery (ed.), Gonococci and meningococci. Kluwer Academic Publishers, Dordrecht, The Netherlands.
45. **Morse, S. A., C. S. Mintz, S. K. Sarafian, L. Bartenstein, M. Bertram, and M. A. Apicella.** 1983. Effect of dilution rate on lipopolysaccharide and serum resistance of *Neisseria gonorrhoeae* grown in continuous culture. Infect. Immun. **41**:74–82.
46. **Norquist, A., J. Davies, L. Norlander, and S. Normark.** 1978. The effect of iron starvation on the outer membrane protein composition of *Neisseria gonorrhoeae*. FEMS Microbiol. Lett. **4**:71–75.
47. **Norrod, E. P.** 1984. A role for sulfite in inducing surface changes in *Neisseria gonorrhoeae*. Can. J. Microbiol. **30**:1297–1301.
48. **Norrod, E. P., and R. P. Williams.** 1978. Growth of *Neisseria gonorrhoeae* in media deficient in iron without detection of siderophore. Curr. Microbiol. **1**:281–284.
49. **Odugbemi, T. O., and S. Hafiz.** 1978. The effects of iron chelators on the colonial morphology of *Neisseria gonorrhoeae*. J. Gen. Microbiol. **104**:165–167.
50. **Payne, S. M., and R. A. Finkelstein.** 1975. Pathogenesis and immunology of experimental gonococcal infection: role of iron in virulence. Infect. Immun. **12**:1313–1318.
51. **Pillon, L., M. Chan, J. Franczyk, and M. Goldner.** 1988. Comparative use of amino acids by three auxotypes of *Neisseria gonorrhoeae*. Antonie van Leeuwenhoek J. Microbiol. **54**:139–148.
52. **Port, J. L., I. W. DeVoe, and F. S. Archibald.** 1984. Sulphur acquisition by *Neisseria meningitidis*. Can. J. Microbiol. **30**:1453–1457.
53. **Powers, C. N., and D. L. Pierson.** 1980. Stabilization and purification of ornithine transcarbamylase from *Neisseria gonorrhoeae*. J. Bacteriol. **141**:544–549.
54. **Schryvers, A. B., and L. J. Morris.** 1988. Identification and characterization of the transferrin receptor from *Neisseria meningitidis*. Mol. Microbiol. **2**:281–288.
55. **Schryvers, A. B., and L. J. Morris.** 1988. Identification and characterization of the human lactoferrin-binding protein of *Neisseria meningitidis*. Infect. Immun. **56**:1144–1149.
56. **Shafer, W. M., and S. A. Morse.** 1987. Cleavage of the protein III and major iron-regulated protein of *Neisseria gonorrhoeae* by lysosomal cathepsin G. J. Gen. Microbiol. **133**:155–162.
57. **Shinners, E. N., and B. W. Catlin.** 1978. Arginine biosynthesis in *Neisseria gonorrhoeae*: enzyme catalyzing the formation of ornithine and citrulline. J. Bacteriol. **136**:131–135.
58. **Shinners, E. N., and B. W. Catlin.** 1982. Arginine and pyrimidine biosynthetic defects in *Neisseria gonorrhoeae* strains isolated from patients. J. Bacteriol. **151**:295–302.
59. **Short, H. B., V. L. Clark, D. S. Kellogg, Jr., and F. E. Young.** 1982. Anaerobic survival of clinical isolates and laboratory strains of *Neisseria gonorrhoeae*: use in transfer and storage. J. Clin. Microbiol. **15**:915–919.
60. **Simonson, C., D. Brener, and I. W. DeVoe.** 1982. Expression of a high-affinity mechanism for acquisition of transferrin iron by *Neisseria meningitidis*. Infect. Immun. **36**:107–113.
61. **Simonson, C., T. Trivett, and I. W. DeVoe.** 1981. Energy-independent uptake of iron from citrate by isolated outer membranes of *Neisseria meningitidis*. Infect. Immun. **31**:547–773.
62. **Tarkka, E., and M. Sarvas.** 1987. Cloning of an outer membrane protein of *Neisseria meningitidis* in *Escherichia coli*. Microb. Pathogen. **3**:445–453.
63. **West, S. E. H., R. Penniall, and P. F. Sparling.** 1988. Polyphosphate-mediated release of iron from transferrin. A possible mechanism for acquisition of iron from transferrin by *Neisseria gonorrhoeae*, p. 837–842. *In* J. T. Poolman, H. C. Zanen, T. F. Meyer, J. E. Heckels, P. R. H. Makela, H. Smith, and E. C. Beuvery (ed.), Gonococci and meningococci. Kluwer Academic Publisher, Dordrecht, The Netherlands.
64. **West, S. E. H., and P. F. Sparling.** 1985. Response of *Neisseria gonorrhoeae* to iron limitation: alterations in expression of membrane proteins without apparent siderophore production. Infect. Immun. **47**:388–394.
65. **West, S. E. H., and P. F. Sparling.** 1987. Aerobactin utilization by *Neisseria gonorrhoeae* and cloning of a genomic DNA fragment that complements *Escherichia coli fhuB* mutations. J. Bacteriol. **169**:3414–3421.
66. **Yancey, R. J., and R. A. Finkelstein.** 1981. Assimilation of iron by pathogenic *Neisseria* spp. Infect. Immun. **32**:592–599.
67. **Yancey, R. J., and R. A. Finkelstein.** 1981. Siderophore production by pathogenic *Neisseria* spp. Infect. Immun. **32**:600–608.
68. **Yeowell, H. N., and J. R. White.** 1982. Iron requirement in the bactericidal mechanism of streptonigrin. Antimicrob. Agents Chemother. **22**:961–968.