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The Meningococcus and Mechanisms of Pathogenicity

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HISTORY AND INTRODUCTION

The meningococcus and meningococcal disease have been curiosities to both scientists and physicians for more than 100 years. Despite the continued high sensitivity of this bacterium to antibiotics, the mortality places meningococcal disease approximately 10th as a cause of death in children in North America. Arresting the growth of the organism in the host by means of antibiotics is, of itself, often inadequate to prevent the progressive pathophysiology and tissue destruction leading to death. To depress further the frequency of mortality from meningococcal disease, a deeper understanding of the disease and of the meningococcus itself is necessary.

A critical shortcoming among those who make an attempt at understanding mechanisms of microbial pathogenicity can be a lack of knowledge about the disease itself. On the other hand, those who are familiar with and study the disease may lack knowledge of the physiology and adaptability of the microbe. This review attempts to present the disease, the microbe, and the dynamic nature of both as they interact during the disease process.

Some aspects of microbial physiology, e.g., intermediary metabolism, are not covered in depth in this review, because the available information in some cases is scanty, or the subject has been covered in other recent reviews. Although it was tempting to review work on the gonococcus, as much of it would certainly apply to the meningococcus, time and space did not permit the inclusion of many of these studies. Therefore, work on the gonococcus was included only where there were direct implications on the meningococcal disease mechanisms or microbial physiology.

Meningococcal disease was first described by Vieusseux in 1805 (286). Two years after his report, an epidemic of "spotted fever" raged through New England, and in that same year heavy losses were sustained in the Prussian Army from the same disease. After the description of these outbreaks, the disease became widely recognized in Europe, in parts of Asia, and in America. In his book on military diseases during the American Civil War, Steiner (270) refers to several reports whose descriptions fit that of meningococcal disease. However, it was not until 1887 that Weichselbaum (291) first described the meningococcus in detail, and Councilman et al. in 1898 (61) found meningococci in 31 of 34 cases of meningitis, firmly establishing this organism as the etiological agent of epidemic cerebrospinal meningitis.

Although much has been written about this gram-negative pathogen and the variety of clinical manifestations of meningococcal disease, little is known of the direct relationship between the physiology and the biochemistry of the microbe, on the one hand, and the pathogenesis of disease on the other. Previous reviews on the

meningococcus and meningococcal disease have dealt primarily with epidemiology (17, 22, 32, 185), pathology (134, 142), physiology of the meningococcus (37, 222, 262), pathogenesis of the disease (19), leukocyte-endotoxin interactions (70), clinical studies (135, 195, 198), genetics (182), ecology of *Neisseria* (127), noncapsular surface antigens (97), and meningococcal vaccines (110, 180). Although the subject of this review does not make it unique among those which have preceded it, with the information of recent years it is possible to take a fresh look at older ideas and a new look at more current ones.

The vast literature on meningococci stems from a curiosity about this bacterium, which harmlessly inhabits the oropharynx of a portion of the normal population without overt symptoms of disease, yet is one of the fastest killers of humans among biological agents. Paradoxically, the meningococcus can cause low-frequency sporadic disease in the population or large and fast-spreading epidemics, such as the recent one in São Paulo, Brazil that lasted from 1970 to 1974 (20, 21). Moreover, the clinical manifestations of meningococcal disease may vary greatly as well, from those associated with chronic meningococcemia at the one extreme (260) to those of fulminant meningococcal disease with associated coagulopathy on the other. The pathological picture also varies widely, depending on the severity of the disease, from relatively insignificant to the involvement of all major organs including the brain.

EPIDEMIOLOGY AND SEROLOGY

The meningococcus has its natural habitat in the mucous membranes of the oropharynx of normal human hosts. Those harboring the organism are usually asymptomatic and are commonly referred to as "carriers." The frequency of carriage in the normal population ranges from 5 to 30% (127) during nonepidemic periods but may approach 100% during epidemics. Before 1950 meningococcal disease occurred in epidemic waves in North America with interepidemic periods of sporadic cases (17, 199). Epidemics often run from 3 to 6 years in duration. During both epidemic and nonepidemic periods, there is a seasonal fluctuation in which the mean monthly incidence is most often at its lowest during the summer and early fall and peaks during February, March, or April in the northern hemisphere. The annual incidence of meningococcal disease in the United States during the years 1968 to 1978 varied from a low of 0.55 cases per 100,000 per year to a high of 1.35 per 100,000 (199). Case-fatality ratios in the United States for meningococcal meningitis and meningococcemia in 1978 were 14 and 25%, respectively. From 1964 to 1968 and from 1972 to date the predominant serogroup isolated from patients has been serogroup B. From 1969 to 1971 serogroup C predominated; serogroup A was only rarely identified (199). The incidence rates during epidemics in the United States before 1950 were less than 15 per 100,000 (199), whereas in the recent epidemic in Norway the overall incidence rate in 1974 and 1975 was high at 26.3 per 100,000 with a peak attack rate of 37.4 per 100,000 cases per year (29). The high attack rate in Norway (150) stands in contrast to those during other recent epidemics in São Paulo, Brazil and Bolton, England, which were 11.3 (20, 68) and 13.7 (90) per 100,000, respectively. Before the advent of sulfonamides, the case-fatality ratio for meningococcal disease was 75 to 90% (19), but with vigorous antimicrobial treatment the case-fatality ratio can be brought below 15%.

Meningococcal disease has been the focus of increasing attention recently due to its changing patterns and the apparent rise in its incidence in several parts of the world where it was not considered previously to be a public health problem. Meningococcal disease continues to be a problem in Africa, and at the same time, deaths due to this disease have risen during the past 10 years in South America, the Middle East, southern Africa, Europe, and Asia (18). Most of these outbreaks around the world are caused by serogroups A and C, but the prevalence of group B is increasing, as evidenced by the epidemics in Bolton (90) and Norway (29).

Neisseria meningitidis has been divided into nine serogroups (A, B, C, D, X, Y, Z, W135, and 29E) on the basis of the immunological specificity of capsular polysaccharides (65). Several articles published recently review the role of these capsular polysaccharides in meningococcal disease (121, 276, 294). In 1968, Frisch (103) observed that the sera from patients with group B disease showed a markedly different bactericidal activity against various group B strains, suggesting that the serum bactericidal activity was directed against antigens other than the capsular polysaccharide common to all group B strains. Antigenic heterogeneity within the group B was also noted by Roberts (249). The meningococci have since been subdivided further into a series of serotypes based upon immunologically distinct outer membrane proteins and lipopolysaccharides (LPS) (306). These noncapsular surface antigens were reviewed recently by Frasch (97). The capsular and noncapsular surface antigens in the immunity to meningococcal disease are reviewed in detail by Gotschlich (121), Triau (276), Weiss and Hill (294), and Frasch (97). Surface antigens can be prepared routinely by a Li^+ extraction of whole cells at

pH 5.8 (50°C). The material released by this method and sedimentable at 100,000 × *g* (2 h) is defined as serotype antigen. With this extraction method 15 separate serotypes have been found. Poolman et al. (240) have studied the outer membrane serotypes of *N. meningitidis* by immunodiffusion and sodium dodecyl sulfate-polyacrylamide gel electrophoresis immunoperoxidase techniques. They have confirmed that serotypes 1, 2, 6, 9, 11, and 12 are protein antigens, whereas serotypes 4, 5, and 8 are LPS. Serotype 2 has been divided into 2a, 2b, and 2c. Serotype 2c was found only in group Y. The existing typing systems (97) are based on group B and C prototype strains, so a higher percentage of B and C are typable than are those from other serogroups. Outer membrane proteins of other neisseriae have been studied by Russell et al. (255). From 30 to 50% of the group B or C isolates are nontypable. This has led to investigations of other methods for further characterization of strains within these serogroups.

Frasch et al. (100) have shown that outer membranes from various serotypes have distinct protein profiles after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Ten distinct gel protein patterns (I through X) have been found among the various serogroups. The serologically nontypable strains exhibit a variety of polyacrylamide gel patterns. Sippel and Quan (265) concluded there was a homogeneity among protein serotype antigens of group A meningococci.

Most serotypes have three or four major proteins in their outer membranes. Using chymotryptic, ^{125}I-peptide mapping, Tsai et al. (277) found that each of the major outer membrane proteins within a strain had markedly different peptide maps. Interestingly, the unique portions of peptides were in the hydrophilic moieties, whereas the peptides common among the strains were in the hydrophobic parts of the proteins.

Further characterization of the serotype system based on the serological diversity among meningococcal LPS was reported by Mandrell and Zollinger (193). With this method LPS-coated sheep erythrocytes were used in a hemagglutination inhibition assay, and eight LPS types were identified. There is more cross-reactivity among the LPS antigens (193) than among the serotype protein antigens (100, 306). Group A meningococcal strains can be distinguished on the basis of either LPS antigens or outer membrane proteins having molecular weights lower than the principal outer membrane proteins (5, 97, 307). Quan and Sippel (245) have recently evaluated a bactericidal assay for serotyping group A organisms.

Zollinger and Mandrell (306) and Jones and Tobin (162) concluded that most group B disease is caused by certain serotypes, mostly type 2. A type 2 protein (141) vaccine protects animals (64) against disease; however, outer membrane proteins from type 2 elicited only low antibody levels in three of five human volunteers, and the antibodies did not have bactericidal activity (308).

From 1964 through 1968 group B meningococci accounted for the majority of disease isolates from United States civilian and military populations, whereas from 1969 to 1971 the majority of isolates were group C. The serotypes during both periods were essentially the same, with serotype 2 accounting for over 50% of disease isolates, whereas non-type 2 strains were found in 78% of the carrier isolates (97, 98, 221). The frequency of serotype 2 isolates from disease-free carriers selected from case contacts or noncontacts was very low.

Aycock et al. (17) pointed out that disease from meningococci was not a simple function of the prevalence of meningococcal carriers. Epidemics have appeared in some populations with a carrier rate similar to that of other populations in which no epidemic ensued. Moreover, epidemics have broken out in populations in which there was no increase in the prevalence of meningococcal carriers in the healthy population. Group-specific carriage rates are of little value in determining the population dynamics of infection by meningococci; however, the serotype-specific carrier rate is a reliable indicator (130).

In the 1960s and early 1970s several meningococcal disease outbreaks occurred in United States military populations. Goldschneider et al. (116, 117) conducted studies on both the organism and host factors associated with the infections. They obtained both cultures and sera from 492 incoming recruits, 54 of whom exhibited bactericidal serum titers of less than 1:4 against the prevalent group C, sulfonamide-resistant case strain, which later was shown to be a serotype 2 (115). Of the 54 individuals, 44 subsequently became carriers, but only 13 were exposed to the group C case strain. Of the six cases of meningococcal disease that occurred in the study group of 492, all were among the 13 susceptibles (as defined by their lack of bactericidal antibody activity) exposed to the virulent strain. Isolates from another military outbreak in 1969 were analyzed by Gold et al. (114); there were 14 cases of group C, 10 of which were serotype 2. The case isolates from a recent outbreak among British military recruits were group B, type 2; however, among the 75% of recruits who came in contact with the group B type 2 cases, 53% became carriers of W135. Approximately one-half of the W135 carriers harbored type 2 strains. In contrast, only 2% of

the case contact carriers were group B serotype 2.

The serotype 2 organisms appear to predominate among the isolates from civilian outbreaks, as well (97). The group C isolates associated with the epidemic in São Paulo, Brazil (1970 to 1972) were predominantly type 2; however, the group A strains from a coincident and continuing epidemic in the same city (1972 to 1974) were nontypable. The outbreak in Alabama reported by Jacobson et al. (155, 156) appears to have been caused by group B serotype 2 organisms. Although the carrier isolates among case contacts were group B, none was serotype 2. The group B isolates obtained during the Bolton, England outbreak were predominantly serotype 15. Broud et al. (40) have concluded recently that in contrast to epidemics which appear to be caused primarily by one serotype, endemic meningococcal disease is caused by a wide distribution of serotypes.

IMMUNITY TO MENINGOCOCCAL DISEASE

Protective antibodies may be acquired either passively by transplacental passage of immunoglobulins or actively as the result of oropharyngeal infection with both capsulated or noncapsulated meningococci (117, 246). Moreover, one cannot rule out subclinical systemic infections as a mechanism for active immunization. The effect of passively acquired immunity is relatively short lived but is effective in protecting infants against meningococcal disease, as evidenced by the low incidence of cases in the first 6 months of life (266). The susceptibility to disease increases progressively during the first 6 months of age (116), which correlates with the progressive decrease in bactericidal activity of sera. The lowest levels of serum bactericidal activity are found between 6 months and 2 years of age, which accounts for the high incidence of meningococcal disease within this age group. Moreover, the progressive reduction in the incidence of disease after 24 months of age correlates with the rise in serum bactericidal activity in children, with adult levels of antibodies developing by the age of 10 years (96).

Protection against meningococcal disease may also result from exposure to cross-reactive antigens from bacteria which bear little or no taxonomic relationship to meningococci. For example, the capsular polysaccharide from *Escherichia coli* strain O7:K1(L):NM is immunologically and chemically indistinguishable from that of the group B meningococcus (171). Neither of these purified capsular polysaccharides induces protective antibodies in human volunteers (171). Several enteric bacteria were found to cross-react serologically with group A and C meningococci, as well as with two types of pneumococci (248). Such cross-reactions were found in both capsular and noncapsular antigens. Furthermore, *Bacillus pumilis* antigens cross-react with those of group A meningococci (248). Very recently, Griffiss and Goroff (131) have found that a naturally occurring galactan, agarose, immunologically cross-reacts with the LPS of a group Y meningococcus, but the same galactan failed to cross-react with heterologous LPS from a group B organism.

The presence of antibodies from cross-reactive antigens could explain the protection in young children against meningococcal disease even though they lack specific bactericidal antibodies to the meningococcus itself. Although meningococcal carriage has been invoked as the mechanism by which natural immunity is acquired against the meningococcus, one could expect children who lack humoral bactericidal antibodies, and therefore specific protection, to develop systemic disease as a result of meningococcal carriage. However, most children between 6 months and 2 years appear to have a general resistance to meningococcal disease (117). It is reasonable that some of this general immunity may come from cross-reactive antibodies to ubiquitous but relatively harmless microbes in the environment.

Natural immunity to the meningococcus is reinforced throughout life by repeated and intermittent carriage of different strains. Although the prevalence of meningococcal carriers in the population is relatively high (5 to 30% during nonepidemic periods), overt meningococcal disease only rarely occurs. Indeed, even during epidemic periods among United States Army recruits when oropharyngeal carriage has reached 95%, the incidence of systemic disease is less than 1% (116). This low frequency of meningococcal disease after oropharyngeal colonization suggests that host rather than parasite factors largely determine its outcome.

The characteristics of the strains carried by adults and children differ markedly. In one study (117), 36 children of 192 tested were found to be meningococcal carriers. Of the 36 strains, 53% were of the lactose-fermenting (*Neisseria lactamica*) variety and only 5 strains were groupable (group B). In contrast, of incoming army recruit populations, 24% were carriers, 67% were groupable (groups B, C, By), and no lactose-positive strains were detected. Of children who acquired *N. lactamica*, more than half exhibited cross-reactive antibodies to groups A, B, and C, as determined by immunofluorescence (109). Only 5% of the children who did not become *N. lactamica* carriers developed detectable antibodies. The conclusion from these studies was

that the population of *Neisseria* carried by children is different from that of adults. The reasons for this difference are not readily apparent but may stem from the variation between adults and children in the oropharyngeal surface environments, i.e., availability of microbial nutrients, nature of microbial attachment sites on host cell surfaces, etc.

Most oropharyngeal carriers not only develop homologous antibodies but also heterologous ones to other meningococcal strains (117). Such heterologous immunity is directed at noncapsular antigens. Several investigators have examined the antibody response to noncapsular antigens induced by carriage of the meningococcus (97, 117, 246, 309). Frasch and Robbins (101, 102) found that serotype 2 antigen was an effective vaccine for protection against meningococcal infection in guinea pigs. Zollinger et al. (309) examined serum antibody responses to group B and C organisms by using a hemagglutination assay. In general, patients with systemic meningococcal disease responded with antibodies against the surface antigens LPS, proteins, and capsular polysaccharides, but group B patients had very low serum antibody response to capsular polysaccharide, although they had strong responses to the other two antigens. Oropharyngeal carriers of group A responded by elaborating antibodies to all three classes of surface antigens, but similar to diseased patients, carriers of group B failed to develop detectable antibodies to the capsular polysaccharide.

In addition, Lowell et al. (188, 189) have demonstrated an antibody-dependent, cell-mediated antibacterial activity in human mononuclear cells. Immune responses to surface proteins other than pili have recently been reviewed by Frasch (97) and, therefore, will not be discussed further here.

OROPHARYNGEAL CARRIAGE

The oropharynx serves as the reservoir for the spread of meningococcal infection within the population; the microorganism is disseminated by means of aerosols (15, 106, 107). Airborne organisms impinge on the mucus-covered surfaces of the oropharyngeal cells, where they adhere and establish a population. As human beings are the only natural host for the meningococcus (127), it seems reasonable to assume that there is some unique property in the human throat, such as a specific receptor, that permits the colonization of the human oropharyngeal mucosa but not that of animals.

Recently Plaut and co-workers (219, 220, 238, 239) described a proteolytic enzyme elaborated by pathogenic neisseriae that cleaves human, but not animal, serum and secretory immunoglobulin A (IgA) of subclasses 1 and 2, although no single meningococcal strain has been identified which will split human IgA of both subclasses. The splitting of the molecule occurs in the hinge region at a critically situated prolyl-threonyl peptide bond to yield an Fab fragment, devoid of antigen-binding capacity, and an Fc fragment. The enzyme is capable of cleaving the antibody whether or not it is bound to its specific antigen. Moreover, the ability to cleave IgA appears to be restricted to human IgA. As secretory IgA is considered an important feature of host defense on mucous membranes, it would be interesting to know the extent to which IgA subclasses 1 and 2 participate in mucosal surface immunity to the meningococcus.

The colonization of the oropharynx by meningococci elicits host antibodies of the three major immunoglobulin classes within 7 to 10 days (117). In many instances such antibodies seem unable to eliminate the homologous strain from the oropharynx, since the host may remain a carrier of a specific strain for weeks or even months. On the other hand, army recruits vaccinated against group C capsular polysaccharide had a marked reduction in carrier acquisition of group C organisms (14, 122, 123). It is possible, although it seems unlikely, that antibodies to capsular polysaccharides are more effective than antibodies to noncapsular antigens in removal of organisms from the oropharynx. As approximately 50% of meningococcal isolates taken from the oropharynx appear to be noncapsulated (102), it is possible that such strains can survive prolonged carriage in the presence of serum antibodies. Ødegaard and Gedde-Dahl (228) found that those harboring gonococci had twice the incidence of meningococcal carriage, suggesting a general susceptibility to the carriage of, or perhaps infection by, pathogenic neisseriae.

The attachment of meningococci to the surface of the oropharyngeal cells has been the center of several studies. Surface pili (fimbriae) of the meningococcus (39, 72, 73, 75, 202, 204) may be required for attachment of organisms to the oropharyngeal surface (63, 97). Salit and Morton (257) and Stephens and McGee (271) found that piliated strains adhered in greater numbers to oropharyngeal cells than did the isogenic, nonpiliated strains. However, the role of pili in oropharyngeal carriage, the effects of secretory antipili antibodies on such carriage, the involvement of pili in twitching motility, and the role of pili in establishing systemic disease have yet to be determined.

Another consideration invariably neglected or ignored is the complex, viscous mucin which continually bathes the surface of the oropharyngeal epithelium. This biochemically complex, highly polar glycoprotein would most likely in-

teract directly with the surfaces of the bacterium and the epithelium during oropharyngeal carriage. The protection it might afford from phagocytosis or the nutrients it might immobilize, e.g., iron, should be a major consideration in the investigation of oropharyngeal ecology. Archibald and DeVoe (11) have reported that iron trapped in mucin is available to the meningococcus, but significant growth occurs only when the "functional siderophores" for iron, citrate, or pyrophosphate are present. Citrate and pyrophosphate are found in body fluids in relatively high amounts (79).

The pili of meningococci, described in several reports (39, 72, 73, 75, 202, 204), have not been tested for their ability to induce protective antibodies. DeVoe and Gilchrist (73) reported that meningococci either from the oropharynx of healthy carriers or from cerebrospinal fluid (CSF) of patients with systemic disease are piliated on initial laboratory culture, but these appendages are generally lost on subsequent subculture on the same medium. Recently McGee et al. (204) discovered that piliation could be maintained on certain rich media. This finding permits experimental studies previously possible only with fresh isolates. The presence of pili on organisms from either carriers or diseased patients suggests a requirement for these structures for survival in the host, whereas they are expendable during in vitro growth. If pili are required for pharyngeal colonization, then the effect of antibodies (especially IgA) against these surface proteins could be of importance. Pili of the meningococcus are reviewed below.

MENINGOCOCCAL DISEASE

According to all data currently available the meningococcus can establish a systemic infection only in normal human hosts that lack serum bactericidal antibodies directed against capsular or noncapsular antigens of the invading strain (97, 116, 117), or in patients deficient in certain complement components. That the bactericidal activity in serum of immune hosts is complement mediated is evidenced by the repeated episodes of meningococcal infections in patients lacking the higher complement components C5, C6, C7, and C8, which are required for immune bacteriolysis (177, 178, 183, 223, 235–237). Moreover, those deficient in IgM (144, 163) appear to be at greater risk from meningococcal infections than normal hosts. The IgM antibody has been identified as the primary one involved in immune bacteriolysis of gram-negative bacteria (88, 209, 210).

As noted above, the carrier acquisition of meningococci alone stimulates the production of antibodies of the three major classes. Griffiss (126) and Griffiss and Bertram (129) have shown that bactericidal activity of IgM and IgG is blocked by IgA. In the initial stages of systemic infection, as the body starts to mount an immune response, the early specific IgA generated permits the growth of the bacterium by blocking immune bacteriolysis. If the meningococcus can cleave and inactivate the human IgA, one would expect the blocking effects of IgA to be minimized. The protease could be detrimental to the survival of the microbe during systemic disease; IgA inhibition of neutrophil chemotaxis is relieved after treatment with IgA-specific protease from *Neisseria gonorrhoeae* (283).

The meningococcus is thought to enter the systemic circulation directly from the oropharynx. This hypothesis is based on the frequent finding of positive blood cultures in patients who have negative CSF cultures. This suggests that the organism must enter the blood stream before it proceeds to the CSF. The establishment of growth in the deeper tissues or systemic circulation of the susceptible host follows the onset of carrier acquisition by only a few days (13, 222), and only rarely do susceptible patients have a prolonged oropharyngeal carriage before the onset of overt signs of disease. The invasion of the blood stream is preceded by an overt oropharyngitis in about one-third of the cases (19), but the role of the meningococcus as a causative agent in pharyngitis has not been determined. There are isolated reports of pure cultures of meningococci in tonsillitis and oropharyngitis (19).

Once the meningococcus has entered the systemic circulation of the susceptible host and has established a population, one of the several faces of meningococcal disease will make itself manifest. Herrick (140) defined three stages of invasion by *N. meningitidis*. He proposed that upon the primary colonization of the oro- or nasopharynx (carrier state) the bacterium enters the blood stream and gives rise to meningococcemia. Concomitantly with the bacteremia, or at various times thereafter, the meningococci metastasize to the skin, eyes, joints, heart, adrenals, or meninges and produce various clinical symptoms. In 90% of cases, meningococcal disease will progress to an inflammation of the leptomeninges (pia and arachnoid) with associated dermal lesions (petechiae or the larger purpura) in approximately half the cases (273). Other forms of meningococcal disease occurring in the remaining proportion of cases have been the subject of a number of investigations (19, 92, 134, 198, 253, 273, 297). In addition to these more well-known forms of meningococcal disease, the meningococcus can be found from time to time in association with diseases in which its role as a primary pathogen is less well docu-

mented. For instance, there are well-substantiated cases of pneumonia in which the meningococcus appeared to be the infectious agent (154, 244). In such cases, the organism is sometimes the only bacterial pathogen in sputum or in lung tissues at autopsy. In several recent reports urethritis caused by *N. meningitidis*, reported earlier by Carpenter and Charles (56), is said to be an ever increasing problem (91, 170, 215).

The spectrum of most meningococcal disease is given in Table 1. The organism is capable of adapting to growth in a variety of host environments. Moreover, the clinical response of the host to meningococcal invasion can vary from a relatively benign picture to the extreme of fulminant meningococcemia with death in hours. The microbial mechanisms in the pathogenicity of the various forms of meningococcal disease have received relatively little attention. It is reasonable to assume that such mechanisms are complex and well adapted to evading the host responses to the invading microbe.

In acute meningococcal infections the disease pursues a variable course. Ordinarily, the bacteremia established by entry of bacteria into the circulation from the oropharynx is followed rapidly by the onset of acute purulent meningitis. In some cases the meninges are not involved but the meningococcemia persists. In either instance eruption of skin lesions is common and is one of the most constant features of the disease (142).

TABLE 1. Various forms of meningococcal disease

Form	Clinical picture	Gross pathology
Common	Pharyngitis (usually subclinical); possible bacteremia; petechiae; meningitis	Purulent leptomeningitis
Chronic meningococcemia	Intermittent fever (every 2 to 3 days); recurrent papules (229, 260); raised erythematous areas; occasional joint swelling (261)	May affect joints, pericardium
Fulminant encephalitis	Sudden onset; vomiting, fever; rapidly progresses to coma; labored breathing; may have petechiae (no purpura); blood pressure normal; meningococci in blood	Brain grossly congested and greatly overweight, purulent meningitis, diffuse capillary thrombosis; small hemorrhages, degeneration of neurons; perivascular leukocytic infiltration.
Fulminant meningococcemia	Pharyngitis; fever; gastrointestinal symptoms; rapid spread of purpura; perivascular collapse; cyanosis	Purpuric hemorrhages in skin; sometimes purulent leptomeningitis; toxic changes in spleen and liver; focal myocardial necrosis; congestion, hemorrhage, edema, capillary thrombosis in lungs; infrequently thrombosis of glomerular capillaries with bilateral cortical necrosis.

PATHOGENESIS OF HEMORRHAGIC PURPURIC SKIN LESIONS IN MENINGOCOCCEMIA

Small hemorrhages in the skin, petechiae, or the larger hemorrhagic lesions, purpura, usually appear within 12 to 36 h after the onset of disease, but in fulminant cases may develop in a few hours (22, 142, 198, 216). Petechial or purpuric lesions are not unique to meningococcal disease or even to gram-negative septicemias but may occur as well in cases of microbial septicemias caused by β-hemolytic streptococci, *Staphylococcus epidermidis*, and *Aspergillus* (216). Nevertheless, the remarkable tendency for hemorrhagic skin lesions in meningococcemia makes them an important feature in this disease (22, 142, 198, 216). Such lesions are observed in approximately half of the cases (19, 134, 273) in the more common forms of meningococcal disease (Table 1), but are present in nearly all cases (80 to 90%) of fulminant meningococcemia (143, 205).

The pathogenesis of the characteristic cutaneous lesions in meningococcal disease has been a curiosity from the time these lesions were first found to be a clinical entity (287). Black-Schaffer and co-workers (28) in 1947 concluded that experimentally produced "meningococcal purpura" in rabbits were cutaneous manifestations of a generalized Shwartzman reaction. A detailed analysis of the pathology of the cutaneous lesions in meningococcemia was carried out by Hill and Kinney (142). The fundamental pathological picture is one of diffuse vascular damage and intravascular coagulation (143). The widespread vascular changes are not restricted to the skin but are found over the serosal and mucosal surfaces, mediastinum, epicardium, endocardium, lungs, liver, kidneys, adrenals, intestines,

and spleen. The vascular changes consist of endothelial damage, inflammation of the vessel walls, necrosis, and thrombosis (142). Early in the development of purpura there is a diffuse dilatation and engorgement of capillaries concomitant with the extravasation of erythrocytes into surrounding tissues (142, 143, 194). Obvious damage to the endothelial lining in capillary vessels is visible. Hill and Kinney (142) reported observing meningococci inside the endothelial cells of sectioned skin. This observation should be viewed with some caution in that the conclusions were based on light-microscopic observations. Margaretten et al. (194) described an electron microscopic study of tissue from a case of fulminant meningococcemia; however, much of the tissue was affected by autolysis.

Superficially, the cutaneous lesions of meningococcal disease and the experimentally induced localized Shwartzman reaction in rabbits appear similar. Indeed, because of the gross visible similarities it has been assumed (66) that the lesions develop by similar mechanisms. The experimentally induced reaction in its classic form shows a conspicuous exudation of acute inflammatory cells, primarily polymorphonuclear leukocytes (PMNs), in the preparatory skin site (195) early after the dermal injection of endotoxin, i.e., long before any hemorrhage (152, 153). On the other hand, high concentrations of exudate cells are not a feature of early pathogenesis of meningococcal purpura (143, 275).

The confusion that arises in distinguishing the pathogenesis of the cutaneous purpura of meningococcemia and the cutaneous Shwartzman reaction stems from the fact that most reports on the histopathology of human lesions are based on autopsy findings. In such specimens the purpura usually show a high concentration of PMNs similar to the picture one observes in the cutaneous Shwartzman reaction. However, an analysis of biopsy material from human purpura during the early stages of development (Fig. 1a) shows capillary dilatation and a conspicuous absence of exudate cells (143, 195). However, the lesion at autopsy (Fig. 1b) is characterized by extravasation of erythrocytes, endothelial necrosis, and a well-developed inflammation (DeVoe and Richardson, unpublished data).

Taken together, all the evidence on the cutaneous purpura of meningococcemia point to a complicated series of reactions. From an analysis of the early histopathological picture from human purpura biopsy specimens, one can see the resemblance to the epinephrine-endotoxic reaction (275) with associated intravascular clotting, a conspicuous absence of exudate cells, extreme vasodilatation, and an extravasation of blood into surrounding tissues. The vasomotor

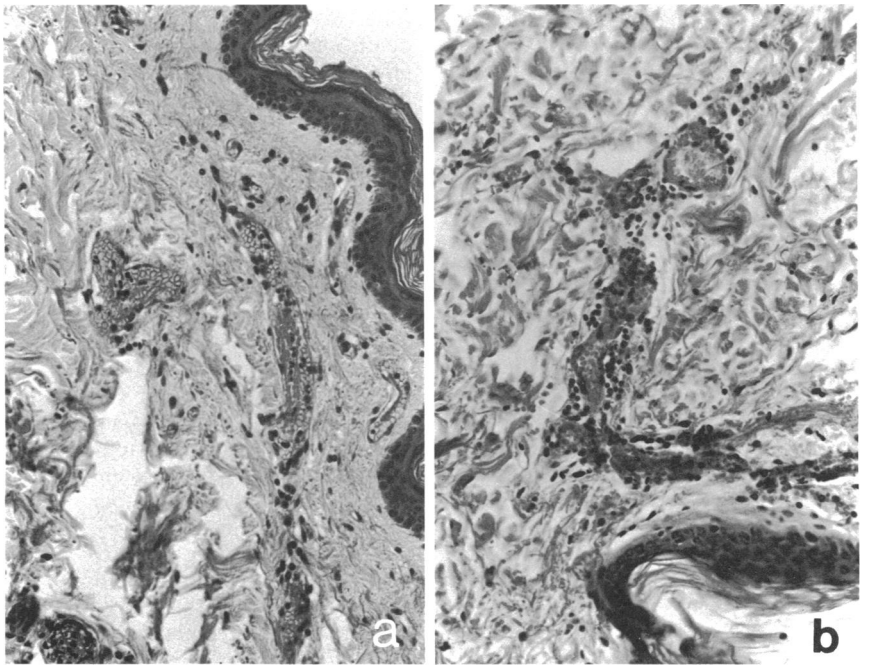

FIG. 1. Light micrographs of sectioned skin purpura from a fatal case of fulminant meningococcemia: (a) biopsy of early purpuric lesion showing congestion but very low numbers of inflammatory cells; (b) purpuric lesion from same patient taken at autopsy. Note large number of inflammatory cells.

dilatation precedes the deposition of thrombi in all organs during the generalized Shwartzman reaction (196, 206, 207), and it seems reasonable that the same would occur in the skin of purpura. As the endothelial cells lining the vessels become progressively more damaged, PMNs sequester in the area, and there is evidence of thrombosis. Margaretten et al. (194) have concluded that the necrotic lesions seen at autopsy in meningococcemia, including bilateral adrenal hemorrhage, are mediated by the blood clotting system and are not due to a direct effect of the meningococcus or its toxin on parenchymal cells. The evidence presented here suggests that the necrosis is brought about by a combination of events which may well include direct action of toxin.

PATHOGENESIS OF SYSTEMIC HEMORRHAGIC LESIONS

The pathogenesis of the hemorrhagic lesions that develop in major organs in meningococcal disease may be the same as that of the cutaneous lesion. These lesions are analyzed at autopsy, so that extrapolations of findings to their pathogenesis can be misleading. For instance, one could presume that the generalized lesions in major organs in meningococcemia are manifestations of a generalized Shwartzman reaction. However, unlike the localized Shwartzman reaction, the generalized one is characterized by the absence of exudate cells in the tissues (143). In contrast, one finds abundant PMNs in autopsy specimens of major organs (particularly lung and liver) from meningococcemia cases, which correlates with the leukocytopenia in severe cases. Therefore, the histopathological picture in major organ tissue from meningococcemia patients appears to be one more closely akin to a series of localized Shwartzman reactions. Hjort and Rapaport (143) conclude that both the generalized and localized Shwartzman reactions operate in meningococcemia, and the relative role of each depends upon the degree of local inflammation, the extent of intravascular coagulation, the functional state of the reticuloendothelial system, and other factors. According to McGehee et al. (205) intravascular coagulation with subsequent deposition of fibrin in small vessels clearly plays a role in the pathogenesis of important clinical manifestations of fulminant meningococcemia.

Hardman (134) studied 200 fatal cases of meningococcal infections from the point of view of the heart, central nervous system, skin, mucous and serous membranes, and adrenal glands. Interestingly, myocarditis, often associated with congestive heart failure, was the most common abnormality observed. Meningitis and mucocutaneous and visceral hemorrhages were also commonly encountered. Adrenal damage, so often mentioned in association with meningococcemia, was encountered least frequently. In contrast, more than 75% of cases had myocarditis, leading Hardman (134) to conclude that myocarditis was a significant factor influencing the fatal outcome of these infections. In an earlier study, Ferguson and Chapman (92) also found that more than 75% of fatal cases had associated myocarditis. Gore and Saphir (120) found myocarditis in 111 of 256 fatal cases of meningococcal disease.

The adrenal gland is affected in 3 to 4% of persons with systemic meningococcal infections. In such cases hemorrhagic destruction of this gland occurs (Waterhouse-Friderichsen syndrome). Ferguson and Chapman (92) concluded that adrenal hemorrhage was only an associated lesion of acute fulminant meningococcemia. Waterhouse-Friderichsen syndrome is apparently more commonly found with *Pseudomonas aeruginosa* infections (196). It is well established that death in fulminant meningococcemia is the result not of adrenal insufficiency but of acute endotoxemia and shock (22, 143, 195, 196, 198).

In a small proportion of cases of fulminant meningococcemic infections the kidney becomes involved. Thrombosis of the renal cortical glomerular capillaries yields to bilateral renal cortical necrosis, the hallmark of the generalized Shwartzman reaction in rabbits. Although the kidneys may be nonfunctional in such cases, renal insufficiency is not a primary factor in death due to endotoxic shock. The detrimental effect on the kidney appears to be secondary to disseminated intravascular coagulation rather than a direct effect of either endotoxin or the meningococcus itself.

The role of the liver in meningococcemia is important both as an endotoxin clearance and detoxifying organ and as a primary responder in endotoxic shock. Existing experimental data indicate that the liver is the most important organ in the clearing and inactivation of endotoxin (281). After fulminant meningococcemia the liver often shows pathology similar to that observed after treatment of experimental animals with large doses of endotoxin. The hepatic sinusoids may appear congested and exhibit a disproportionately high concentration of sequestered PMNs, and small foci of necrosis may appear in such sinusoids as well as parenchymal tissue (70). Kupffer cells, the sessile phagocytic cells lining the sinusoids, appear swollen or show signs of deterioration or necrosis. In severe meningococcemia involving shock, the total volume of blood in the circulation remains unchanged, i.e., there is no significant loss of blood into the tissues (198). The venous return to the heart is diminished, however, due to the

pooling of blood in the liver (134) and mesenteric vessels. Consequently, this reduction in blood volume is an important factor in decreased cardiac output and hypotension.

Experimentally, the introduction of endotoxin into rats produces an increase in plasma thromboxane levels (59). In view of the evidence that thromboxane A2 is a potent vasoconstrictor (258), its presence in plasma during endotoxic shock appears to be an important factor. Evidence to date suggests that thromboxane released during the endotoxemia associated with gram-negative septicemias, e.g., meningococcemia, may be a primary factor leading to shock. The levels of thromboxane in plasma during meningococcemia have not been reported but would provide useful information in the pathogenesis of meningococcal shock. Serotonin (5-hydroxytryptamine), another vasoconstrictor, is released from human platelets treated with endotoxin (137). Salari et al. (256) recently found that meningococcal endotoxin initially stimulates lipooxygenase activity in human PMNs, but after 1 h this activity is inhibited. The consequence of such inhibition is the suppression of leukotriene B_4 synthesis and two other monohydroxy mediators that are produced from the same pathway. The loss of leukotriene B_4 deprives the leukocytes of a strong chemokinetic and chemotactic factor.

There is some evidence to suggest that the coagulopathic manifestations of endotoxemia are due, at least in part, to a direct action of endotoxin on the components of the autonomic nervous system (82, 151). This hypothesis was pursued in the past (see comments by Hopkins and LaPlane [151]) by James Reilly. Ducker and Simmons (82) reported that endotoxin may act directly on the central nervous system to produce remote pathological events. They found that endotoxin introduced into the lateral and third ventricles of dogs produced a massive hemorrhagic pulmonary edema, subendocardial hemorrhages, visceral congestion, and adrenal hemorrhage. These experimental pathological changes were remarkably similar to those of patients dying of meningococcal septicemia. Unlike systemic endotoxic shock, central nervous system-administered endotoxin does not produce alterations in serum enzymes, blood glucose, plasma volume, or extracellular fluid volume (82).

PATHOGENESIS OF MENINGOCOCCAL MENINGITIS

The CSF serves as an excellent culture medium for a number of bacteria. Within hours of its inoculation with bacteria an inflammatory reaction in the leptomeninges becomes evident. Inflammation of the leptomeninges in meningococcal disease is characterized by an inflammatory exudate consisting primarily of PMNs. In meningococcal meningitis the pathological picture will vary with the duration and severity of the inflammatory process. The hallmark of full-blown meningitis is a creamy pus in the subarachnoid space (253). There appears to be no direct invasion of cerebral tissue itself by the exudate, per se, but there are serious changes in the underlying brain. In the extreme, the brain reacts by becoming intensely edematous and congested (19, 253). Most of the nervous system lies outside the brain parenchyma, and the pressure on the brain parenchyma itself is normally low. With edema from intracerebral swelling, the efficiency of venous drainage is hampered, adding to the stagnation and stasis of blood. Therefore, the increased pressure on the brain is not due to an increase in the volume of CSF. This condition is not unique to meningococcal meningitis or that from other gram-negative bacteria but also occurs with *Streptococcus pneumoniae* meningitis as well (253). With fulminant meningococcal meningitis, cerebral findings may consist essentially of severe hyperemia and tissue swelling, with the addition of petechial hemorrhage suggesting endotoxin involvement. After entry of meningococci into the CSF, the outcome is almost invariably fatal without antimicrobial therapy. Therefore, without vigorous antimicrobial treatment in cases of meningitis and acute meningococcal septicemia, one must conclude that the body cellular and humoral defenses are nearly helpless against this organism.

Early workers studied meningococcal meningitis intensively, but with the advent of sulfonamides and antibiotics, wide-spread interest in studies on experimental meningitis disappeared. Among the early workers who investigated the pathogenesis of meningitis were the well-known investigators Councilman, Flexner, and Weed. Weed and colleagues found that removing small amounts of CSF during experimental bacteremia led to the development of meningitis (288, 289). Meningitis could not be produced even with twice the intravenous bacterial inoculum if CSF were not removed. From results in these experiments, Weed postulated that the occurrence of meningitis was facilitated in nature by transient lowering of CSF pressure. This hypothesis has no basis in experimental data, but the role of a pressure differential between blood and CSF is supported indirectly by other workers. It is well established that the endothelial portion of the CSF barrier, i.e., the endothelial blood vessels, is susceptible to experimental breakdown or intervention by certain vasotoxic agents (e.g., nickel chloride, mercuric chloride, lysolectin, or

endotoxin [84, 164, 230]). Such modifications in the increased permeability of the barrier can be measured by the extravasation of intravenous sodium fluorescein dye or the protein horseradish peroxidase into the cerebral parenchyma (133, 135, 230, 282). The question of the passage of proteins into the CSF from the blood is relevant in that the increase in CSF proteins during bacterial meningitis is a characteristic feature (81).

Van Deurs (282) has shown by electron microscopy that during induced acute hypertension in rats the endothelial cells lining neuroarterioles become phagocytic and engulf membrane vesicles containing horseradish peroxidase. After phagocytosis the enzyme appears in the brain parenchymal tissues. During meningococcal septicemia the endotoxin in the blood stream, which causes the release of serotonin (137) from platelets, could produce vasoconstriction leading to a transitory localized or generalized hypertension. Such hypertension could lead to the phagocytosis of bacteria by endothelial cells on the CSF-blood barrier and their translocation into the CSF. Although there is no direct evidence that the meningococci reach the CSF via the blood stream, the frequency with which meningeal infection is preceded or accompanied by bacteremia suggests that the blood stream is an important vehicle by which meningococci, and other bacteria, reach the CSF. Gregorius et al. (125) injected *Staphylococcus aureus* and *Clostridium perfringens* cultures (10^9 organisms) into the carotid artery of rabbits; within 15 min the bacteria could be cultured from the CSF. Moreover, the intracarotid inoculation produced an early and marked extravasation of fluorescein dye into the brain. Unfortunately, the data do not distinguish between the effects from the bacterium, the culture supernatant fluid, or the hyper- or hypotonicity of the culture medium.

The microscopic signs of inflammation during meningitis can vary greatly in meningococcal disease. Classically one observes gram-negative diplococci in and outside of PMNs; however, in practice the numbers of bacteria can range from undetectable to numerous, and PMNs may be found in numbers as low as 1 or as high as 50,000 per m^3. Furthermore, there appears to be no relationship between the clinical outcome of the disease and the number of leukocytes present (81). CSF protein (normal is 15 to 45 mg/100 ml) is usually in the range of 50 to 400 mg but can reach 1,000 mg per 100 ml.

Only a few workers have studied meningococcal meningitis. Flexner in the early 1900s (95) experimented with meningococcal meningitis in monkeys. Branham and co-workers in the 1930s (33–36) also experimented with meningococcal meningitis but with guinea pigs and rabbits. None of these experimental models was representative of the events that occur in human disease. The establishment of an infection in animals took large numbers of meningococci. Moreover, Branham and co-workers (33–36) found that the induction of inflammatory exudates and the damage to the meninges after injection by the intracisternal route could have been due to toxic products of the bacterium rather than to the intact organism. They found that purulent meningitis occurred with the injection not only of live bacteria but also with heat-killed ones. It seems likely that the heat-stable toxic component in these meningococcal cultures was endotoxin.

ANIMAL MODELS FOR MENINGOCOCCAL INFECTION

Several animal models have been used for the study of meningococcal disease. Each provides certain advantages over others, and each simulates to a limited extent some aspect of the disease. None of the models completely satisfies all the criteria required of an ideal animal model. Harter and Petersdorf (135) have listed the criteria for an ideal animal infection model as follows: (i) the portal of entry and route of dissemination of the organism must be similar to those in humans; (ii) the bacterium must be virulent for humans as well as the experimental animal; (iii) the course of disease must be relatively predictable; (iv) the disease must be reproducible within the limits of biological variation; (v) the lesions in the experimental infection must be morphologically similar to those in humans; and (vi) the techniques involved must be relatively simple. One could add to this list a seventh point that the pathophysiological events must be similar to those in humans, particularly if the animals are to be used for detailed studies on pathogenic mechanisms. Unfortunately, it is unlikely that any one animal model can meet all these requirements for studies on meningococcal disease.

One of the early animal models for meningococcal infections was the monkey (95). The mouse model of Miller (213) reported in 1933 has survived without modification to the present time. In this model meningococci are suspended in sterile hog gastric mucin and injected intraperitoneally into mice. Mice injected with virulent strains (214) died of meningococcal septicemia in 16 to 48 h depending on the dose size. The minimum fatal dose in virulent strains was as low as 10 organisms, whereas relatively avirulent strains had minimum fatal doses of from 100 to several thousands of organisms (214). Virulent strains suspended in saline (i.e., no mucin) were unable to produce a fatal infection in mice

unless 10^8 microorganisms or more were applied in the infecting dose. Such numbers were not far below the lethal dose from heat-killed organisms, suggesting that the high numbers of saline-suspended organisms killed mice by intoxication rather than by infection. Interestingly, the presence of sterile mucin in the peritoneal cavity also enhanced infection by the intravenous route, probably due to the high levels of iron in the mucin (see reference 54).

The possibility that the mucin protected against phagocytosis was considered by Miller and Castles (214). Virulent strains were not found in phagocytes in the peritoneal exudates of infected mucin-treated mice, whereas the avirulent strains were readily phagocytosed in the presence of mucin. Therefore, it seems unlikely that the mucin, itself, provides protection against phagocytosis. This same model was used successfully for mouse protection tests with immune anti-meningococcal sera. The method was standardized by Branham and Pittman (37). The basic problem with the model for the mouse protection test is that the effects of mucin in the immune response and clearance are not defined.

The requirement for mucin in the mouse model of Miller and Castles (214) remained unexplained until Calver et al. (54) in 1976 found that mucin may owe at least part of its infection-promoting power to its high concentration of iron. Powell et al. (241) indirectly confirmed these findings: "sub-standard" mucin lots used in *Salmonella*-infected mice became acceptable with the addition of appropriate concentrations of iron. Calver et al. (54) were able to infect mice with low numbers of virulent meningococci by substituting various forms of iron for the mucin itself. The Calver model has the distinct advantage of not requiring commercial preparations of hog gastric mucin, which can vary greatly in their infection-promoting power from one lot to the next.

Holbein et al. (149) found many of the forms of iron used by Calver et al. (54) to be highly toxic to mice, especially to the reticuloendothelial system. Holbein et al. (149) found that iron sulfate, ferric ammonium citrate, and iron sorbitol citrate were each too toxic in mice for use as an infection-enhancing agent. Iron dextran, on the other hand, displayed a low toxicity even at the highest dosage tested (1.6 g/kg) and had the same advantages as the original mucin-mouse model of Miller (213). Moreover, animals that die of infection exhibit pathological features similar to those described for human meningococcemia (92). The Holbein model is able to resolve the relative virulence between meningococci, and relative virulence among strains appears to parallel that of human infections. Disease isolates, especially type 2 strains, are highly virulent, whereas the carrier strains are relatively avirulent (149). The requirement for relatively high doses of iron (250 mg/kg), as dextran, in the Holbein model remains unexplained, but it is reasonable that it is somehow related to the nature of the iron dextran molecule itself. Holbein has suggested that the answer may lie in the fact that iron from dextran does not enter the physiological iron pools as quickly as other forms of iron. Holbein (145) recently reported an iron-controlled infection in the mouse without the addition of exogenous iron (see Iron and Virulence, below).

Attempts to propagate meningococci in germ-free animals (guinea pigs, rats, mice) have been unsuccessful; all animals cleared infections well (M. Pollard and J. P. Doll, Bacteriol. Proc., p. 68–69, 1969). An antecedent pulmonary viral infection was tested for its effect on resistance of mice to infection by meningococcal aerosols (118). The viral infection transiently depressed the murine anti-meningococcal response. There is some evidence to support antecedent viral infections as a factor in meningococcal disease (175, 302).

Chicken embryos have been used for the study of neisserial virulence, endotoxic activity, and passive immunity by several workers (45–47, 49, 80, 93, 94, 99, 278–280). Within 18 to 24 h after the injection of virulent meningococci into a 12-day-old embryo, bacteria are found localized in the brain. When antibodies specific for the infecting strain were mixed with meningococci before infection, the pathogenic effects of the bacterium were neutralized (278). The rabbit eye has also been used as an infection site to study meningococcus-host interactions (242, 243). Payne and Finkelstein (231) were able to divide pathogenic neisseriae into various levels of virulence based on the ability of the bacteria to acquire iron in chick embryos. Recently, Finkelstein and Yancey (94) have used the same model to test the ability of siderophores from meningococci and gonococci to enhance virulence of relatively avirulent colonial type 3 gonococci.

DeVoe and co-workers (70, 77, 78) used rabbits to test products produced from the PMN degradation of meningococci (69, 76) in combination with endotoxin. Minute quantities of PMN-degraded meningococci rendered rabbits highly susceptible to intravascular coagulation and shock by amounts of endotoxin that are normally not lethal in previously untreated animals. Native outer membrane, in the form of membranous blebs (71) (Fig. 2), was equally as effective as purified endotoxin in the reaction. The histopathological picture in rabbits was one resembling that in patients having died in shock from fulminant meningococcemia. PMN-degrad-

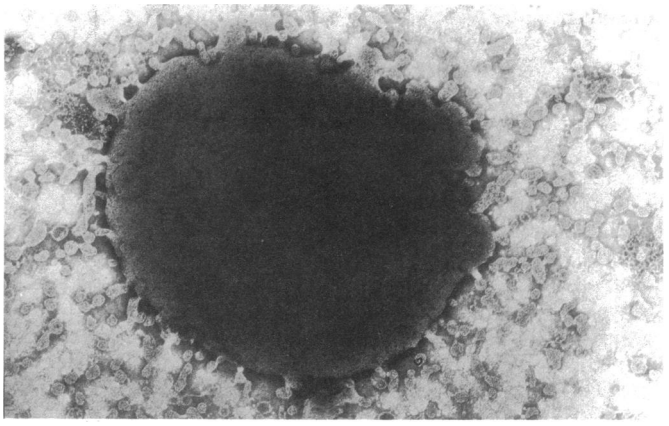

FIG. 2. Release of outer membrane blebs from a meningococcus taken from an actively growing culture. Negative stain; ×40,000. From DeVoe and Gilchrist (71).

ed, particulate meningococcal material was responsible for the depletion of the reserve of mature PMNs and for the destruction of much of the macrophage population in the animals. The destruction or severe compromise of the two lines of cell defense in the rabbit from minute quantities of PMN-degraded meningococci have significant implications. Not only would such animals be severely hampered in their ability to detoxify endotoxin, but they would also be compromised in their ability to fight infection. Evidence for such host compromise was shown (78) when a latent protozoan infection in the rabbits flared into a nosematosis after animals were injected with PMN-degraded meningococci. The overwhelming bacteremia and intoxication in fulminating meningococcemia is evidence of the fact that such patients are incapable of fighting infection or detoxifying the endotoxin released from the bacterium. The studies of DeVoe (70) on endotoxin-PMN interactions have been discussed in a recent report.

MENINGOCOCCAL PHYSIOLOGY AND PATHOGENESIS OF DISEASE

In their simplest form the requirements for microbial survival upon entry into body fluids (for information on growth of meningococci in human serum, see Holbein [148]) or tissues do not differ greatly from those any microbe faces in a new environment except that the animal host, as opposed to a lake, for example, has a preprogrammed response against invading organisms. Successful pathogens, such as the meningococcus, must have mechanisms to overcome such responses of the host. The fatality rate in meningococcal disease before the age of antibiotic or sulfanamide therapy was sufficiently high (75 to 90%) for one to conclude that the meningococcus does indeed possess the required mechanisms to overcome or evade all host defenses.

Most literature treats virulence of the meningococcus as a fixed property in given strains. Virulent strains are considered to be those which in low numbers cause disease in some animal model, whereas avirulent strains are those that are either incapable of producing disease in an animal model or require relatively large numbers of organisms to do so. The observation that most hosts can harbor "virulent" serotypes of meningococci in the oropharynx but show no overt signs of disease is interpreted in the framework of a strong host defense (bactericidal antibody) specifically directed at, or cross-reacting with, the meningococcus. On the other hand, during epidemics only certain serotypes are routinely isolated from diseased patients, suggesting that such microbes possess certain fixed virulence properties. The evidence in support of both serum bactericidal host resistance and the virulence associated with specific serotypes is convincing and has gained general acceptance. In contrast, the spectrum of manifestations of meningococcal disease during epidemics has not been adequately explained. It is possible that certain patients have little or no defense whatever against the invading meningococci and, therefore, fall victim to fulminant meningococcemia, whereas others with stronger defenses have less severe forms of the disease. The past histories of those with fulminant disease do not support the conclusion, however, that they were unable to defend against bacterial infections in general. Therefore, one must consider either a transitory anergy, such as that brought on in some patients by surgery or traumatic injury (208), or a more virulent organism

that can evade host defenses, obtain required nutrients from the host more efficiently, or both.

There is no evidence to support a transient state of anergy associated with the onset of meningococcemia. However, there is evidence that environmental changes around the meningococcus can greatly influence the metabolism and phenotypic expression of the organism. Brener et al. (38) grew a strain of meningococcus at its lowest tolerable pH (6.6) and under conditions of iron starvation. The phenotype of the meningococci grown at low pH and limited for iron in Mueller-Hinton broth changed dramatically as compared to those grown under routine iron-sufficient conditions at neutral pH. The relative virulence index for the mouse increased 1,200-fold from a 50% lethal dose of 3,600 to one of 4 organisms (38). In vitro experiments showed the bacteria to be resistant to phagocytosis by both mouse and rabbit PMNs, whereas the same strain grown conventionally was readily phagocytosed (Brener and DeVoe, unpublished data). In addition, the colonial morphology of this organism changed from an M1 type (see reference 75 for discussion of colonial types), exhibited by cells grown at neutral pH on Mueller-Hinton agar, to a rough M3 colonial type when grown at low pH (6.6). The rationale for these experiments was based upon the low pH associated with pharyngitis, a frequent consequence of heavy pharyngeal carriage of meningococci (19), and large quantities of lactoferrin, an iron scavenger, released to the environment from PMNs during inflammation (284). It is noteworthy that the pH of the CSF decreases during meningitis (181) as well. There is also some indirect evidence for meningococcal phenotypic shifts in vivo. Griffiss et al. (128) reported a recurrent infection in a child who developed bactericidal antibody against the invading strain. In the recurrence the serotype and serogroup were identical, leading the authors to suggest that in vivo alterations in the meningococcus were the result of environmental changes in the host.

There are, no doubt, other phenotypic changes that occur as a result of iron starvation or low pH or both. Morse and Hebeler (218) found in gonococci that the tricarboxylic acid cycle is active only when cells are grown at low pH (6.0), and that cells preferentially metabolize glucose via the pentose phosphate pathway. The same cells grown at pH 7.2 or 8.0 metabolize glucose primarily by the Entner-Doudoroff pathway. Norqvist et al. (224) reported that starvation of gonococci for iron induced the synthesis of an outer membrane protein in the molecular weight range of the ones reported by Simonson et al. (264) and Brener et al. (38) in iron-starved meningococci. Richardson and Sadoff (247) have found capsule production on gonococci when they are grown on an agar medium in the presence of a lactic acid-producing bacterium. Cells grown at low pH may be more stable and less susceptible to autolysis because the peptidoglycan is less prone to hydrolysis at low pH (290). However, the most dramatic phenotypic change expressed after growth of meningococci in iron-limited media was the expression of a highly efficient mechanism to remove iron from human transferrin, the only ready source of iron in the blood (263). The expression of the iron-uptake mechanism correlated with the appearance of a 69,000-dalton protein in the outer membrane. This and other meningococcal iron uptake mechanisms are discussed in Iron and Virulence, below.

The metabolic responses of pathogenic neisseriae to the low pH and low iron are substantial. All of the changes reported to date would contribute to the survival potential in the host. It would appear that the organisms respond metabolically to the inflammatory reaction in the host by becoming hardier and more virulent.

IRON AND VIRULENCE

With a simple defined medium (*Neisseria* defined medium) Archibald and DeVoe (9) were able to grow meningococci in continuous culture in a chemostat in which the generation time could be controlled by either oxygen or iron limitation. As the level of iron in the medium was lowered below 1 μM, generation times became inversely related to the amount of iron in the medium. Cells could be maintained at equilibrium in the chemostat with iron as low as 0.1 μM with a generation time of 164 min, as opposed to one of 55 min in chemostat cultures in which iron was in excess. Cells grown under iron-deficient conditions responded with an increased oxygen consumption per cell doubling, a decrease in total cellular catalase activity, and a reduction in all forms of cellular iron, e.g., cytochromes and nonheme iron proteins. At iron levels below 0.08 μM cells in the chemostat were unable to divide. Therefore, the minimum iron required for cellular division was established at 0.08 μM.

Archibald and DeVoe (11) found that iron-deprived, chemostat-grown cells could use citrate-complexed iron as readily as iron in the form of an inorganic salt. The uptake of iron from citrate or inorganic salts proceeded in two stages: an energy-independent, very rapid initial uptake, followed by a slower, energy-dependent incorporation of the iron (12). Subsequently, Simonson et al. (264) showed that the citrate was not taken up concomitantly with the iron. Isolated outer membranes (in which the respiratory capacity was completely absent) from iron-starved cells were also capable of removing iron

from citrate. After isolated outer membranes removed the radioactive iron from citrate, the radioactivity was found in association with the 36,000-dalton major outer membrane protein (264). Whether this protein was directly involved in the removal of iron from the citrate or was merely a step in the processing of iron after its uptake is unknown.

Certain microorganisms have been shown to acquire their iron through the production of low-molecular-weight iron-binding compounds known collectively as siderophores (176). These compounds fall generally into two major chemical classes: the hydroxamates and the phenolates. A number of workers have shown that the production of siderophores by pathogenic organisms correlates with their ability to infect animals or grow in serum (48, 94, 176, 252, 292, 293). Until recently siderophore production among the neisseriae has gone undetected (11, 225, 293). Very recently, Yancey and Finkelstein (299, 300) reported low levels of dihydroxamate-type chelators in supernatant fluids of both gonococci and meningococci grown in batch culture for extended periods of time. They have proposed that the low amounts of siderophore found in culture fluids may be due to the fact that it is cell associated and that the siderophore is released to the medium in late cultures as a result of cell lysis (300). If such siderophores are cell surface associated and released to culture fluids only upon cell lysis, this could account for their remaining undetectable in culture fluids from other laboratories (10, 11, 293).

In view of the small amount of cell-free siderophore produced by the pathogenic neisseriae (300), it seems unlikely that the release of such compounds into the blood stream would be an advantage to these organisms. On the other hand, a system for iron acquisition or mobilization which utilizes plentiful iron-binding compounds produced by the host would provide a highly efficient, energy-saving method for obtaining this vital nutrient. If the meningococcal chelator reported by Yancey and Finkelstein (299) is cell associated, as they suggest, the iron on molecules, such as citrate or pyrophosphate, could be readily removed and incorporated into cells by such siderophores. Archibald and DeVoe (11) presented results which suggest that citrate and pyrophosphate molecules, available in body fluids at concentrations of approximately 120 and 200 μM, respectively, act as functional siderophores in that they are capable of mobilizing iron from host molecules not directly subject to iron removal by meningococci. Such a mechanism could be particularly useful in purpuric lesions where there is hemostasis and destruction of host tissues.

Most iron in the vertebrate host is retained inside cells in association with storage molecules (e.g., ferritin) or functional molecules (e.g., cytochromes, hemoglobin, and nonheme iron proteins). In a survey which included 20 meningococcal strains chosen for their wide range of virulence in mice, Archibald and DeVoe (11) tested the ability of these organisms to utilize iron complexed to a number of organic acids, various host molecules, and siderophores from other microorganisms.

Meningococci are able to use various forms of iron as their sole source of iron. The effects of certain iron complexes on the growth of *N. meningitidis* are as follows. Microbial Fe siderophores, precursors, or related substances that do not support growth include: desferal, ferrichrome, rhodotorulic acid, enterochelin, 2,3-dihydroxybenzoate, dihydroxyphenylalanine, and glutamate monohydroxamate (11). Microbial Fe siderophores, precursors, or related substances that do support growth are: meningobactin (301), gonobactin (301), and salicylhydroxamate (11). Synthetic iron chelators that support growth include: diethylenetriamine pentaacetate, *N*-(2-acetamido)-iminodiacetate, ethylenediaminetetraacetate, nitrilotriacetate, and ethyleneglycol-bis(β-aminoethylether)-*N*,*N*′-tetraacetate. Organic acid-iron complexes that act as functional siderophores for meningococci include citrate, *cis*-asconitate, isocitrate, ketomalonate, malate, oxalate, pyruvate, adenosine di- or triphosphate, and glycerophosphate (11). Meningococci will grow with the animal iron molecules hog gastric mucin (11), myoglobin (11), hemoglobin (11), transferrin (10, 212, 263), and lactoferrin (211) as sole sources of iron, but growth will not be supported by cytochrome *c* (11), ferritin (ethylenediaminetetraacetate dialyzed) (11, 53), or conalbumin (10, 231).

Free iron, i.e., uncomplex iron, in the normal host is maintained at extremely low levels, $<10^{-18}$ M (47, 289), far below the 8×10^{-8} M established as a minimum to sustain cell division of meningococci (9). Calver et al. (53) found that ferritin, a source of iron in very low concentrations in serum but found in abundance in cells as a iron storage form, not only does not support growth of the meningococci but inhibits their growth.

The glycoprotein transferrin (80,000 daltons) is present in serum in relatively high concentrations (2.5 mg/ml; 32 μM) and under normal conditions is maintained at 30% saturation (two iron sites; 18 μM Fe^{3+}). However, the Fe^{3+} on transferrin is held tightly ($K_a = 10^{37}$) and is not readily available to microorganisms that lack special mechanisms for its acquisition. However, Archibald and DeVoe (10) showed that me-

ningococci can obtain iron from transferrin for growth, but if the Fe-transferrin were separated from the cells by a dialysis membrane with a 12,000-dalton exclusion limit, cells were unable to grow. As mentioned above, Brener et al. (38) found that meningococci grown under low pH and iron deprivation greatly increased in virulence, and Simonson et al. (263) found that these iron-starved cells expressed a mechanism on the cell surface that specifically recognized the glycoprotein transferrin. Once the transferrin was recognized the iron was removed by a mechanism requiring a functioning respiratory chain. This iron acquisition system is heat sensitive (60°C, 5 min), trypsin sensitive, sensitive to inhibitors of the respiratory chain, specific for transferrin, and saturable at concentrations of transferrin approximating half that found in normal serum. Moreover, the initial rates of iron uptake from transferrin increase with the onset of iron deprivation of meningococci until 4 h, at which time the uptake rate is maximal (264). Using plate assays, Mickelsen and Sparling found that all meningococci and gonococci were able to scavenge iron from transferrin (212) and lactoferrin (211), whereas most commensal neisseriae were not.

The rapidly accumulating data indicate that the ability of meningococci to obtain iron from transferrin may be an essential feature of their pathogenic nature. These microorganisms have apparently evolved a mechanism specifically aimed at the acquisition of iron from this host molecule. The mechanism does not require cell-free siderophores but depends on the recognition of the transferrin molecule itself at the microbial cell surface. Whether siderophores associated with the meningococcal surface are involved in the removal of iron from transferrin is unknown.

Holbein (147) has reported an interesting series of experiments in mice. The type 2 meningococcus, M1011, injected intraperitoneally without mucin or other adjuncts, rapidly produced a bacteremia that peaked by 6 h with approximately 10^5 colony-forming units per ml of blood (145). Subsequently, viable counts in the blood decreased rapidly until by 18 h animals completely cleared infections. The clearance of meningococci from the blood corresponded to a concomitant decrease in serum transferrin iron, although the amount of transferrin in the blood remained constant throughout the course of the experiment (24 h). When iron dextran was used as an adjunct to infection, the outcome of the experiment, i.e., death or clearance of bacteria, was directly related to the amount of iron on the dextran: increases in iron prolonged infections to death. Moreover, the iron from the dextran appeared on serum transferrin, which was then used by meningococci to support growth in the blood stream (145). In animals that received no iron dextran, the injection of human iron transferrin into the animals prolonged infections until the animals died (146). From these results Holbein (145) hypothesized that *N. meningitidis* obtains iron for growth from the transferrin pool.

Van Snick et al. (284) have postulated that as a part of the inflammatory process PMNs in the peripheral circulation excrete lactoferrin which removes iron from transferrin. The iron on the lactoferrin, according to the proposal, is then sequestered in the liver where it is inaccessible to microorganisms. Recent experiments of Letendre and Holbein (E. D. Letendre and B. E. Holbein, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, CCI, II 14, p. 90) suggest quite a different mechanism. They have found that the transferrin iron in mouse serum has a 50% turnover rate of approximately 1 h. During meningococcal infection in mice the iron removal rate from transferrin is normal; however, the transferrin is not reloaded in the process. Therefore, the end result is a rapid net loss of serum transferrin iron.

RESPIRATORY CHAIN

The ability of cells to produce energy is vital to their existence. Therefore, the mechanisms by which pathogenic organisms produce energy from host nutrients during infection and disease is central to an understanding of the microbial survival mechanisms in the host. A case in point is the necessity for a functional respiratory chain for the uptake of iron from host transferrin (10, 263) as well as for the active accumulation of iron from other molecules including citrate (11, 12, 264).

The respiratory chain of the neisseriae has been the subject of a number of investigations (165–167, 174, 269, 295, 302–304). Some of these investigations have concentrated primarily on the taxonomic significance of tetramethylphenylenediamine-oxidase activity of these organisms (86, 119, 165–167), whereas other investigators have studied the interrelationships of respiratory chain components (166, 174, 295, 302–304). The major oxidases in meningococci are cytochrome types *o* and *a*, which bind CO (304) and are inhibited by cyanide, azide, and hydroxylamine (302). A third oxidase, which is associated with cysteine-oxidase activity, is sensitive to cyanide but completely unaffected by azide (303). The electron transport chain (Fig. 3) of the meningococcus proposed by Yu and DeVoe (303) is branched. Interestingly, the

tetramethylphenylenediamine-oxidase activity, which has become a taxonomic feature of the neisseriae, involves only two cytochromes in the proposed scheme: cytochromes c_{552} and *o*. The cytochrome *a* oxidase predominates in early-log-phase cultures where dissolved oxygen levels are high (302). As cultures progress into late log phase, where dissolved oxygen is greatly reduced, the cytochrome *o* oxidase predominates.

DeVoe and Gilchrist (74) reported high specific activity of ascorbate–tetramethylphenylenediamine-oxidase activity in outer membrane blebs which were devoid of measurable dehydrogenase or oxidase activities for succinate or reduced nicotinamide adenine dinucleotide (cytoplasmic membrane markers). The relationship of this activity to the scheme presented in Fig. 3 is unknown.

A soluble *c'* cytochrome was also found (304) in the meningococcal cell-free supernatant fraction (365,000 × *g*, 3 h). This cytochrome bound CO when reduced and NO in both the reduced and oxidized state. The function or association of this cytochrome with the membrane-associated electron transport chain is unknown.

SULFUR METABOLISM

L-Cysteine oxidase activity in neisseriae was first reported by Tauber and Russell (274). The enzyme was localized in the membranous fraction of meningococci by Yu and DeVoe (303). This oxidase activity was limited to L-cysteine among the L-amino acids, unlike the broad-spectrum L-amino acid oxidases described for other bacteria (58, 60, 83, 232). The membrane-associated cysteine oxidase may be of special importance to cells because of the theoretical ability of the cells to produce energy merely by the oxidation of cysteine to cystine at their membrane surfaces. Electrons from such oxidation could enter the electron transport chain and translocate protons from within the cell to the exterior. It is not known whether this oxidase functions as a part of a transport mechanism for cysteine-cystine or exists merely as a device to provide energy without the necessity for intermediary catabolism of substrates.

DeVoe and co-workers (FEMS Microbiol. Lett., in press) have recently found thiosulfate reductase activity in cell-free soluble extracts of meningococci. This activity is not sensitive to oxygen in the absence of substrate but is irreversibly lost when oxygen is present during the enzymatic reduction of thiosulfate. The role of this oxygen-labile enzyme activity in this "obligate aerobe" is interesting. Whether such activity is involved in an assimilatory or a respiratory reduction is unknown at this time. Le Faou (179) reported a thiosulfate reductase in *N. gonorrhoeae* that compensates for the cysteine requirement in that organism (57). It was generally accepted that all *N. gonorrhoeae* strains have an

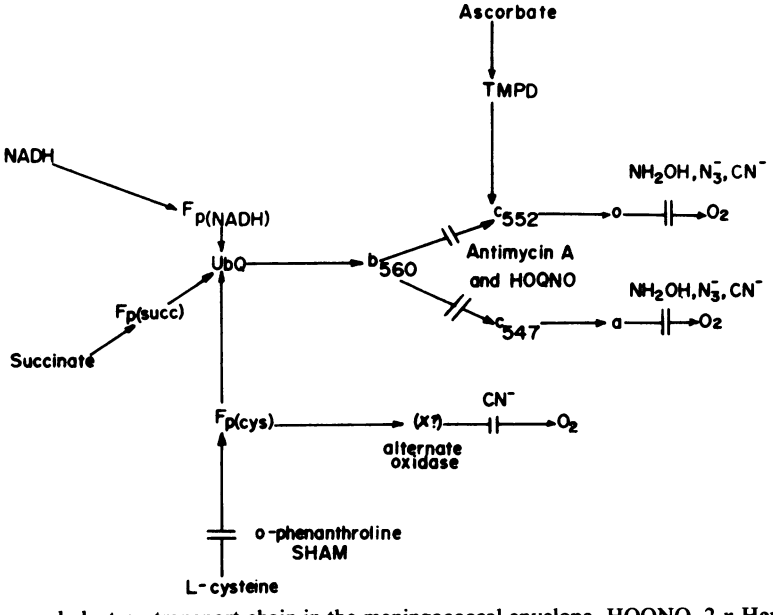

FIG. 3. Proposed electron transport chain in the meningococcal envelope. HOQNO, 2-*n*-Heptyl-4-hydroxyquinoline-n-oxide; SHAM, salicylhydroxamate; UbQ, Ubiquinone. From Yu and DeVoe (303).

absolute requirement for cysteine or cystine (55, 57), whereas cysteine is not an absolute requirement for *N. meningitidis*, *N. lactamica*, or nonpathogenic neisseriae (57, 201). Port and DeVoe (unpublished data) have found that several organic (cysteine, glutathione, lanthionine) and inorganic forms of sulfur (SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, $S_2O_4^{2-}$, $S_2O_6^{2-}$, SCN^-, HSO_3^-) will serve as sole sources of sulfur for growth of meningococci in a defined medium. The form or forms most commonly used in host fluids is, as yet, unknown.

CARBON DIOXIDE REQUIREMENT

Two enzymes have been described for *Neisseria* species that are known to assimilate CO_2. Carbonic anhydrase, which catalyzes the hydration of CO_2 to H^+ and HCO_3^-, was found in bacteria by Veitch and Blankenship (285) and is present in most neisseriae (259). The second enzyme, phosphoenolpyruvate carboxylase, a cytoplasmic enzyme, has been reported for pathogenic *Neisseria* species (62, 138, 168). The 30,000-molecular-weight carbonic anhydrase from *Neisseria sicca* was studied in detail by Adler et al. (4) and Brundell et al. (41), and their findings suggested that the enzyme is surface bound. MacLeod and DeVoe (190) have shown that the enzyme is rather loosely associated with the cytoplasmic membrane. The inhibition of carbonic anhydrase with the sulfonamide acetazolamide can be reversed with an excess of CO_2 or HCO_3^- but not with *p*-aminobenzoate. The requirement for CO_2 in the neisseriae, including the gonococcus is discussed further by Morse et al. (217).

The role of carbonic anhydrase in the metabolism of the neisseriae is not known. The inhibition of this enzyme by acetazolamide prevents growth of the organisms, including the meningococcus, indicating that a vital cellular function has been interrupted. It seems highly unlikely that the cell would require HCO_3^- merely for its carbon when such cells live in an environment abundant in organic nutrients, including CO_2 and HCO_3^-. Rather, the usefulness of this membrane-bound enzyme could lie in its involvement in the regulation of ion movement across the cytoplasmic membrane or in its ability to concentrate either protons or bicarbonate ions at the cell membrane. Without further research, the role of this vital and interesting enzyme in the neisseriae remains a matter for speculation.

CARBOHYDRATE METABOLISM

The intermediary metabolism of the neisseriae has been reviewed recently in detail by Morse et al. (217). These authors have covered publications relating to the metabolism of glucose, pentose, lactate, pyruvate, acetate, and tricarboxylic acid intermediates.

MENINGOCOCCAL SURFACE CAPSULES

Capsules produced by *N. meningitidis* are chemically and immunologically distinct for each serogroup (Table 2). The surface polysaccharides of groups A and C have provided practical, effective vaccines against meningococcal disease in children and adults (110). Roberts (250) reported that the anticapsular antibodies in serum against group A and C polysaccharides are responsible for most of the opsonic activity against meningococci. Kabat et al. (169) in 1945 attempted to induce in human subjects antibody to meningococcal capsular polysaccharides, but failed, probably because of the small molecular weight of their preparations (<50,000 daltons), which failed to stimulate antibody. Gotschlich et al. (124) developed an extraction and purification method that yielded polysaccharides from groups A, B, and C with molecular weights of >100,000. Although the polysaccharides from groups A and C have proven highly antigenic in humans (30, 31, 111–113, 122, 191, 192, 233), the group B meningococcal polysaccharide is a poor immunogen in human volunteers (121, 298). The capsular polysaccharide from group Y meningococci was found to produce high levels of bactericidal activity in humans (89).

The immunological response of human subjects to polysaccharides of groups A and C depends on a number of factors, e.g., age, dose level, number of injections of the vaccine, prior exposure to cross-reacting antigens, and the molecular weight of the polysaccharide preparation (110). Studies on the efficacy of these vaccines are reviewed by Gold and Lepow (110). The group C vaccine is effective in children older than 2 years and in adults (14, 16, 108).

Masson et al. (197) have studied the importance of the capsule of group B meningococci in their virulence for mice. Wild type cells and spontaneous isogenic mutants deficient in polysaccharide production were examined for their virulence. The loss of polysaccharide biosynthetic capability in mutants caused a dramatic loss in virulence which could be regained upon reversion to the wild-type phenotype.

LIPOPOLYSACCHARIDE (ENDOTOXIN)

The LPS (endotoxin) of the meningococcus resides primarily in the outer membrane (71, 305). During log-phase in vitro growth, the meningococcus oversynthesizes its outer membrane relative to the remainder of the organism,

TABLE 2. Capsular polysaccharides of the meningococci

Serogroup	Components[a]	Structural repeating unit	Reference
A (homopolymer)	ManNAc, phosphate, NAc and OAc	ManNAc-(1-P $\xrightarrow{\alpha}$ 6)--- 3 OAc[b]	52, 124, 187, 251
B (homopolymer)	NeuNAc	NeuNAc-(2 $\xrightarrow{\alpha}$ 8)---	25, 124, 136, 157, 186
C (homopolymer)	NeuNAc, OAc	NeuNAc-(2 $\xrightarrow{\alpha}$ 9)--- 7 8 OAc OAc[c]	25, 124, 157, 186, 251
W-135 (disaccharide repeating unit)	Gal, NeuNAc	6-D-Gal(1 $\xrightarrow{\alpha}$ 4)-NeuNAc(2 $\xrightarrow{\alpha}$ 6)---	26, 157
X	GlcNAc, phosphate	DOGlcNAc(1-P $\xrightarrow{\alpha}$ 4)---	8, 50, 51, 52, 251
Y (Bo) (disaccharide repeating unit)	Glc, NeuNAc, OAc	6-D-Glc(1 $\xrightarrow{\alpha}$ 4)-NeuNAc(2 $\xrightarrow{\alpha}$ 6) O-Ac[d]	7, 26, 251
Z (monosaccharide-glycerol repeating unit)	GalNAc, glycerol phosphate	D-GalNAc, (1 $\xrightarrow{\alpha}$ 1')-glycerol-(3'-P $\rightarrow$ 4)---	160
29-e (disaccharide repeating unit)	GalNAc, KDO, OAc	D-GalNAc(1 $\xrightarrow{\beta}$ 7)-KDO(2 $\xrightarrow{\alpha}$ 3)--- 4,5 O-Ac	23, 24

[a] The abbreviations used are: Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine (2-acetamido-2-deoxy-D-glucose); KDO, 3-deoxy-D-manno-octulosonic acid; ManNAc, *N*-acetylmannosamine (2-acetamido-2-deoxy-D-mannose); NeuNAc, *N*-acetyl neuraminic acid (sialic acid); OAc, *O*-acetylated; NAc, *N*-acetylated; phosphate, phosphodiester linkage.

[b] Group A is substituted with *O*-acetyl at C_3 on ca. 70% of the ManNAc-P residues.

[c] Group C is substituted on C7 or C8 with 1 mol of *O*-acetyl per mol of sialic acid. One-quarter of the sialyl residues are not acetylated. Some di-*O*-acetylated (C7 and C8) may exist.

[d] The Y polysaccharide contains 1.3 mol of *O*-acetyl per NeuNAc residue. The most probable site for acetylation is C3, C4, or C7 (26). ^{13}C nuclear magnetic resonance studies have shown that the serogroup By polysaccharide is identical to serogroup Y although Bo contains 1.8 mol of *O*-acetyl per mol of NeuNAc (26).

with the result that bleblike structures of the outer membrane are formed and released from the outer surface into the surrounding medium (Fig. 2). Such blebs take bizarre shapes depending on the strain, and they range from small round vesicles of approximately 25 nm in diameter to long tubelike structures extending for several cell diameters. The continuous release of endotoxin may play an important role in the severe endotoxic reaction of meningococcemia.

The endotoxin from the outer membrane of the meningococcus has been studied for its toxic properties (66, 67, 77 78, 275), antigenic properties (5, 307), and biochemistry (6, 100, 132, 158, 159, 184). A study on the composition of LPS from *Neisseria perflava*, *N. sicca*, and *Branhamella catarrhalis* (1–3) revealed marked differences from organism to organism among the *Neisseriaceae*. Furthermore, the cultural conditions themselves influenced the composition of

LPS (200). In contrast, the composition of LPS from various *N. gonorrhoeae* strains (268) shows that all had the same sugars and fatty acids. Perry et al. (234) found that cells from T_1 and T_4 colonial types of the gonococci both have "R"-type LPS, i.e., lacked an "O" antigenic polysaccharide chain as part of the LPS. Subsequently, Jennings et al. (158) reported that the LPS from representatives of all meningococcal serogroups also have R-type LPS despite their smooth colonial character and proven virulence. Whether this will prove to be the case in strains taken from patients with systemic disease remains to be seen. The glycoses found in the meningococcal core LPS were glucose, galactose, glucosamine, heptose, and 2-keto-3-deoxyoctonate (158).

There are enzymes in meningococcal and gonococcal sonic extracts that release these core sugars from LPS (6). Although the sugars present in LPS from various meningococci are identical, they can be separated into three groups on the basis of their galactose-glucose ratios. Typical are the cores of groups A, C and 29-e, which have galactose-glucose ratios of 1:2, 2:2, and 2:1, respectively. The major fatty acids in the lipid A portion of the LPS of serogroups A, B, X, and Y are β-hydroxylauric, β-hydroxymyristic, and lauric acid (158, 159). The hydroxy-fatty acids are released only after rigorous acid hydrolysis, suggesting that they are in an amide linkage to glucosamine in the lipid A. The protein associated with meningococcal LPS (159) is considered an integral part of the LPS. Several marked differences are found in the amino acid composition of proteins from the various serogroups. For example, serogroup A LPS-protein is low in arginine, whereas methionine is a minor constituent of X and Y in comparison with A and B (159). The significance of such differences is unknown, but they might be expected to affect immunological properties of such antigenic molecules.

In summary, the LPS of the neisseriae appear to have the same core and lipid A components as the *Enterobacteriaceae* but appear to lack completely the O-antigen side chains (1, 3, 158, 184, 200, 268). Davis et al. (67) indirectly confirmed the close structural relationship of the meningococcal endotoxin and that from *Escherichia coli*. Rabbit anti-*E. coli* J5 (a rough mutant of *E. coli* 0111 without an O-antigenic side chain) serum was found to protect against both the localized and generalized Shwartzman reaction in rabbits that were treated with meningococcal LPS. The "rough" nature of meningococcal LPS could be related to the severe endotoxic reactions frequently associated with systemic meningococcal disease but infrequently found in those involving other gram-negative organisms. Without the high-molecular-weight O antigen one would expect the lipid A, the toxic moiety of endotoxin (85), to be less inclined to remain in body fluids and more inclined to home in membranes, e.g., those of platelets and PMNs where there are abundant sites for LPS binding (267). This explanation is almost certainly an oversimplification for the "enhanced" endotoxin reaction in meningococcemia. It is unlikely that native endotoxin ever exists as a molecule stripped of all other outer membrane components. The subject of "free endotoxin," i.e., endotoxin released from various gram-negative bacteria, is reviewed by Russell (254).

PILI

The piliation on the gonococcus is correlated with colonial type and virulence (161, 172, 173, 272). Unfortunately, the same is not true for the meningococcus. There is little or no correlation between colonial morphology and piliation among meningococci, in general (75, 202).

The first mention in the literature that meningococci possess pili was in a report by Jephcott et al. (161). Subsequently, Froholm et al. (104) found pili on transformation-competent strains of meningococci but not on incompetent ones. In a survey of meningococcal prototype strains, DeVoe and Gilchrist (72) detected pili on only three organisms; however, the pili were found in low numbers (one or two per cell) on only 5% of cells in the population. In contrast, nearly all cells of a nonprototype strain (ATCC 13090) had pili on their surfaces regardless of the medium on which they were grown (72). This same strain was subsequently found by Brener et al. (39) to have two colonial types, large and small, that except for their size were indistinguishable. The large colony contained cells with large-diameter pili (4.5 nm), whereas the small colonies contained cells with predominately smaller diameter pili (2.5 nm). DeVoe and Gilchrist (73) reported that all meningococci isolated from CSF of patients or the oropharynx of carriers were piliated on initial isolation. During subsequent laboratory subculture on the same medium, cells lost their pili. A similar finding was recently reported by Salit and Morton (257). Pili have also been observed on nonpathogenic neisseriae (202, 296). McGee et al. (204) have discovered that the composition of the medium, itself, is responsible for the maintenance of pili on subcultured meningococci. The best medium was chocolate agar supplemented with 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) without antibiotics.

The regulation of pili production by nutrients opens an interesting area for study, especially in view of recent in vivo findings (87, 226) indicating that gonococci are not piliated in urethral exudates or biopsy samples from cervical gonorrhea. One is left with the question of whether certain body fluids or nutrients in the human host regulate the synthesis of pili on the neisseriae, or whether such structures are a "laboratory phenomenon confined to growth on artificial media or in organ culture models," as concluded by Evans (87). The answer is probably more complex than current literature would suggest. DeVoe and Gilchrist (75) have found a substrain of group B meningococcus, SD1C, that is piliated on all media and is associated with a specific rough colonial type. A substrain of a group A organism (SP3428) is also constitutively piliated. Unlike the strain ATCC 13090, in which all known substrains are piliated, the SD1C and SP3428 parent strains are not piliated on Mueller-Hinton agar. Holbein and DeVoe (unpublished data) found that the nonpiliated parent strain of SP3428 when first grown on Trypticase soy agar became piliated, but on subsequent transfers on the same medium, once again lost its pili. Brener and DeVoe (unpublished data) found that the piliated strain ATCC 13090 became fully nonpiliated when grown at the low pH of 6.6 in buffered Mueller-Hinton broth. Moreover, the raising of the growth temperature from 37 to 41°C permitted growth, but cells ceased production of pili. Even when transferred to permissive pH and temperature, several generations occurred before this strain regenerated pili on its surface.

The repeating subunit of gonococcal pili has a molecular weight of approximately 18,000 (43). There is considerable antigenic heterogeneity among different gonococcal pili strains (42, 44, 226). The amino-terminal sequences of the pilus subunit for the gonococcus, the meningococcus (139), and *Moraxella nonliquifaciens* (105) are nearly identical (Fig. 4), although the pili used for these studies were antigenically distinct. The preservation of the specific amino acid sequence of the amino terminus containing the unusual amino acid *N*-methylphenylalanine, among five antigenically dissimilar pili, implies a role for the *N*-terminal sequence in pilus function.

Pili may aid in the attachment to host cell surfaces. Salit and Morton (257) and McGee and Stephens (203) have reported the adherence of meningococci to pharyngeal cells; however, meningococci adhere poorly to HeLa cells, and their adherence is not inhibited by mannose (63, 257). With the advancement from McGee et al. (204) with regard to media required for continuous pili production, it should be possible to characterize in some detail meningococcal pili and their role in bacterial growth and possibly pathogenesis of meningococcal disease.

SUMMARY

Many questions regarding the interaction of the meningococcus with its host remain unanswered. Specific adherence of meningococci to host tissues, properties of invasiveness, paracrine and endocrine hormone interactions with the host in response to endotoxin, and pathogenesis of meningitis are ony a few of the exciting areas for future research.

A major question remains unanswered in the field of epidemiology: of the bacteria that cause meningitis, why should the meningococcus be the only one associated with large epidemics? There is nothing now known about this organism that could account for this unique property. Moreover, the pattern of epidemics does not fit

	1				5					10					15
NEISSERIA:[A]	MePhe	Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Ile	Ala	Ile	Val	Gly	Ile
MORAXELLA:[B]	X -	Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Ile	Ala	Ile	Ile	Gly	Ile
E. COLI:[C]	Ala	Ala	Thr	Thr	Val	Asn	Gly	Gly	Thr	Val	His	Phe	Lys	Gly	Glu
					20					25				29	
NEISSERIA:	Leu	Ala	Ala	Val	Ala	Leu	Pro	Ala	Tyr	Gln	Asp	Tyr	Thr	Ala	
MORAXELLA:	Leu	Ala	Ala	Ile	Ala	Leu	Pro	Ala	Tyr	Gln	Asp	Tyr	Ile	Ala	
E. COLI:	Val	Val	Asn	Ala	Ala	?	Ala	Val	Asp	-	-	-	-	-	

FIG. 4. The amino acid sequence of the pili protein of (A) four strains of *N. gonorrhoeae* and of *N. meningitidis* strain ATCC 13090 (from Hermodson et al. [139]). (B) The sequence in pili protein of *M. nonliquifaciens* (from Froholm and Sletten [105]). (C) Amino acid sequence of the pili protein of *E. coli* (139).

the classic cycle for attack of nonimmune susceptible hosts in a population. The periodicity of meningococcal disease is notably different in character from that of the immunizing infections of childhood we have observed in the past.

The physiology of the meningococcus must be explored further to uncover pathogen-related properties. The evidence to date indicates that inside the cytoplasmic membrane there is little that contributes to the uniquely pathogenic traits of the bacterium. It is reasonable that the cytoplasmic membrane, cell wall, and surface components will be the areas in which most physiology related to pathogenic mechanisms will be found.

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LITERATURE CITED

1. **Adams, G. A.** 1971. Structural investigations on a cell-wall lipopolysaccharide from *Neisseria sicca*. Can. J. Biochem. **49**:243–250.
2. **Adams, G. A., M. Kates, D. H. Shaw, and M. Yaguchi.** 1968. Studies on the chemical constitution of cell-wall lipopolysaccharides from *Neisseria perflava*. Can. J. Biochem. **46**:1175–1184.
3. **Adams, G. A., T. G. Tornabene, and M. Yaguchi.** 1969. Cell wall lipopolysaccharide from *Neisseria catarrhalis*. Can. J. Microbiol. **15**:365–374.
4. **Adler, L., J. Brundell, S. O. Falkbring, and P. O. Nyman.** 1972. Carbonic anhydrase from *Neisseria sicca* strain 6021. I. Bacterial growth and purification of the enzyme. Biochim. Biophys. Acta **284**:298–310.
5. **Apicella, M. A.** 1979. Lipopolysaccharide-derived serotype polysaccharides from *Neisseria meningitidis* Group B. J. Infect. Dis. **140**:62–72.
6. **Apicella, M. A., J. F. Breen, and N. C. Gagliardi.** 1978. Degradation of the polysaccharide component of gonococcal lipopolysaccharide by gonococcal and meningococcal sonic extracts. Infect. Immun. **20**:228–234.
7. **Apicella, M. A., and J. A. Robinson.** 1970. Physiochemical properties of *Neisseria meningitidis* group C and Y polysaccharide antigens. Infect. Immun. **2**:392–397.
8. **Apicella, M. A., and J. A. Robinson.** 1972. Physiochemical properties of *Neisseria meningitidis* group X polysaccharide antigen. Infect. Immun. **6**:773–778.
9. **Archibald, F. S., and I. W. DeVoe.** 1978. Iron in *Neisseria meningitidis*: minimum requirements, effects of limitation, and characteristics of uptake. J. Bacteriol. **136**:35–48.
10. **Archibald, F. S., and I. W. DeVoe.** 1979. Removal of iron from human transferrin by *Neisseria meningitidis*. FEMS Microbiol. Lett. **6**:159–162.
11. **Archibald, F. S., and I. W. DeVoe.** 1980. Iron acquisition by *Neisseria meningitidis* in vitro. Infect. Immun. **27**:322–334.
12. **Archibald, F. S., C. Simonson, and I. W. DeVoe.** 1981. Comparison of iron binding and uptake from FeCl and Fe-citrate by *Neisseria meningitidis*. Can. J. Microbiol. **27**:1066–1070.
13. **Artenstein, M. S.** 1967. Studies on meningococcal meningitis, p. 260–292. *In* Department of Bacteriology, Annual report, vol. 2. Walter Reed Army Institute of Research, Washington, D.C.
14. **Artenstein, M. S., R. Gold, J. G. Zimmerly, F. A. Wyle, H. Schneider, and C. Harkins.** 1970. Prevention of meningococcal disease by group C polysaccharide vaccine. N. Engl. J. Med. **282**:417–420.
15. **Artenstein, M. S., W. S. Miller, T. H. Lamson, and L. Brandt.** 1968. Large-volume air sampling for meningococci and adenoviruses. Am. J. Epidemiol. **87**:567–577.
16. **Artenstein, M. S., P. E. Winter, R. Gold, and C. D. Smith.** 1974. Immunoprophylaxis of meningococcal infection. Mil. Med. **139**:91–95.
17. **Aycock, W. L., J. H. Mueller, and F. B. Carroll.** 1950. Meningococcus carrier rates and meningitis incidence. Bacteriol. Rev. **14**:115–160.
18. **Aymes, G., J. Etienne, E. C. Gotschlich, M. R. Hilleman, I. Joo, R. Netter, J. B. Robbins, and N. Vedros.** 1976. Cerebrospinal meningitis control. World Health Organization report, ser. no. 588. World Health Organization, Geneva.
19. **Banks, H. S.** 1948. Meningococcosis. A protean disease. Lancet **ii**:635–640, 677–681.
20. **Bastos, C. deO., A. deE. Taunnay, A. daC. Tiriba, and P. A. A. Galvao.** 1975. Meningitis meningococia en Sao Paulo, Brasil. Inf. Prelim. Bol. Of. Sanit. Panam. (Washington) **79**:54–62.
21. **Bastos, C. deO., A. deE. Taunnay, P. A. A. Galvao, A. daC. Tiriba, P. A. Saraiva, I. deO. Castro, and A. V. Lomar.** 1973. Meningites Consideracoes gerais sobre 15,067 casos internados no hospital "Emilio Ribas" durante o quindenio 1958–1972. Ocorrencia, etiologia e letalidade. Rev. Assoc. Med. Brasil **19**:451–456.
22. **Bell, W. E., and D. L. Silber.** 1971. Meningococcal meningitis: past and present concepts. Mil. Med. **136**:601–611.
23. **Bhattacharjee, A. K., H. J. Jennings, and C. P. Kenny.** 1974. Characterization of 3-deoxy-D-manno-octulosonic acid as a component of the capsular polysaccharide antigen from *Neisseria meningitidis* serogroup 29-e. Biochem. Biophys. Res. Commun. **61**:489–493.
24. **Bhattacharjee, A. K., H. J. Jennings, and C. P. Kenny.** 1978. Structural elucidation of the 3-deoxy-D-manno-octulosonic acid containing meningococcal 29-e capsular polysaccharide antigen using ^{13}C nuclear magnetic resonance. Biochemistry **17**:645–651.
25. **Bhattacharjee, A. K., H. J. Jennings, C. P. Kenny, A. Martin, and I. C. P. Smith.** 1975. Structural determination of the sialic acid polysaccharide antigens of *Neisseria meningitidis* serogroups B and C with ^{13}C-nuclear magnetic resonance. J. Biol. Chem. **250**:1926–1932.
26. **Bhattacharjee, A. K., H. J. Jennings, C. P. Kenny, A. Martin, and I. C. P. Smith.** 1976. Structural determination of the polysaccharide antigens of *Neisseria meningitidis* serogroups Y, W-135 and Bo. Can. J. Biochem. **54**:1–8.
27. **Blacklow, R. S., and L. Warren.** 1962. Biosynthesis of sialic acid by *Neisseria meningitidis*. J. Biol. Chem. **237**:3520–3526.
28. **Black-Schaeffer, B., T. G. Hiebert, and G. P. Kerby.** 1947. Experimental study of purpuric meningococcemia in relation to the Shwartzman Phenomenon. Arch. Pathol. **43**:28–54.
29. **Bøvre, K., E. Holten, H. Vik-Mo, A. Brondbo, D. Bratlid, P. Bjark, and P. J. Moe.** 1977. *Neisseria meningitidis* infections in Northern Norway: an epidemic in 1974–1975 due mainly to Group B organisms. J. Infect. Dis. **135**:669–672.
30. **Brandt, B. L., and M. S. Artenstein.** 1975. Duration of antibody responses after vaccination with group C *Neisseria meningitidis* polysaccharide. J. Infect. Dis. **131**:S69–S72.
31. **Brandt, B. L., M. S. Artenstein, and C. D. Smith.** 1973. Antibody responses to meningococcal polysaccharide vaccines. Infect. Immun. **8**:590–596.

32. **Branham, S. E.** 1956. Milestones in the history of the meningococcus. Can. J. Microbiol. **2**:175–188.
33. **Branham, S. E., and R. D. Lillie.** 1932. Notes on experimental meningitis in rabbits. Public Health Rep. **47**:1683–1686.
34. **Branham, S. E., and R. D. Lillie.** 1932. Observations on experimental meningitis in rabbits. Public Health Rep. **47**:2137–2150.
35. **Branham, S. E., R. D. Lillie, and A. M. Pabst.** 1937. Experimental meningitis in guinea pigs. Public Health Rep. **52**:1135–1142.
36. **Branham, S. E., and A. M. Pabst.** 1937. Serum studies in experimental meningitis. Lack of protection for rabbits and guinea pigs. Public Health Rep. **52**:1143–1150.
37. **Branham, S. E., and M. Pittman.** 1940. Recommended procedure for mouse protection test in evaluation of antimeningococcus serum. Public Health Rep. **55**:2340–2346.
38. **Brener, D., I. W. DeVoe, and B. E. Holbein.** 1981. Increased virulence of *Neisseria meningitidis* after in vitro iron-limited growth at low pH. Infect. Immun. **33**:59–66.
39. **Brener, D., J. E. Gilchrist, and I. W. DeVoe.** 1977. Relationship between colonial variation and pili morphology in a strain of *Neisseria meningitidis*. FEMS Microbiol. Lett. **2**:157–161.
40. **Broud, D. D., J. M. Griffiss, and C. J. Baker.** 1979. Heterogeneity of serotypes of *Neisseria meningitidis* that cause endemic disease. J. Infect. Dis. **140**:465–470.
41. **Brundell, J., S. O. Falkbring, and P. O. Nylan.** 1972. Carbonic anhydrase from *Neisseria sicca* strain 6021. II. Properties of the purified enzyme. Biochim. Biophys. Acta **284**:311–323.
42. **Buchanan, T. M.** 1975. Antigenic heterogeneity of gonococcal pili. J. Exp. Med. **141**:1470–1475.
43. **Buchanan, T. M.** 1977. Surface antigens: pili, p. 255. *In* R. B. Roberts (ed.), The gonococcus. Wiley, New York.
44. **Buchanan, T. M., and W. A. Pearce.** 1976. Pili as a mediator of the attachment of gonococci to human erythrocytes. Infect. Immun. **13**:1483–1489.
45. **Buddingh, G. J., and A. D. Polk.** 1939. Experimental meningococcus infection of the chick embryo. J. Exp. Med. **70**:485–493.
46. **Buddingh, G. J., and A. D. Polk.** 1939. The pathogenesis of meningococcus meningitis in the chick embryo. J. Exp. Med. **70**:499–512.
47. **Buddingh, G. J., and A. D. Polk.** 1939. A study of passive immunity of meningococcus in the chick embryo. J. Exp. Med. **70**:513–520.
48. **Bullen, J. J., H. J. Rogers, and E. Griffiths.** 1978. Role of iron in bacterial infection. Curr. Top. Microbiol. Immunol. **80**:1–35.
49. **Bumgarner, L. R., and R. A. Finkelstein.** 1973. Pathogenesis and immunology of experimental gonococcal infection: virulence of colonial types of *Neisseria gonorrhoeae* for chick embryos. Infect. Immun. **8**:919–924.
50. **Bundle, D. R., H. J. Jennings, and C. P. Kenny.** 1973. An improved procedure for the isolation of meningococcal polysaccharide antigens and the structural determination of the antigen from serogroup X. Carbohydr. Res. **26**:268–270.
51. **Bundle, D. R., H. J. Jennings, and C. P. Kenny.** 1974. Studies on the group-specific polysaccharide of *Neisseria meningitidis* serogroup X and an improved procedure for its isolation. J. Biol. Chem. **249**:4797–4801.
52. **Bundle, D. R., I. C. P. Smith, and H. J. Jennings.** 1974. Determination of the structure and conformation of bacterial polysaccharides by carbon 13 nuclear magnetic resonance. Studies on the group-specific antigens of *Neisseria meningitidis* serogroups A and X. J. Biol. Chem. **249**:2275–2281.
53. **Calver, G. A., C. P. Kenny, and D. J. Kushner.** 1979. Inhibition of the growth of *Neisseria meningitidis* by reduced ferritin and other iron-binding agents. Infect. Immun. **25**:880–890.
54. **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.
55. **Carifo, K., and B. W. Catlin.** 1973. *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. Appl. Microbiol. **26**:223–230.
56. **Carpenter, C. M., and R. Charles.** 1942. Isolation of meningococcus from the genitourinary tract of seven patients. Am. J. Public Health **32**:640–643.
57. **Catlin, B. W.** 1973. Nutritional profiles 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.
58. **Chen, S. S., J. H. Walgate, and J. A. Duerre.** 1971. Oxidative deamination of sulfur amino acids by bacterial and snake venom L-amino oxidase. Arch. Biochem. Biophys. **146**:54–63.
59. **Cook, J. A., W. C. Wise, and P. V. Halushka.** 1980. Elevated thromboxane levels in the rat during shock: protective effects of imidazole, 13-azaprostanoic acid, or essential fatty acid deficiency. J. Clin. Invest. **65**:227–230.
60. **Coudert, M., and J. P. Vandecasteele.** 1975. Characterization and physiological function of a soluble L-amino acid oxidase in Corynebacterium. Arch. Microbiol. **102**:151–153.
61. **Councilman, W. T., F. B. Mallory, and J. H. Wright.** 1898. Epidemic cerebro-spinal meningitis and its relation to other forms of meningitis, p. 1–178. Report of the State Board of Health of Massachusetts, Boston.
62. **Cox, D. L., and C. L. Baugh.** 1977. Carboxylation of phosphoenolpyruvate by extracts of *Neisseria gonorrhoeae*. J. Bacteriol. **129**:202–206.
63. **Craven, D. E., and C. E. Frasch.** 1978. Pili and nonpili mediated adherence of *Neisseria meningitidis* and its relationship to invasive disease, p. 250–252. *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.
64. **Craven, D. E., and C. E. Frasch.** 1979. Protection against group B meningococcal disease: evaluation of serotype 2 protein vaccines in a mouse bacteremia model. Infect. Immun. **26**:110–117.
65. **Craven, D. E., C. E. Frasch, L. F. Mocca, F. B. Rose, and R. Gonzalez.** 1979. Rapid serogroup identification of *Neisseria meningitidis* by using antiserum agar: prevalence of serotypes in a disease-free military population. J. Clin. Microbiol. **10**:302–307.
66. **Davis, C. E., and K. Arnold.** 1974. Role of meningococcal endotoxin in meningococcal purpura. J. Exp. Med. **140**:159–171.
67. **Davis, C. E., E. J. Ziegler, and K. F. Arnold.** 1978. Neutralization of meningococcal endotoxin by antibody to core glycolipid. J. Exp. Med. **147**:1007–1017.
68. **De Morais, J. S., R. S. Munsford, J. B. Risi, E. Antezana, and R. A. Feldman.** 1974. Epidemic disease due to serogroup C *Neisseria meningitidis* in Sao Paulo, Brazil. J. Infect. Dis. **129**:568–571.
69. **DeVoe, I. W.** 1976. Egestion of degraded meningococci by polymorphonuclear leukocytes. J. Bacteriol. **125**:258–266.
70. **DeVoe, I. W.** 1980. The interaction of polymorphonuclear leukocytes and endotoxin in meningococcal disease—a short review. Can. J. Microbiol. **26**:729–740.
71. **DeVoe, I. W., and J. E. Gilchrist.** 1973. Release of endotoxin in the form of cell wall blebs during in vitro growth of *Neisseria meningitidis*. J. Exp. Med. **138**:1156–1167.
72. **DeVoe, I. W., and J. E. Gilchrist.** 1974. Ultrastructure of pili and annular structures on the cell wall surface of *Neisseria meningitidis*. Infect. Immun. **10**:872–876.

73. **DeVoe, I. W., and J. E. Gilchrist.** 1975. Pili on meningococci from primary cultures of nasopharyngeal carriers and cerebrospinal fluid of patients with acute disease. J. Exp. Med. **141**:297–305.
74. **DeVoe, I. W., and J. E. Gilchrist.** 1976. Localization of tetramethylphenylenediamine-oxidase in the outer cell wall layer of *Neisseria meningitidis*. J. Bacteriol. **128**:144–148.
75. **DeVoe, I. W., and J. E. Gilchrist.** 1978. Piliation and colonial morphology among laboratory strains of meningococci. J. Clin. Microbiol. **7**:379–384.
76. **DeVoe, I. W., J. E. Gilchrist, and D. W. Storm.** 1973. Ultrastructural studies on the fate of group B meningococci in human peripheral blood leukocytes. Can. J. Microbiol. **19**:1355–1359.
77. **DeVoe, I. W., and F. Gilka.** 1976. Disseminated intravascular coagulation in rabbits: synergistic activity of meningococcal endotoxin and materials egested from leucocytes containing meningococci. J. Med. Microbiol. **9**:451–458.
78. **DeVoe, I. W., F. Gilka, J. E. Gilchrist, and E. Yu.** 1977. Pathology in rabbits treated with leukocyte-degraded meningococci in combination with meningococcal endotoxin. Infect. Immun. **16**:271–279.
79. **Diem, K., and C. Lentner (ed.).** 1970. Documenta Geigy Scientific Tables. Ciba-Geigy Ltd., Basle, Switzerland.
80. **Diena, B. B., G. Laverge, A. Ryan, F. Ashton, and R. Wallace.** 1976. Transmission of immunity of *Neisseria gonorrhoeae* from vaccinated hens to embryos. Immunol. Commun. **5**:69–73.
81. **Dodge, P. R., and M. N. Swartz.** 1965. Bacterial meningitis—a review of selected aspects. N. Engl. J. Med. **272**:842–848.
82. **Ducker, T. B., and R. L. Simmons.** 1968. The pathogenesis of meningitis. Arch. Neurol. **18**:123–128.
83. **Duerre, J. A., and S. Chakrabarty.** 1975. L-Amino acid oxidases of *Proteus rettgeri*. J. Bacteriol. **121**:656–663.
84. **Eckman, P. A., W. M. King, and J. G. Brunson.** 1958. Studies on the blood brain barrier. I. Effects produced by a single injection of gram-negative endotoxin on the permeability of the cerebral vessels. Am. J. Pathol. **34**:631–643.
85. **Elin, R. J., and S. M. Wolff.** 1976. Biology of endotoxin. Annu. Rev. Med. **27**:127–141.
86. **Ellingworth, S., J. W. McLeod, and J. W. Gordon.** 1929. Further observations on the oxidation by bacteria of compounds of the para-phenylene diamine series. J. Pathol. Bacteriol. **32**:173–184.
87. **Evans, B. A.** 1977. Ultrastructural study of cervical gonorrhea. J. Infect. Dis. **136**:248–255.
88. **Evans, R. T., S. Spaeth, and S. E. Mergenhagen.** 1966. Bactericidal antibody in mammalian serum to obligatory anaerobic gram-negative bacteria. J. Immunol. **97**:112–119.
89. **Farquhar, J. D., W. A. Hankins, A. N. DeSanctis, J. L. DeMelo, and D. P. Metzgar.** 1977. Clinical and serological evaluation of purified polysaccharide vaccines prepared from *Neisseria meningitidis* group Y. Proc. Soc. Exp. Biol. Med. **155**:453–455.
90. **Farries, J. S., W. Dickson, E. Greenwood, T. R. Malhotra, J. D. Abbot, and D. M. Jones.** 1975. Meningococcal infections in Bolton, 1971–1974. Lancet **ii**:118–121.
91. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Isolation of *Neisseria meningitidis* from the genitourinary tract and anal canal. J. Clin. Microbiol. **2**:178–182.
92. **Ferguson, J. H., and O. D. Chapman.** 1948. Fulminating meningococcic infections and the so-called Waterhouse-Friderichsen syndrome. Am. J. Pathol. **24**:763–795.
93. **Finkelstein, R. A.** 1964. Observations on mode of action of endotoxin in chick embryos. Proc. Soc. Exp. Biol. Med. **115**:702–707.
94. **Finkelstein, R. A., and R. J. Yancey.** 1981. Effect of siderophores on virulence of *Neisseria gonorrhoeae*. Infect. Immun. **32**:609–613.
95. **Flexner, S.** 1907. Experimental cerebrospinal meningitis in monkeys. J. Exp. Med. **9**:142–166.
96. **Frasch, C. E.** 1977. Role of protein serotype antigens in protection against disease due to *Neisseria meningitidis*. J. Infect. Dis. **136**(Suppl):S84–S90.
97. **Frasch, C. E.** 1979. Non-capsular surface antigens of *Neisseria meningitidis*, p. 304–337. *In* L. Weinstein and B. N. Fields (ed.), Seminars in infectious disease, vol. II. Stratton Intercontinental Medical Book Corp., New York.
98. **Frasch, C. E., and S. S. Chapman.** 1973. Classification of *Neisseria meningitidis* Group B into distinct serotypes. III. Application of a new bactericidal inhibition technique to distribution of serotypes among cases and carriers. J. Infect. Dis. **127**:149–154.
99. **Frasch, C. E., R. M. McNelis, and E. C. Gotschlich.** 1976. Strain specific variation in the protein and lipopolysaccharide composition of the group B meningococcal outer membrane. J. Bacteriol. **127**:973–981.
100. **Frasch, C. E., L. Parkes, R. M. McNelis, and E. C. Gotschlich.** 1976. Protection against group B meningococcal disease. I. Comparison of group-specific and type-specific protection in the chick embryo model. J. Exp. Med. **144**:319–329.
101. **Frasch, C. E., and J. D. Robbins.** 1978. Protection against group B meningococcal disease. II. Infection and resulting immunity in a guinea pig model. J. Exp. Med. **147**:619–628.
102. **Frasch, C. E., and J. D. Robbins.** 1978. Protection against group B meningococcal disease. III. Immunogenicity of serotype 2 vaccines and specificity of protection in a guinea pig model. J. Exp. Med. **147**:629–644.
103. **Frisch, A. W.** 1968. The meningococcidal power of heparinized blood for group B organisms. Am. J. Clin. Pathol. **50**:221–228.
104. **Froholm, L. O., K. Jyssum, and K. Bøvre.** 1973. Electron microscopical and cultural features of *Neisseria meningitidis* competence variants. Acta Pathol. Microbiol. Scand. Sect. B **81**:525–537.
105. **Froholm, L. O., and K. Sletten.** 1977. Purification and N-terminal sequence of a fimbrial protein from *Moraxella nonliquefaciens*. FEBS Lett. **73**:29–32.
106. **Glover, J. A.** 1918. "Spacing out" in prevention of military epidemics of cerebro-spinal fever. Br. Med. J. **11**:509–512.
107. **Glover, J. A.** 1920. Cerebrospinal fever: studies in the bacteriology, preventive control and specific treatment of cerebrospinal fever among the military forces 1915–1919, p. 133. *In* Med. Res. Council Spec. Res. Series no. 50, Section 20. His Majesty's Stationery Office, London.
108. **Gold, R., and M. S. Artenstein.** 1971. Meningococcal infections. 2. Field trial of group C meningococcal polysaccharide vaccine in 1969–1970. Bull. W.H.O. **45**:279–282.
109. **Gold, R., I. Goldschneider, M. L. Lepow, T. F. Draper, and M. Randolph.** 1978. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. J. Infect. Dis. **137**:112–121.
110. **Gold, R., and M. L. Lepow.** 1977. Present status of polysaccharide vaccines in the prevention of meningococcal disease. Adv. Pediatr. **23**:71–93.
111. **Gold, R., M. L. Lepow, I. Goldschneider, T. F. Draper, and E. C. Gotschlich.** 1978. Antibody responses of human infants to three doses of group A *Neisseria meningitidis* polysaccharide vaccine administered at two, four and six months of age. J. Infect. Dis. **138**:731–735.
112. **Gold, R., M. L. Lepow, I. Goldschneider, T. F. Draper, and E. C. Gotschlich.** 1979. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. J. Infect. Dis. **140**:690–697.
113. **Gold, R., M. L. Lepow, I. Goldschneider, and E. C. Gotschlich.** 1977. Immune response of human infants to polysaccharide vaccines of groups A and C *Neisseria meningitidis*. J. Infect. Dis. **136**:S31–S35.

114. **Gold, R., J. L. Winkelhake, R. S. Mars, and M. S. Artenstein.** 1971. Identification of an epidemic strain of group C *Neisseria meningitidis* by bactericidal serotyping. J. Infect. Dis. **124**:593–597.
115. **Gold, R., and F. A. Wyle.** 1970. New classification of *Neisseria meningitidis* by means of bactericidal reactions. Infect. Immun. **1**:479–484.
116. **Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J. Exp. Med. **129**:1307–1326.
117. **Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. II. Development of natural immunity. J. Exp. Med. **129**:1327–1348.
118. **Goldstein, E., W. C. Buhles, T. G. Akers, and N. Vedros.** 1972. Murine resistance to inhaled *Neisseria meningitidis* after infection with an encephalomyocarditis virus. Infect. Immun. **6**:398–402.
119. **Gordon, J., and J. W. McLeod.** 1928. The practical application of the direct oxidase reaction in bacteriology. J. Pathol. Bacteriol. **31**:185–190.
120. **Gore, I., and O. Saphir.** 1947. Myocarditis. A classification of 1,402 cases. Am. Heart J. **34**:827–830.
121. **Gotschlich, E. C.** 1976. Unresolved problems in the prevention of meningococcal meningitis, p. 91–99. *In* R. F. Beers and E. G. Bassett (ed.), The role of immunological factors in infections, allergic and autoimmune processes. Raven Press, New York.
122. **Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. IV. Immunogenicity of Group A and Group C meningococcal polysaccharides in human volunteers. J. Exp. Med. **129**:1377–1384.
123. **Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. V. The effect of immunization with meningococcal group C polysaccharide on the carrier state. J. Exp. Med. **129**:1385–1395.
124. **Gotschlich, E. C., T.-Y. Liu, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B, and group C meningococcal polysaccharides. J. Exp. Med. **129**:1349–1365.
125. **Gregorius, F. K., B. L. Johnson, Jr., W. E. Stern, and W. J. Brown.** 1976. Pathogenesis of hematogenous bacterial meningitis in rabbits. J. Neurosurg. **45**:561–567.
126. **Griffiss, J. M.** 1975. Bactericidal activity of meningococcal antisera: blocking by IgA of lytic antibody in human convalescent sera. J. Immunol. **114**:1779–1784.
127. **Griffiss, J. M., and M. S. Artenstein.** 1976. The ecology of the genus *Neisseria*. Mount Sinai J. Med. **43**:746–761.
128. **Griffiss, J. M., R. M. Bannatyne, M. S. Artenstein, and C. S. Anglin.** 1974. Recurrent meningococcal infection with an antigenically identical strain. J. Am. Med. Assoc. **229**:68–70.
129. **Griffiss, J. M., and M. A. Bertram.** 1977. Immunoepidemiology of meningococcal disease in military recruits. II. Blocking of serum bactericidal activity by circulating IgA early in the course of invasive disease. J. Infect. Dis. **136**:733–739.
130. **Griffiss, J. M., D. D. Broud, and M. S. Artenstein.** 1977. Immunoepidemiology of meningococcal disease in military recruits. I. A model for serogroup independency of epidemic potential as determined by serotyping. J. Infect. Dis. **136**:176–186.
131. **Griffiss, J. M., and D. K. Goroff.** 1981. Immunological cross-reaction between a naturally occurring galactan, agarose, and an LPS locus for immune lysis of *Neisseria meningitidis* by human sera. Clin. Exp. Immunol. **43**:20–27.
132. **Hackenthal, E.** 1969. Distribution of keto-deoxyoctonic acid and sialic acid in *Neisseria meningitidis*. J. Immunol. **102**:1099–1102.
133. **Hansson, H. A., B. Johansson, and C. Blomstrand.** 1975. Ultrastructural studies on cerebrovascular permeability in acute hypertension. Acta Neuropathol. **32**:187–198.
134. **Hardman, J. M.** 1968. Fatal meningococcal infections: The changing pathologic picture in the 60's. Mil. Med. **133**:951–964.
135. **Harter, D. H., and R. G. Petersdorf.** 1960. A consideration of the pathogenesis of bacterial meningitis: review of experimental and clinical studies. Yale J. Biol. Med. **32**:280–309.
136. **Haverkamp, J., H. L. C. Meuzelaar, E. C. Beuvery, M. Boonekamp, and R. H. Tiesjema.** 1980. Characterization of *Neisseria meningitidis* capsular polysaccharides containing sialic acid by pyrolysis mass spectrometry. Anal. Biochem. **104**:407–418.
137. **Hawiger, J., A. Hawiger, S. Steckley, S. Timmons, and C. Cheng.** 1977. Membrane changes in human platelets induced by lipopolysaccharide endotoxin. Br. J. Haematol. **35**:285–299.
138. **Hebeler, B. H., and S. A. Morse.** 1976. Physiology and metabolism of pathogenic *Neisseria*: tricarboxylic acid cycle activity in *Neisseria gonorrhoeae*. J. Bacteriol. **128**:192–201.
139. **Hermodson, M. A., K. S. C. Chen, and T. M. Buchanan.** 1978. Neisseria pili proteins: amino-terminal amino acid sequences and an identification of an unusual amino acid. Biochemistry **17**:442–445.
140. **Herrick, W. W.** 1919. Extrameningeal meningococcus infections. Arch. Intern. Med. **23**:409–418.
141. **Hill, J. C., and E. Weiss.** 1974. Protein fraction with immunogenic potential and low toxicity isolated from the cell wall of *Neisseria meningitidis* group B. Infect. Immun. **10**:605–615.
142. **Hill, W. R., and T. D. Kinney.** 1947. The cutaneous lesions in acute meningococcemia. J. Am. Med. Assoc. **134**:513–518.
143. **Hjort, P. F., and S. I. Rapaport.** 1964. The Shwartzman reaction: pathogenetic mechanisms and clinical manifestations. Annu. Rev. Med. **16**:135–168.
144. **Hobbs, J. R., R. D. G. Milner, and P. J. Watt.** 1967. Gamma-M deficiency predisposing to meningococcal septicaemia. Br. Med. J. **4**:583–586.
145. **Holbein, B. E.** 1980. Iron-controlled infection with *Neisseria meningitidis* in mice. Infect. Immun. **29**:886–891.
146. **Holbein, B. E.** 1981. Enhancement of *Neisseria meningitidis* infection in mice by addition of iron bound to transferrin. Infect. Immun. **34**:120–125.
147. **Holbein, B. E.** 1981. Differences in virulence for mice between disease and carrier strains of *Neisseria meningitidis*. Can. J. Microbiol. **27**:738–741.
148. **Holbein, B. E.** 1981. Growth and surface binding of proteins by Neisseria meningitidis in normal human serum. Curr. Microbiol. **6**:213–216.
149. **Holbein, B. E., K. W. F. Jericho, and G. C. Likes.** 1979. *Neisseria meningitidis* infections in mice: influence of iron, variations in virulence among strains, and pathology. Infect. Immun. **24**:545–551.
150. **Holten, E.** 1979. Serotypes of *Neisseria meningitidis* isolated from patients in Norway during the first six months of 1978. J. Clin. Microbiol. **9**:186–188.
151. **Hopkins, D. A. B., and R. Laplane.** 1978. James Reilly and the autonomic nervous system. A prophet unheeded? Ann. R. Coll. Surg. Engl. **60**:108–116.
152. **Horn, R. G.** 1973. Evidence for participation of granulocytes in the pathogenesis of the generalized Shwartzman reaction: a review. J. Infect. Dis. **128**:S134–S143.
153. **Horn, R. G., and R. D. Collins.** 1968. Studies on the pathogenesis of the Generalized Shwartzman Reaction. The role of granulocytes. Lab. Invest. **18**:101–107.
154. **Jacobs, S. A., and C. W. Norden.** 1974. Pneumonia caused by *Neisseria meningitidis*. J. Am. Med. Assoc. **227**:67–68.
155. **Jacobson, J. A., T. J. Chester, and D. W. Fraser.** 1977. An epidemic of disease due to serogroup B *Neisseria meningitidis* in Alabama: report of an investigation and community-wide prophylaxis with a sulfonamide. J. Infect. Dis. **136**:104–108.

156. **Jacobson, J. A., and C. E. Frasch.** 1978. Meningococcal disease in Alabama. J. Infect. Dis. **137**:375.
157. **Jennings, H. J., A. K. Bhattacharjee, D. R. Bundle, C. P. Kenny, A. Martin, and I. C. P. Smith.** 1977. Structures of the capsular polysaccharides of *Neisseria meningitidis* as determined by ^{13}C-nuclear magnetic resonance spectroscopy. J. Infect. Dis. **136**:S78–S83.
158. **Jennings, H. J., A. K. Bhattacharjee, L. Kenne, C. P. Kenny, and G. Calver.** 1980. The R-type lipopolysaccharides of *Neisseria meningitidis*. Can. J. Biochem. **58**:128–136.
159. **Jennings, H. J., G. B. Hawes, G. A. Adams, and C. P. Kenny.** 1973. The chemical composition and serological reactions of lipopolysaccharides from serogroups A, B, X, and Y *Neisseria meningitidis*. Can. J. Biochem. **51**:1347–1354.
160. **Jennings, H. J., K.-G. Rosell, and C. P. Kenny.** 1979. Structural elucidation of the capsular polysaccharide antigen of *Neisseria meningitidis* serogroup Z using ^{13}C-nuclear magnetic resonance. Can. J. Biochem. **57**:2902–2907.
161. **Jephcott, A. E., A. Reyn, and A. Birch-Anderson.** 1971. Brief report: *Neisseria gonorrhoeae*. III. Demonstration of presumed appendages to cells from different colony types. Acta Pathol. Microbiol. Scand. Sect. B **79**:437–439.
162. **Jones, D. M., and B. M. Tobin.** 1976. Serotypes of group B meningococci. J. Clin. Pathol. **29**:746–748.
163. **Jones, D. M., B. M. Tobin, and A. Butterworth.** 1973. Three cases of meningococcal infection in a family, associated with a deficient immune response. Arch. Dis. Child. **48**:742–743.
164. **Joó, F.** 1971. Increased production of coated vesicles in the brain capillaries during enhanced permeability of the blood-brain barrier. Br. J. Exp. Pathol. **52**:646–649.
165. **Jurtshuk, P., Jr., and D. N. McQuitty.** 1976. Survey of oxidase-positive and -negative bacteria using a quantitative Kovacs oxidase test. Int. J. Syst. Bacteriol. **26**:127–135.
166. **Jurtshuk, P., Jr., and T. W. Milligan.** 1974. Quantitation of the tetramethyl-*p*-phenylenediamine oxidase reaction in *Neisseria* species. Appl. Microbiol. **28**:1079–1081.
167. **Jurtshuk, P., Jr., T. J. Mueller, and W. C. Acord.** 1975. Bacterial terminal oxidases. Crit. Rev. Microbiol. **3**:399–468.
168. **Jyssum, K., and S. Jyssum.** 1962. Phosphoenolpyruvic carboxylase activity in extracts from *Neisseria meningitidis*. Acta Pathol. Microbiol. Scand. **54**:412–424.
169. **Kabat, E. A., H. Kaiser, and H. Sikorski.** 1944. Preparation of the type-specific polysaccharide of the type I meningococcus and a study of its effectiveness as an antigen in human beings. J. Exp. Med. **80**:229–307.
170. **Karolus, J. J., A. L. Gandelman, and B. A. Nolan.** 1980. Urethritis caused by *Neisseria meningitidis*. J. Clin. Microbiol. **12**:284–285.
171. **Kasper, D. L., J. L. Winkelhake, W. D. Zollinger, B. L. Brandt, and M. S. Artenstein.** 1973. Immunochemical similarity between polysaccharide antigens of *Escherichia coli* O7:K1(L): NM and group B *Neisseria meningitidis*. J. Immunol. **110**:262–268.
172. **Kellogg, D. S., Jr., I. R. Cohen, L. C. Norins, A. L. Schroeter, and G. Reising.** 1968. *Neisseria gonorrhoeae*. II. Colony variation and pathogenicity during 35 months in vitro. J. Bacteriol. **96**:596–605.
173. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274–1279.
174. **Kenimer, E. A., and D. F. Lapp.** 1978. Effect of selected inhibitors on electron transport in *Neisseria gonorrhoeae*. J. Bacteriol. **134**:537–545.
175. **Lal, H. B., T. K. Narayan, S. L. Kalra, and R. Lal.** 1963. Simultaneous outbreaks of influenza and meningitis. J. Indian Med. Assoc. **40**:113–115.
176. **Lankford, C. E.** 1973. Bacterial assimilation of iron. Crit. Rev. Microbiol. **2**:273–331.
177. **Lee, T. J., R. Snyderman, J. Patterson, A. S. Rauchbach, J. D. Folds, and W. J. Yount.** 1979. *Neisseria meningitidis* bacteremia in association with deficiency of the sixth component of complement. Infect. Immun. **24**:656–660.
178. **Lee, T. J., P. D. Utsinger, R. Snyderman, W. J. Yount, and P. F. Sparling.** 1978. Familial deficiency of the seventh component of complement associated with recurrent bacteremic infections due to Neisseria. J. Infect. Dis. **138**:359–368.
179. **Le Faou, A.** 1981. Étude du métabolisme des formes minérales du soufre chez *Neisseria gonorrhoeae*: mise en évidence d'une thiosulfate réductase. C. R. Acad. Sci. Paris **292**:851–856.
180. **Lepow, M. L., and R. Gold.** 1974. Current status of vaccines against the meningococcus. Prevent. Med. **3**:449–455.
181. **Levinson, A.** 1917. The hydrogen-ion concentration of cerebrospinal fluid. Studies in meningitis. J. Infect. Dis. **21**:556–557.
182. **Lie, S.** 1965. On the genetics of *Neisseria meningitidis*. Aas Wahls Boktyrkkeri, Oslo.
183. **Lim, D., A. Gewurz, T. F. Lint, M. Ghaze, B. Sepheri, and H. Gewurz.** 1976. Absence of the sixth component of complement in a patient with repeated episodes of meningococcal meningitis. J. Pediatr. **89**:42–47.
184. **Limjuco, G. A., Y. D. Karkhanis, J. Y. Zeltner, R. Z. Maigetter, J. J. King, and D. J. Carlo.** 1978. Studies on the chemical composition of lipopolysaccharide from *Neisseria meningitidis* group B. J. Gen. Microbiol. **104**:187–191.
185. **Littlejohns, W. A.** 1976. The enigma of the meningococcus. J. Antimicrob. Chemother. **3**:9–16.
186. **Liu, T.-Y., E. C. Gotschlich, F. T. Dunne, and E. K. Jonssen.** 1971. Studies on the meningococcal polysaccharides. II. Composition and chemical properties of the group B and group C polysaccharide. J. Biol. Chem. **246**:4703–4712.
187. **Liu, T.-Y., E. C. Gotschlich, E. K. Jonssen, and J. R. Wysocki.** 1971. Studies on the meningococcal polysaccharides. I. Composition and chemical properties of the group A polysaccharides. J. Biol. Chem. **246**:2849–2858.
188. **Lowell, G. H., L. F. Smith, M. S. Artenstein, G. S. Nash, and R. P. MacDermott, Jr.** 1979. Antibody-dependent cell-mediated antibacterial activity of human mononuclear cells. I. K lymphocytes and monocytes are effective against meningococci in cooperation with human immune sera. J. Exp. Med. **150**:127–137.
189. **Lowell, G. H., L. F. Smith, J. M. Griffiss, B. L. Brandt, and R. P. MacDermott, Jr.** 1980. Antibody-dependent mononuclear cell-mediated antimeningococcal activity. Comparison of the effects of convalescent and postimmunization immunoglobulins G, M, and A. J. Clin. Invest. **66**:260–267.
190. **MacLeod, M. N., and I. W. DeVoe.** 1981. Localization of carbonic anhydrase in the cytoplasmic membrane of *Neisseria sicca* (strain 19). Can. J. Microbiol. **27**:87–92.
191. **Mäkelä, P. H., H. Käyhty, P. Weckström, A. Sivonen, and O. V. Renkonen.** 1975. Effect of group A meningococcal vaccine in army recruits in Finland. Lancet **ii**:883–886.
192. **Mäkelä, P. H., H. Peltola, H. Käyhty, H. Jousimies, D. Pettay, E. R. Voslahti, A. Sivonen, and O. V. Renkonen.** 1977. Polysaccharide vaccines of group A *Neisseria meningitidis* and *Haemophilus influenzae* type b: a field trial in Finland. J. Infect. Dis. **136**:S43–S50.
193. **Mandrell, R. E., and W. D. Zollinger.** 1977. Lipopolysaccharide serotyping of *Neisseria meningitidis* by hemagglutination inhibition. Infect. Immun. **16**:471–475.
194. **Margaretten, W., I. Csarossy, and D. G. McKay.** 1967. An electron microscope study of a case of meningococcemia in man. Am. J. Dis. Child. **114**:268–277.
195. **Margaretten, W., and A. J. McAdams.** 1958. An appraisal of fulminant meningococcemia with reference to the Shwartzman Phenomenon. Am. J. Med. **25**:868–876.

196. **Margaretten, W., H. Nakai, and B. H. Landing.** 1963. Septicemic adrenal hemorrhage. Am. J. Dis. Child. **105**:346–351.
197. **Masson, L., B. E. Holbein, and F. E. Ashton.** 1982. Virulence linked to polysaccharide production in serogroup B *Neisseria* meningitidis. FEMS Microbiol. Lett. **13**:187–190.
198. **May, C. D.** 1960. Circulatory failure (shock) in fulminant meningococcal infection. Pediatrics **25**:316–328.
199. **May, C. W.** 1980. Microbial diseases: notes, reports, summaries, trends, p. 138. William Kaufmann Inc., Los Altos, Calif.
200. **McDonald, I. J., and G. A. Adams.** 1971. Influence of cultural conditions on the lipopolysaccharide composition of *Neisseria sicca*. J. Gen. Microbiol. **65**:201–207.
201. **McDonald, I. J., and K. G. Johnson.** 1975. Nutritional requirements of some non-pathogenic *Neisseria* grown in simple synthetic media. Can. J. Microbiol. **21**:1198–1204.
202. **McGee, Z. A., R. R. Dourmashkin, J. G. Gross, J. B. Clark, and D. Taylor-Robinson.** 1977. Relationship of pili to colonial morphology among pathogenic and nonpathogenic species of *Neisseria*. Infect. Immun. **15**:594–600.
203. **McGee, Z. A., and D. S. Stephens.** 1981. Attachment of *Neisseria meningitidis* to human mucosal surfaces: influence of receptor cell. J. Infect. Dis. **143**:525–532.
204. **McGee, Z. A., C. H. Street, C. L. Chappell, E. S. Cousar, F. Morris, and R. G. Horn.** 1979. Pili of *Neisseria meningitidis*: effect of media on maintenance of piliation, characteristics of pili, and colonial morphology. Infect. Immun. **24**:199–201.
205. **McGehee, W. G., S. I. Rapaport, and P. F. Hjort.** 1967. Intravascular coagulation in fulminant meningococcemia. Ann. Intern. Med. **67**:250–260.
206. **McKay, D. G., and J. C. Merriam.** 1960. Vascular changes induced by bacterial endotoxin during generalized Shwartzman reaction. Am. Med. Assoc. Arch. Pathol. **69**:524–530.
207. **McKay, D. G., and F. J. Row.** 1960. The effect on the arterial vascular system of bacterial endotoxin in the generalized Shwartzman reaction. Lab. Invest. **9**:117–126.
208. **McLoughlin, G. A., A. Wu, I. Saporoschetz, R. Nimberg, and J. A. Mannick.** 1979. Correlation between anergy and a circulating immunosuppressive factor following major surgical trauma. Ann. Surg. **190**:297–304.
209. **Michael, J. G., and F. S. Rosen.** 1963. Association of "natural" antibodies to gram-negative bacteria with the γ-macroglobulins. J. Exp. Med. **118**:619–626.
210. **Michael, J. G., J. L. Whitby, and M. Landy.** 1962. Studies on natural antibodies to gram-negative bacteria. J. Exp. Med. **115**:131–146.
211. **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.
212. **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.
213. **Miller, C. P.** 1933. Experimental meningococcal infection in mice. Science **78**:340–341.
214. **Miller, C. P., and R. Castles.** 1936. Experimental meningococcal infection in the mouse. J. Infect. Dis. **58**:263–279.
215. **Miller, M. A., P. Millikin, P. S. Griffin, R. A. Sexton, and M. Yousaf.** 1979. *Neisseria meningitidis* urethritis—a case report. J. Am. Med. Assoc. **242**:1651–1656.
216. **Minna, J. D., S. J. Robboy, and R. W. Colman.** 1974. Disseminated intravascular coagulation in man, p. 3–18. Charles C Thomas Publisher, Springfield, Ill.
217. **Morse, S. A., A. F. Cacciapuoti, and P. Lysko.** 1979. Physiology of *Neisseria gonorrhoeae*. Adv. Microb. Physiol. **20**:251–320.
218. **Morse, S. A., and B. H. Hebeler.** 1978. Effect of pH on the growth and glucose metabolism of *Neisseria gonorrhoeae*. Infect. Immun. **21**:87–95.
219. **Mulks, M. H., and A. G. Plaut.** 1978. IgA protease production as a characteristic distinguishing pathogenic from harmless Neisseriaceae. N. Engl. J. Med. **299**:973–976.
220. **Mulks, M. H., A. G. Plaut, H. A. Feldman, and B. Frangione.** 1980. IgA proteases of two distinct specificities are released by *Neisseria meningitidis*. J. Exp. Med. **152**:1442–1447.
221. **Munsford, R. S., C. M. Patton, and G. W. Gorman.** 1975. Epidemiological studies of serotype antigens common to groups B and C *Neisseria meningitidis*. J. Infect. Dis. **131**:286–290.
222. **Murray, E. G. D.** 1929. The meningococcus. Special report series no. 124, Privy Council, Medical Research Council. His Majesty's Stationery Office, London.
223. **Nicholson, A., and I. H. Lepow.** 1979. Host defense against *Neisseria meningitidis* requires a complement-dependent bactericidal activity. Science **25**:298–299.
224. **Norqvist, 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.
225. **Norrod, P., and R. P. Williams.** 1978. Growth of *Neisseria gonorrhoeae* in media deficient in iron without detection of siderophores. Curr. Microbiol. **1**:281–284.
226. **Novotny, P., J. A. Short, and P. D. Walker.** 1975. An electron microscope study of naturally occurring and cultured cells of *Neisseria gonorrhoeae*. J. Med. Microbiol. **8**:413–427.
227. **Novotny, P., and W. H. Turner.** 1975. Immunological heterogeneity of pili of *Neisseria gonorrhoeae*. J. Gen. Microbiol. **89**:87–92.
228. **Ødegaard, K., and T. W. Gedde-Dahl.** 1979. Frequency of simultaneous carriage of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Br. J. Vener. Dis. **55**:334–335.
229. **Ognibene, A. J., and W. R. Dito.** 1964. Chronic meningococcemia. Further comments on the pathogenesis of associated skin lesions. Arch. Intern. Med. **114**:29–32.
230. **Olsson, Y., and K. A. Hossmann.** 1970. Fine structural localization of exudated protein tracers in the brain. Acta Neuropathol. **16**:103–116.
231. **Payne, S. M., and R. A. Finkelstein.** 1978. The critical role of iron in host-bacterial interactions. J. Clin. Invest. **61**:1428–1440.
232. **Pelmont, J., G. Arland, and A. M. Rossat.** 1972. L-amino acid oxidases of *Proteus mirabilis*. General properties. Biochimie **54**:1359–1374.
233. **Peltola, H., P. H. Mäkelä, H. Käthty, H. Jousimies, E. Herra, K. Hällström, A. Sivonen, O. Renkonen, O. Pettay, V. Karanko, P. Ahvonen, and S. Sarna.** 1977. Clinical efficacy of meningococcus Group A capsular polysaccharide vaccine in children three months to five years. N. Engl. J. Med. **297**:686–691.
234. **Perry, M. B., V. Daoust, K. G. Johnson, B. B. Diena, and F. E. Ashton.** 1978. Gonococcal R-type lipopolysaccharides, p. 101–107. *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.
235. **Peter, G., M. B. Weigert, A. R. Bissel, R. Gold, D. Kreutzer, and R. H. McLean.** 1981. Meningococcal meningitis in familial deficiency of the fifth component of complement. Pediatrics **67**:882–886.
236. **Petersen, B. H., J. A. Graham, and G. F. Brooks.** 1976. Human deficiency of the eighth component of complement. The requirement of C8 for serum *Neisseria gonorrhoeae* bactericidal activity. J. Clin. Invest. **57**:283–290.
237. **Petersen, B. H., T. J. Lee, R. Snyderman, and G. F. Brooks.** 1979. *Neisseria meningitidis* and *Neisseria gonorrhoeae* bacteremia associated with C6, C7 or C8 deficiency. Ann. Intern. Med. **90**:917–920.
238. **Plaut, A. G., J. V. Gilbert, M. S. Artenstein, and J. D.**

Capra. 1975. *Neisseria gonorrhoeae* and *Neisseria meningitidis*: extracellular enzyme cleaves human immunoglobulin A. Science **190**:1103–1105.

239. **Plaut, A. G., J. V. Gilbert, and R. Wistar, Jr.** 1977. Loss of antibody activity in human immunoglobulin A exposed to extracellular immunoglobulin A proteases of *Neisseria gonorrhoeae* and *Streptococcus sanguis*. Infect. Immun. **17**:130–135.
240. **Poolman, J. T., C. T. P. Hopman, and H. C. Zanen.** 1980. Immunochemical characterization of *Neisseria meningitidis* serotype antigens by immunodiffusion and SDS-polyacrylamide gel electrophoresis immunoperoxidase techniques and the distribution of serotypes among cases and carriers. J. Gen. Microbiol. **116**:465–473.
241. **Powell, C. J., Jr., C. R. Desett, J. P. Lowenthal, and S. Berman.** 1980. The effect of adding iron to mucin on the enhancement of virulence for mice of *Salmonella typhi* strain TY2. J. Biol. Stand. **8**:79–85.
242. **Pribnow, J. F., J. M. Hall, D. Bradley, and N. A. Vedros.** 1971. Cellular response of the rabbit eye to primary intravitreal injection of *Neisseria meningitidis*. Infect. Immun. **3**:739–746.
243. **Pribnow, J. F., J. M. Hall, R. H. Stewart, and N. Vedros.** 1973. The ocular inflammation produced following intravitreal injection of a stable L-phase variant of *Neisseria meningitidis*. Can. J. Ophthalmol. **8**:361–365.
244. **Putsch, R. W., J. D. Hamilton, and E. Wolinsky.** 1970. *Neisseria meningitidis*, a respiratory pathogen? J. Infect. Dis. **121**:48–54.
245. **Quan, A. L., and J. E. Sippel.** 1979. Evaluation of bactericidal assay in serotyping *Neisseria meningitidis* Group A. Can. J. Microbiol. **25**:929–931.
246. **Reller, L. B., R. R. MacGregor, and H. N. Beaty.** 1973. Bactericidal antibody after colonization with *Neisseria meningitidis*. J. Infect. Dis. **127**:56–62.
247. **Richardson, W. P., and J. C. Sadoff.** 1977. Production of a capsule by *Neisseria gonorrhoeae*. Infect. Immun. **15**:663–664.
248. **Robbins, J. B., R. Schneerson, T. Y. Liu, M. S. Schiffer, G. Schiffman, R. L. Myerowitz, G. H. McCracken, Jr., I. Ørskov, and F. Ørskov.** 1974. Cross-reacting bacterial antigens and immunity to disease caused by encapsulated bacteria, p. 218–241. *In* E. Neter and F. Milgram (ed.), The immune system and infectious diseases. S. Karger, Basel.
249. **Roberts, R. B.** 1967. The interaction *in vitro* between group B meningococci and rabbit polymorphonuclear leukocytes. Demonstration of type specific opsonins and bactericidins. J. Exp. Med. **126**:795–819.
250. **Roberts, R. B.** 1970. The relationship between group A and group C meningococcal polysaccharides and serum opsonins in man. J. Exp. Med. **131**:499–513.
251. **Robinson, J. A., and M. A. Apicella.** 1970. Isolation and characterization of *Neisseria meningitidis* groups A, C, X, and Y polysaccharide antigens. Infect. Immun. **1**:8–14.
252. **Rogers, H. J.** 1973. Iron-binding catechols and virulence of *Escherichia coli*. Infect. Immun. **7**:445–456.
253. **Rorke, L. B., and F. W. Pitts.** 1963. Purulent meningitis: the pathologic basis of clinical manifestations. Clin. Pediatr. **2**:64–71.
254. **Russell, R. R. B.** 1976. Free endotoxin—a review. Microbios Lett. **2**:125–135.
255. **Russell, R. R. B., K. G. Johnson, and I. J. McDonald.** 1975. Envelope proteins in *Neisseria*. Can. J. Microbiol. **21**:1519–1534.
256. **Salari, S. H., I. W. DeVoe, and W. S. Powell.** 1982. Inhibition of leukotriene B_4 synthesis in human polymorphonuclear leukocytes after exposure to meningococcal lipopolysaccharide. Biochem. Biophys. Res. Commun. **104**:1517–1524.
257. **Salit, I. E., and C. Morton.** 1981. Adherence of *Neisseria meningitidis* to human epithelial cells. Infect. Immun. **31**:430–435.
258. **Samuelsson, B., M. Goldyne, E. Granström, M. Hamberg, S. Hammarström, and C. Malmsten.** 1978. Prostaglandins and thromboxanes. Annu. Rev. Biochem. **47**:997–1029.
259. **Sanders, E., and T. H. Maren.** 1967. Inhibition of carbonic anhydrase in *Neisseria*: effects on enzyme activity and growth. Mol. Pharmacol. **3**:204–215.
260. **Sanders, F. T., and R. M. Gomez.** 1968. Chronic meningococcemia. Mil. Med. **133**:918–920.
261. **Schaad, U. B.** 1980. Arthritis in disease due to *Neisseria meningitidis*. Rev. Infect. Dis. **2**:880–887.
262. **Scherp, H. W.** 1955. *Neisseria* and neisserial infection. Annu. Rev. Microbiol. **9**:319–334.
263. **Simonson, C., D. Brener, and I. W. DeVoe.** 1982. Expression of a high-affinity mechanism for the acquisition of transferrin iron by *Neisseria meningitidis*. Infect. Immun. **36**:107–113.
264. **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**:547–553.
265. **Sippel, J. E., and A. Quan.** 1977. Homogeneity of protein serotype antigens in *Neisseria meningitidis* Group A. Infect. Immun. **16**:623–627.
266. **Smith, E. S.** 1954. Purulent meningitis in infants and children: a review of 409 cases. J. Pediatr. **45**:425–436.
267. **Springer, G. F., and J. C. Adye.** 1975. Endotoxin-binding substances from human leukocytes and platelets. Infect. Immun. **12**:978–986.
268. **Stead, A., J. S. Main, M. E. Ward, and P. J. Watt.** 1975. Studies on lipopolysaccharides isolated from strains of *Neisseria gonorrhoeae*. J. Gen. Microbiol. **88**:123–131.
269. **Steel, K. J.** 1961. The oxidase reaction as a taxonomic tool. J. Gen. Microbiol. **25**:297–306.
270. **Steiner, P. E.** 1968. Disease in the civil war. Natural biological warfare in 1861–1865. Charles C Thomas Publisher, Springfield, Ill.
271. **Stephens, D. S., and Z. A. McGee.** 1981. Attachment of *Neisseria meningitidis* to human mucosal surfaces: influence of pili and type of receptor cell. J. Infect. Dis. **143**:525–532.
272. **Swanson, J., S. J. Kraus, and E. C. Gotschlich.** 1971. Studies on gonococcus infection. I. Pili and zones of adhesion: their relation to gonococcal growth patterns. J. Exp. Med. **134**:886–906.
273. **Swartz, M. N., and P. R. Dodge.** 1965. Bacterial meningitis—a review of selected aspects. N. Engl. J. Med. **272**:725–730.
274. **Tauber, H., and H. Russell.** 1962. Enzymes of *Neisseria gonorrhoeae* and other *Neisseria*. Proc. Soc. Exp. Biol. Med. **110**:440–443.
275. **Thomas, L.** 1956. The role of epinephrine in the reactions produced by the endotoxins of gram-negative bacteria. J. Exp. Med. **104**:865–880.
276. **Triau, R.** 1978. Development of meningococcal vaccines, p. 255–273. *In* A. Voller and H. Friedman (ed.), New trends and developments in vaccines. University Park Press, Baltimore.
277. **Tsai, C.-H., C. E. Frasch, and L. F. Mocca.** 1981. Five structural classes of major outer membrane proteins in *Neisseria meningitidis*. J. Bacteriol. **146**:69–78.
278. **Ueda, K., B.B. Diena, and L. Greenberg.** 1969. The chick embryo neutralization test in the assay of meningococcal antibody. I. Infection of the embryo with *Neisseria meningitidis*. Bull. W.H.O. **40**:235–240.
279. **Ueda, K., B. B. Diena, H. Sata, and L. Greenberg.** 1969. The chick embryo neutralization test in the assay of meningococcal antibody. II. Response of the embryo to the meningococcal endotoxin and to infection. Bull. W.H.O. **40**:241–244.
280. **Ueda, K., B. B. Diena, R. Wallace, H. Sato, and L. Greenberg.** 1971. The chick embryo neutralization test in the assay of meningococcal antibody. III. Dynamics and specificity of the sero-protection test. Rev. Can. Biol. **30**:1–9.
281. **Utili, R., C. O. Abernathy, and H. J. Zimmerman.** 1977.

Minireview. Endotoxin effects on the liver. Life Sci. 20:553–568.
282. Van Deurs, B. 1976. Observations on the blood-brain barrier in hypertensive rats, with particular reference to phagocytic pericytes. J. Ultrastruct. Res. 56:65–77.
283. Van Epps, D. E., A. Plaut, G. M. Bernier, and R. C. Williams, Jr. 1980. IgA paraprotein inhibition of human neutrophil chemotaxis. Reduced activity following treatment with IgA-specific protease from *Neisseria gonorrhoeae*. Inflammation 4:137–144.
284. Van Snick, J. L., P. L. Masson, and J. F. Heremans. 1974. The involvement of lactoferrin in the hyposideremia of acute inflammation. J. Exp. Med. 140:1068–1084.
285. Veitch, F. P., and L. C. Blankenship. 1963. Carbonic anhydrase in bacteria. Nature (London) 197:76–77.
286. Vieusseux, G. 1805. Mémoire sur la maladie qui a regné à Genève au printemps de 1805. J. Med. Chi. Pharm. 11:163.
287. Voeleker, A. F. 1894. Pathological report. Middlesex Hosp. Rep. 12:279. (Cited in W. R. Hill and T. D. Kinney, J. Am. Med. Assoc. 134:513–518, 1947.)
288. Weed, L. H., P. Wegeforth, J. B. Ayer, and J. D. Felton. 1919. The production of meningitis by release of cerebrospinal fluid during an experimental septicemia: preliminary note. J. Am. Med. Assoc. 72:190–193.
289. Weed, L. H., P. Wegeforth, J. B. Ayer, and L. A. Felton. 1920. A study of experimental meningitis. IV. The influence of certain experimental procedures upon the production of meningitis by intravenous inoculation. Monogr. Rockefeller Inst. Med. Res. 12:57–112.
290. Wegener, W. S., B. H. Hebeler, and S. A. Morse. 1977. Cell envelope of *Neisseria gonorrhoeae*: relationship between autolysis in buffer and the hydrolysis of peptidoglycan. Infect. Immun. 18:210–219.
291. Weichselbaum, A. 1887. Ueber die Aetiologie der akuten Meningitis cerebrospinalis. Fortschr. Med. 5:573–583.
292. Weinberg, E. D. 1975. Metal starvation of pathogens by hosts. Bioscience 25:314–318.
293. Weinberg, E. D. 1978. Iron and infection. Microbiol. Rev. 42:45–66.
294. Weiss, E., and J. C. Hill. 1975. Immunization against *Neisseria* infection, p. 423–440. *In* E. Neter and F. Milgrom (ed.), The immune system and infectious diseases. 4th Int. Convoc. Immunol., Buffalo, N.Y., 1974. Karger, Basel.
295. Winter, D. B., and S. A. Morse. 1975. Physiology and metabolism of pathogenic *Neisseria*: partial characterization of the respiratory chain of *Neisseria gonorrhoeae*. J. Bacteriol. 123:631–636.
296. Wistreich, G. A., and R. F. Baker. 1971. The presence of fimbriae (pili) in three species of *Neissera*. J. Gen. Microbiol. 65:167–173.
297. Wolf, R. E., and C. A. Birbara. 1968. Meningococcal infections at an army training center. Am. J. Med. 44:239–255.
298. Wyle, F. A., M. S. Artenstein, B. L. Brandt, E. C. Tramont, D. L. Kasper, P. L. Altieri, S. L. Berman, and J. P. Lowenthal. 1972. Immunologic response of man to group B meningococcal polysaccaride vaccines. J. Infect. Dis. 126:514–522.
299. Yancey, R. J., and R. A. Finkelstein. 1981. Assimilation of iron by pathogenic *Neisseria* spp. Infect. Immun. 32:592–599.
300. Yancey, R. J., and R. A. Finkelstein. 1981. Siderophore production by pathogenic *Neisseria* spp. Infect. Immun. 32:600–608.
301. Young, L. S., F. M. LaForce, J. J. Head, J. C. Feeley, and J. V. Bennett. 1972. A simultaneous outbreak of meningococcal and influenza infections. N. Engl. J. Med. 287:5–9.
302. Yu, E. K. C., and I. W. DeVoe. 1980. Terminal branching of the respiratory electron transport chain in *Neisseria meningitidis*. J. Bacteriol. 142:879–887.
303. Yu, E. K. C., and I. W. DeVoe. 1981. L-Cysteine oxidase activity in the membrane of *Neisseria meningitidis*. J. Bacteriol. 145:280–287.
304. Yu, E. K. C., I. W. DeVoe, and J. E. Gilchrist. 1979. A soluble CO- and NO-binding c-type cytochrome in *Neisseria meningitidis*. Curr. Microbiol. 142:879–887.
305. Zollinger, W. D., D. L. Kasper, B. J. Vetri, and M. S. Artenstein. 1972. Isolation and characterization of a native cell wall complex from *Neisseria meningitidis*. Infect. Immun. 6:835-851.
306. Zollinger, W. D., and R. E. Mandrell. 1977. Outer membrane protein and lipopolysaccharide serotyping of *Neisseria meningitidis* by inhibition of a solid-phase radioimmunoassay. Infect. Immun. 18:424–433.
307. Zollinger, W. D., and R. E. Mandrell. 1980. Type-specific antigens of group A *Neisseria meningitidis* lipopolysaccharide and heat-modifiable outer-membrane proteins. Infect. Immun. 28:451–458.
308. Zollinger, W. D., R. E. Mandrell, P. Altieri, S. Berman, J. Lowenthal, and M. S. Artenstein. 1978. Safety and immunogenicity of a *Neisseria meningitidis* Type 2 protein vaccine in animals and humans. J. Infect. Dis. 137:728–739.
309. Zollinger, W. D., C. L. Pennington, and M. S. Artenstein. 1974. Human antibody response to three meningococcal outer membrane antigens: comparison by specific hemagglutination assays. Infect. Immun. 10:975–984.