OCCASIONAL REVIEW
THE PATHOGENICITY OF HAEMOPHILUS INFLUENZAE

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Problems about the pathogenicity of an organism can be classified as: (1) clinical and epidemiological—what diseases does it cause or take part in, and when? (2) research (with prophylactic and therapeutic implications)—how does it do so? Category (1) answers for Haemophilus influenzae began in 1892, with Pfeiffer’s claim that it was the cause of influenza; but the true picture of the range of pathogenic activities of this species became clear in the period 1930–1960. Category (2) answers have in general been more recent, and are still far from complete (as is the case with most pathogens); but a fascinating array of contributions has appeared in the past few years.

What and when?

To discuss the pathogenicity of H. influenzae as though the species were in this respect a single entity would be to confuse things that differ widely. It has been clear for over 50 years (Pittman, 1931), but not known as widely as it should have been, that a minority of H. influenzae strains have polysaccharide capsules, and that one of the six capsular types (type b) differs in pathogenicity from the rest of the species at least as markedly as do streptococci of Lancefield’s group A from other β-haemolytic streptococci. The main facts about the distribution and pathogenicity of capsulated and non-capsulated strains of H. influenzae are summarised in the table. The species has no non-human hosts.

Capsular type b

The 2–4% carriage rates for H. influenzae type b listed in the table represent the findings of numerous surveys, each of which was a “snapshot” of the distribution of the organism in one community at one time. These give no information about the dynamics of carriage. Rates in the range 2–4% would be found, for example, in a community of which each member carried the organism for about 2 months during a 5-year period. There is some evidence (e.g., Turk, 1963) that the duration of carriage of H. influenzae type b by individual children is often of that order. Moreover, when Sell, Turner and Federspiel (1973) studied 55 children through their first 5 years of life, culturing nasopharyngeal swabs mostly at 3-month intervals, they isolated H.
TABLE
Carriage and pathogenicity of H. influenzae

<table>
<thead>
<tr>
<th>Strains</th>
<th>Common nasopharyngeal carriage rates*</th>
<th>Principal manifestations of pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsulated, type b</td>
<td>2–4%</td>
<td>Meningitis, epiglottitis, pneumonia, suppurative arthritis, osteitis, otitis media, cellulitis, pericarditis. Patients are usually young children. Infections commonly bacteraemic.</td>
</tr>
<tr>
<td>Capsulated, other types</td>
<td>1–2%</td>
<td>Rarely incriminated as pathogens, but all 5 types have sometimes caused diseases as above.</td>
</tr>
<tr>
<td>Non-capsulated</td>
<td>50–80%</td>
<td>Exacerbations of chronic bronchitis, etc. Also otitis media, conjunctivitis, paranasal sinusitis. Patients are commonly adults. Infections rarely bacteraemic.</td>
</tr>
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* Carriage rates vary widely between communities and with time. In general they are higher among children than among adults.


*H. influenzae* type b at some time from 38.5% of the children. More frequent swabbing might have given a higher figure, and culture of a single nasopharyngeal swab on a non-selective culture medium is not a sensitive means of detecting all carriers; so it is not unreasonable to interpret their findings as supporting the concept that most children carry *H. influenzae* type b at some time during the first 5 years of life. This concept fits in well with the classical findings of Fothergill and Wright (1933), that bactericidal activity against *H. influenzae* type b is common in the blood of neonates, declines during the early weeks of life, is uncommon for the next 2 or 3 years, but is in most cases restored by the fifth year. It does not seem necessary to invoke colonisation by *Escherichia coli* K100 or other cross-reacting bacteria as an explanation for the high frequency of antibodies to *H. influenzae* type b polysaccharide in blood samples from older children and adults (Bradshaw et al., 1971).

Fortunately, most of these encounters with *H. influenzae* type b are asymptomatic, or at most are associated only with minor upper-respiratory-tract symptoms. Of the unlucky few children who develop serious illness, most get meningitis; and for the sake of simplicity the epidemiology of this condition will be discussed before considering the other manifestations of bacteraemic *H. influenzae* type b disease, even though these can co-exist with meningitis in the same patient or in associated patients. Maternal antibodies are presumably responsible for the infrequency of haemophilus meningitis in the first 2 months of life; but most cases occur during the first 2 years, and nearly all by the age of 4 years, with a few in later childhood (Wood et al., 1982) and throughout adult life (Spagnuolo et al., 1982). In Britain, where *Neisseria meningitidis* shares with *H. influenzae* type b most of the responsibility for bacterial meningitis in young children, the best available estimate of a child's risk of getting haemophilus meningitis during the first 5 years of life is 1 in 1500 (Goldacre, 1976). In the USA, where
haemophilus meningitis is by far the commonest form of bacterial meningitis overall, despite its restricted age-range, the risk that a child will suffer from it during the first 5 years of life is about 1 in 500 (based on data summarised by Ward et al., 1981) in the country as a whole, but is very much higher in the Navajo tribe of American Indians (Coulehan et al., 1976) and among Alaskan Eskimos (Ward et al., 1981). In the latter population the risk was found to be about 1 in 50, with nearly all cases occurring in the first year of life, though still with relative sparing of neonates. Ward et al. (1981) suggested that these findings were not due to special virulence of the local type-b strains or to any special susceptibility of Eskimo children, but to greater exposure of the children to the organism early in life, before they have developed immunity. (The Alaskan survey produced many other interesting findings about H. influenzae type b disease, but they are not strictly relevant here.)

Although there are no true epidemics of haemophilus meningitis, and most cases are sporadic with no history of direct or indirect connection with others, two kinds of case-association do occur. One of these, familiar to many paediatricians and bacteriologists, becomes apparent when a hospital admits within 2 or 3 weeks as many children with haemophilus meningitis as might be expected there in 6 months or a year. They do not come from any particular part of the neighbourhood or have any other apparent connections. Are such “outbreaks” due to the temporary presence in the neighbourhood of a specially virulent H. influenzae strain? Alternatively, is there a particular virus or other transient factor at work in the neighbourhood at that time, increasing the likelihood that children who happen to be carrying H. influenzae type b will develop meningitis? More precise “finger-printing” of the bacterial isolates—e.g., by outer-membrane protein (OMP) typing (Loeb and Smith, 1980; Gulig, Frisch and Hansen, 1983)—should decide between these alternatives before long.

A less obvious kind of case-association is secondary illness among family or other close contacts. As recently as 1967 I could find only six examples of intra-family spread (Turk and May, 1967), even though it was known by then that high rates of nasopharyngeal carriage of H. influenzae type b are common among the patients’ close contacts (Good et al., 1943; Turk, 1963), and that children with haemophilus meningitis tend to have more siblings than do children with other forms of meningitis or admitted to hospital for other conditions (Ounsted, 1950, 1951). It took a nation-wide survey in the USA (Ward et al., 1979) to establish that the likelihood of a case of haemophilus meningitis occurring in a household within 30 days of the occurrence of an index case was much higher (the authors’ calculation was 585 times higher) than in the general population. Secondary spread in day-care centres was already well documented (for references see Ward et al., 1979), and the new attitude to the epidemiology of haemophilus meningitis was embodied by Glode et al. (1980) in the title of their paper, in which they described it as “a contagious disease of children”. OMP typing has confirmed that secondary cases of this kind are caused by the strains causing their respective index cases (Loeb and Smith, 1980; Barton, Granoff and Barenkamp, 1983; Kaplan and Mason, 1983), and there is therefore no need to postulate any other factor to explain these case-associations. However, it remains to be established to what extent the increased incidence of illness among family contacts is due to their being unduly susceptible (Granoff et al., 1983) or excessively exposed to the organism, or whether it is an indication that the strains involved are of unusual virulence—perhaps developed, as Ounsted (1950) suggested, during rapid passage
through the family. That there may be strains of *H. influenzae* type b which lack virulence is suggested by the finding that nearly all of 19 infants present in or admitted to a Jamaican orphanage during a 5-month period carried an organism of this capsular type but none became ill, even though their ages and general condition might have been expected to make them highly susceptible (Turk, 1963). Mpairwe (1970) and others have made similar observations elsewhere.

Meningitis is the commonest manifestation of *H. influenzae* type b bacteraemia, but there are a number of others, of which the most important are listed in the table. Combinations of these manifestations may occur in one patient—notably *otitis media* with meningitis—or within a group of related cases. The age-incidence of most of them is similar to that of haemophilus meningitis, but *epiglottitis* is an exception. This condition was well-known in North America over 40 years ago (Alexander, Ellis and Leidy, 1942), and occurs there with about one-third of the frequency of haemophilus meningitis (Dajani, Asmar and Thirumoorthi, 1979). In Britain, though well described in the 1950s (Jones and Camps, 1957), it was rarely recognised until the 1970s (Andrew, Tandon and Turk, 1968; Russell, 1971; Addy, Ellis and Turk, 1972), but it now seems likely that here also its incidence is about one-third of that of haemophilus meningitis. Epidemiologically its most distinctive feature is that the affected children are on average older than those with other *H. influenzae* type b infections—nearly all are over one year old and mostly between 2 and 7 years. This means that they are more likely to have younger siblings, of appropriate age to become secondary cases of *H. influenzae* type b disease; and although there are as yet no studies of this aspect of epiglottitis comparable to those of meningitis, personal experience (some of it reported by Addy *et al.*, 1972) suggests that the child with epiglottitis is a source of significant infection in his siblings more often than is the child with haemophilus meningitis. There is as yet no explanation of the relatively late age-incidence of epiglottitis, which also stands out from the other manifestations of *H. influenzae* type b bacteraemia in being a condition almost specific to this pathogen (at least when the patients are children), whereas other bacterial species can cause meningitis, pneumonia, etc. In the Alaskan survey (Ward *et al.*, 1981) no cases of epiglottitis were seen, presumably because virtually all of the children had acquired natural immunity before reaching the age-range for that disease.

**Pneumonia** caused by *H. influenzae* type b is usually lobar or segmental, and is similar to such pneumonia caused by pneumococci in its clinical features (apart from age-range) and its radiological appearances.

*H. influenzae* bacteraemia does not always give rise to localised disease, or even to illness sufficient to require hospital admission (Marshall, Teele and Klein, 1979). Indeed, we have no idea in what proportion of children the first encounter with this organism is followed by a low-grade undetected bacteraemia. Four cases in my own experience lead to the speculation that sometimes trauma converts such a bacteraemia into overt localised disease. They were: a child who crushed her finger in a door, without any break of the skin, and developed *H. influenzae* type b suppurative arthritis of the relevant interphalangeal joint (Miller and Turk, 1965); a child who developed epiglottitis about 12 h after his mother had with difficulty dislodged a piece of peanut candy from his throat; and two children admitted to hospital for head injuries (without fractures) following falls, and found some hours after admission to be suffering from haemophilus meningitis.
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As well as being components of some of the bacteraemic illnesses, local *H. influenzae* type b infections such as otitis media and conjunctivitis sometimes occur without systemic spread. Far more often, however, these purely local *H. influenzae* infections are caused by non-capsulated strains.

*Capsular types other than b*

On rare occasions each of the other five capsular types (a and c–f) has been incriminated as causing the sort of systemic illnesses that are far more commonly due to type b; indeed, a few such illnesses are caused by non-capsulated strains. It has been suggested that types a and c may have particular relationships to paranasal sinusitis (Holdaway and Turk, 1967; Turk and Cruickshank, 1981), but in general 'non-b' capsulated strains can be regarded as non-pathogens.

*Non-capsulated strains*

Upper respiratory tract carriage of non-capsulated *H. influenzae* is common (see table), and it is likely that everyone carries them from time to time (Blackburn et al., 1930). They can, therefore, be regarded as part of man's normal nasopharyngeal flora. Nevertheless, in this and many other countries they are responsible for far more illness and medical expense than are type-b strains, for reasons outlined in the next paragraph.

*Chronic bronchial disease.* Much of what we know about the pathogenicity of *H. influenzae* in the bronchi was established by May in the 1950s and 1960s (see Turk and May, 1967; May, 1972). A brief summary will suffice here. In health the bronchi are self-sterilising; but in chronic bronchitis and related diseases they lose this capacity and are liable to bacterial colonisation, mainly by non-capsulated *H. influenzae* and by pneumococci. So long as the sputum remains mucoid these organisms are present rather infrequently and are rarely numerous, and in such circumstances they are not to be regarded as pathogens. They have nothing to do with the aetiology of chronic bronchitis, and are not responsible for initiating acute exacerbations. However, they are nearly always responsible for the sputum becoming purulent in an acute exacerbation or being persistently purulent in more advanced disease, and for the associated impairment of respiratory function and systemic illness. Antibacterial treatment—aimed chiefly at the haemophili because they are the cause of the trouble more often than the pneumococci and are also more difficult to control or eradicate—will in many cases improve the patients' breathing and general health, at least for a time, but relapse and reinfection are common. Non-capsulated *H. influenzae* strains are therefore responsible, wherever chronic bronchitis is common, for a great deal of ill-health, lost working capacity and expenditure on antibacterial drugs. Their involvement in bronchiectasis and cystic fibrosis is similar, though in these conditions they have additional bacterial collaborators. How often such bronchial infections extend to lung parenchyma is not clear, because most of the papers that claim *H. influenzae* (non-capsulated or of undetermined capsular status) as the cause of pneumonia are invalidated by the authors' failure to allow for the frequency
with which this species is present in the nasopharynx (and thus can contaminate sputum) and in diseased bronchi.

**Conjunctivitis.** It is now over a century since Koch (1883) wrote the first description of an organism now classified in the genus *Haemophilus*. He saw it in Egypt, as small gram-negative bacilli in pus from cases of conjunctivitis. It is still arguable whether the "Koch-Weeks bacillus" deserves the status of a separate species *H. aegyptius* or should be included in *H. influenzae* (Kilian, 1976; Mazloum et al., 1982). It is clear, however, that both this organism and some that are indisputably *H. influenzae* have been involved in epidemics of conjunctivitis in the southern USA (Davis and Pittman, 1950) and in North Africa (Huet, 1956); this is the only form of *H. influenzae* infection to occur in epidemics. In Britain, haemophilus conjunctivitis occurs as sporadic cases; the strains involved are "ordinary" *H. influenzae*, usually non-capsulated (Ingham and Turk, 1969); and in some cases there is underlying nasolachrymal duct obstruction, with liability of the infection to recur until this is corrected.

**Female genital tract and neonatal infections.** Colonisation of the healthy vagina by *H. influenzae* is rare, as judged from results obtained by procedures that should reliably have detected its presence except in small numbers (Khuri-Bulos and McIntosh, 1975). Sometimes, however, this species is responsible for cases of salpingitis, tubo-ovarian abscess or other infection of the non-pregnant female genital tract, usually in the presence of intra-uterine devices or of previous infective or other damage. Perinatal infections also occur, involving the mother or the baby or both, with or without bacteraemia, and with turbid foul-smelling amniotic fluid as a common presenting feature. Even when such gynaecological or perinatal infections are bacteraemic, the organisms are more often non-capsulated than of type b (Khuri-Bulos and McIntosh, 1975; Albritton, Hammond and Ronald, 1978).

**Other sites.** Those who look carefully are liable to find *H. influenzae* in unusual sites—even in faeces (Palmer, 1981), though this finding may reflect merely the organism's ability to survive the journey from the nasopharynx through the alimentary tract. Urinary-tract infection with this species, reported first by Albright, Dienes and Sulkowitch in 1938 and by various others since, is usually associated with the presence of calculi or anatomical abnormality of the urinary tract. While it is too rare to justify routine use of special culture media for urine (Schuit, 1979), it should be remembered as a possibility when a patient with appropriate predisposing factors has "sterile" purulent urine. Underlying damage or disease has also been a feature of nearly all reported examples of *H. influenzae* infection of the biliary tract (e.g., Arnau de Bolos et al., 1982; also type b strains, Pallares et al., 1983); this localisation is made less surprising by the finding of Sa Pereira et al. (1981) that bile contains all factors necessary for the growth of *H. influenzae*. The reader will doubtless have noticed the recurring theme of damaged or blocked tubes throughout our discussion of the pathogenicity of non-capsulated *H. influenzae*. It seems that they need some such setting to be able to cause trouble. However, damaged heart valves do not offer them much scope; the closely related species that live in the mouth more often than the nasopharynx, *H. parainfluenzae* and *H. aphrophilus*, are involved in endocarditis more often than *H. influenzae*. More general underlying damage, such as immune deficiency or debilitating disease, can provide the basis for opportunist infections by *H. influenzae* (capsulated or non-capsulated), as also for those by many other species.
"The influenza bacillus"

We cannot altogether ignore Pfeifer's claim (1892) that "in every case of influenza a similar type of bacillus was found in the characteristic purulent sputum... in uncomplicated cases in absolutely pure growth and in almost incredible numbers". The organism that he grew from these sputa (1893)—by adding blood to his culture medium, which may have been the first use of blood agar—was undoubtedly what we now call *H. influenzae*. Not till 40 years later did the discovery of the influenza viruses by Smith, Andrewes and Laidlaw (1933) cause the "influenza bacillus" to be discredited as such, with subsequent diversion of attention to the forms of pathogenicity that we have already considered. But was Pfeiffer wrong? It is still possible that his bacillus did play an important role, in collaboration with the influenza viruses, in some outbreaks of human influenza between 1889 and 1919, as undoubtedly did a very closely similar bacillus in swine influenza, which was first recognised in the USA in 1918 during the human influenza pandemic. This topic was more fully discussed by Turk and May (1967).

How?

The results of an encounter between a potential pathogen and a potential host depend, of course, on the properties and behaviour of both parties; but here we are concerned with those of the potential pathogen, and host factors will be mentioned only incidentally.

Bacteraemic infections

Animal experiments were disappointing as sources of information about the pathogenesis of bacteraemic *H. influenzae* type b infections, until the introduction of the infant rat model (Moxon *et al.*, 1974). This has been shown to reflect faithfully much of what happens in human infections with this organism. When inoculated in adequate numbers intranasally (the "natural" route) into 5-day-old rats, *H. influenzae* type b causes bacteraemia in most of them, usually with meningitis and other localised infections. The bacteria penetrate the nasal mucosa and enter the circulation, and the meningitis is the result of blood-borne dissemination, not of direct spread through the cribriform plate (Ostrow *et al.*, 1979). In this context, therefore, pathogenicity must include ability (i) to colonise the nasal mucosa; (ii) to penetrate the mucosa and reach the circulation (presumably as passengers in or on host cells—Rubin and Moxon, 1983); (iii) to survive (and probably to multiply) in the blood; and (iv) to cause inflammation of the meninges and in the other "target" areas. Information is accumulating rapidly about the roles of various bacterial components as virulence factors, and it is simpler to consider these components one by one than to deal directly with the sequence of events that we have just outlined.

Capsules. The predominant importance of the type-b capsule in the pathogenicity of *H. influenzae* has been recognized for over 50 years (Pittman, 1931) and has been evident throughout this review. The capsules of all six capsular types of this species consist of two-sugar polysaccharides, but the capsular material of type b, polyribosylribitol phosphate (PRP), is unique among them in that both of its sugars are pentoses,
whereas all of the others contain hexoses. The problem of understanding the strong association between PRP and the ability to cause serious illness is made all the more intriguing by the occasional exception; for, as we have already seen, typical "H. influenzae type b bacteraemic disease" is sometimes caused by strains with no capsules or with capsules of types other than b. It may be that some (or all) of the non-capsulated strains incriminated in such situations are type-b variants such as those described by Catlin (1970) and by Buckmire (1976, 1982), which contain PRP but do not (probably cannot) excrete it as capsule and so are not recognised as type b by the usual procedures for detecting capsulated strains. However, this is made less likely by the finding of Zwahlen, Winkelstein and Moxon (1983a) that production of small amounts of type-b capsular material does not enhance virulence as tested in complement-depleted infant rats; and in any case there remains in connection with human disease the problem that capsulated strains of types other than b can exhibit pathogenicity characteristic of type b on rare occasions—far too rare to be merely a reflection of their relatively low nasopharyngeal carriage rates.

Early studies of the animal pathogenicity of H. influenzae established that non-capsulated variants derived from type-b strains in the laboratory were, like naturally occurring non-capsulated strains, less virulent than the parent type-b strains when injected into rabbits (Pittman, 1931), mice (Chandler, Fothergill and Dingle, 1937) and chick embryos (Buddingh, 1956). Wright and Ward (1932) showed that a capsulated strain (presumably type b, as it came from a human case of meningitis) was much less susceptible to the killing action of normal rabbit blood than was a non-capsulated variant derived from it. Buddingh (1963) showed that the amount of capsular material made by type-b strains determined how virulent they were to chick embryos. The situation became somewhat more complicated when Stillman (1944) and Leidy et al. (1960) found that capsulated strains of types other than b could be virulent for mice; and Leidy et al. also showed that when they used type-b DNA to confer type-b capsules on two non-capsulated strains—one derived from a type-b strain and the other from a type-d strain that was highly virulent to mice—the type-b-capsulated derivative of the type-d strain was the more virulent of the two in further mouse experiments. From this it was clear that, at least in infections of mice, virulence could depend on the somatic as well as the capsular composition of the H. influenzae.

More recently Moxon and Vaughn (1981) reported some important studies on the effects of capsulation on the pathogenicity of H. influenzae to young rats (infants, except in the experiments summarised under (1) below, for which technical factors made it necessary to use 3-month-old rats). Their main findings were:

(1) After intravenous inoculation in doses of c.10^5 organisms their non-capsulated strains and those of capsular types other than a or b disappeared rapidly from the circulation; their type-a strain was cleared more slowly; and only their type-b strain produced a persistent bacteraemia.

(2) After peritoneal inoculation in doses of c.10^3 or c.10^7 organisms, their non-capsulated strains were non-invasive, but each of their capsulated strains caused bacteraemia and meningitis in at least some animals—though only the type-b strain did so in every test animal, even with the lower dose.

(3) After intranasal inoculation, type-b-capsulated and type-d-capsulated transformants of the same non-capsulated strain were about equally efficient in establishing
persistent nasopharyngeal colonisation, and both were far more efficient than the parent non-capsulated strain, but only the type-b transformant caused bacteraemia and meningitis.

These findings indicated that possession of either a type-b or a type-d capsule aided nasopharyngeal colonisation, but that only possession of a type-b capsule enhanced the organism's ability to invade the blood stream, to persist there and to cause meningitis. However, Roberts, Stull and Smith (1981), using infant rats of the same breed, found that type-b and type-d strains (natural or transformants) were comparable in their ability to cause bacteraemia when given intranasally, but that the type-b strains were the more virulent when given subcutaneously. The discrepancies may have been due to differences of bacterial strains and of intranasal inoculation technique.

The overall conclusion from these rat experiments, probably applicable also to human infections, is that PRP contributes to the virulence of \textit{H. influenzae} by improving its chances of colonising and invading susceptible hosts and of reaching the meninges and other sites of local infection. It has long been assumed that the type-b capsule protects the organism against phagocytosis, as happens with pneumococci (Wood, 1960); but I am not aware of any formal proof that this is true of \textit{H. influenzae}, or that PRP gives more protection against phagocytosis than do the capsular polysaccharides of the other five types. Work on phagocytosis of this species has been concerned with showing that naturally occurring or vaccine-induced antibodies to PRP promote phagocytosis of type-b organisms—presumably reducing them to the status of non-capsulated strains (Newman, Waldo and Johnston, 1973, give relevant references). However, it has long been clear that type-b capsule can protect against hazards other than phagocytosis. As mentioned in the clinical section of this review, Fothergill and Wright (1933) found that the blood of most young children lacked the bactericidal action against \textit{H. influenzae} type b which they found in the blood of most neonates, older children and adults. Their additional finding that is relevant here was that even those blood samples that failed to kill their type-b strain did kill a non-capsulated variant of it—presumably because of its lack of capsular protection. Ward and Wright (1932) had shown that this bactericidal activity did not require the presence of host cells but was complement-dependent. Much more recently Sutton, Schneerson and Robbins (1982) published data suggesting that type-b strains of \textit{H. influenzae} are the most invasive members of the species because PRP gives them better protection against complement than do the capsules of other types.

\textit{Pili (fimbriae).} The ability of \textit{H. influenzae} to adhere to human epithelial cells has become a subject of research in the past few years because of its possible relevance to nasopharyngeal colonisation. The human cells used in the tests have been buccal or oropharyngeal, presumably because these are easily collected. Lewis and Dajani (1980) and Lampe \textit{et al.} (1982) found that non-capsulated \textit{H. influenzae} strains adhered to such cells but capsulated strains did not. In contrast, Guerina \textit{et al.} (1982) found that some type-b strains were capable of adhering when first isolated, and that they could select out adherent populations from other strains which had appeared non-adherent on first isolation as judged by their usual test. The selection was achieved by adding human red blood cells to suspensions of the bacteria and then by centrifugation removing the red cells and any bacteria adherent to them. They also demonstrated that adherence depends on possession of pili. Pichichero \textit{et al.} (1982)
found that by such means they could select piliated subpopulations from any of their type-b strains, but that pili and capacity to adhere were soon lost on subsequent culture in the absence of red cells—except by two human nasopharyngeal type-b strains that were heavily piliated and exceptionally adherent to human epithelial cells on first isolation and were stable in that condition. They postulated that potential piliation may be general among *H. influenzae* type b, that piliation may be important in achieving nasopharyngeal colonisation, but that subsequent reversion to the non-piliated state may assist invasion of the host by reducing the organism's liability to phagocytosis. An extension of this work is reported on pp 109–118 of this issue (Pichichero, 1984). Meanwhile, Kaplan, Mason and Wiedermann (1983) have reported that the heavily piliated nasopharyngeal isolates that they obtained from three children with haemophilus meningitis were no better at colonising the noses of infant rats than were the non-piliated isolates from the cerebrospinal fluids of the same children. However, because their piliated isolates adhered well to human but not to rat epithelial cells, it would seem that in this particular context the infant rat model may not give a valid picture of what happens in human disease. All isolates from the blood or cerebrospinal fluids of their rats were non-piliated, whatever their state when inoculated intranasally, and the authors agree with Pichichero et al. (1982) that loss of pili may facilitate invasion of the host.

**IgA proteases.** Another topic that has attracted attention recently because of its possible relevance to nasopharyngeal colonisation is the production of IgA1 proteases by *H. influenzae* (Male, 1979; Kilian, Mestecky and Schrohenloher, 1979; Mulks et al., 1980). Other bacteria known to produce such enzymes include pneumococci, meningococci and gonococci. All workers in this field have been interested in the idea that ability to destroy the host's secretory IgA might facilitate mucosal colonisation. Mulks et al. (1982) found some correlation between capsular type and the type of protease produced, but there is nothing to suggest that type-b strains have any special ability in this respect, and in any case most victims of invasive *H. influenzae* type b disease lack specific antibodies at the time of infection.

**Phenotypic change while in the host's blood.** Shaw et al. (1976) reported the paradox of bacteraemia occurring in children whose sera were bactericidal to their own type-b strains when tested *in vitro*. They found that this bactericidal action occurred when they used broth cultures of the organisms in their tests, but not if the organisms were incubated for 30 min in rat serum before the tests were set up. Presumably human serum had conferred similar protection *in vivo*. Anderson et al. (1980) and Inzana and Anderson (1982) showed that protection could be conferred by incubating the organisms in dialysate of human serum, that it was associated with (and presumably due to) marked reduction in binding of the organisms to antibodies against their lipopolysaccharides (LPS), but that the LPS composition of the organisms appeared to be unchanged and that the detectable alteration in their composition was the presence of some additional proteins, which possibly interfered with the binding of antibodies to LPS. It remains to be determined whether ability to undergo this phenotypic change while in the host is linked to pathogenicity.

**Lipopolysaccharide.** *H. influenzae* LPS differs somewhat in composition from those of enterobacteria, but shares their endotoxic activity (Flesher and Insel, 1978; Tuyau and Sims, 1983). There is sufficient electrophoretic heterogeneity among LPS from different strains of *H. influenzae* type b to allow subtyping of that serotype.
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(Inzana, 1983; van Alphen et al., 1983). Zwahlen, Winkelstein and Moxon (1983b), in studies using various capsulated transformants of a single non-capsulated strain, found that they differed from one another in LPS as well as capsular composition. They suggested that "genes determining the structure of the capsule and the LPS of H. influenzae may be linked or shared". From this it might follow that the pathogenicity of type-b strains is in part due not to PRP but to the corresponding LPS. In work as yet not published but mentioned in the above paper, Zwahlen and his colleagues found that alteration of the LPS of a type-b strain by transformation with cloned DNA produced an organism of attenuated virulence, associated with increased sensitivity to the bactericidal action of serum. Thus LPS composition may have an important influence on survival of type-b strains in the blood-stream. Quite apart from this, LPS is the only bacterial component that we have discussed which has known toxic properties, and it is therefore reasonable to postulate that its composition may be found to be related to the production of local inflammation in the meninges and elsewhere.

Outer membrane proteins. As we have seen, OMP analysis has proved valuable in epidemiological studies of H. influenzae type b infections; and it may well prove a basis for new vaccines. There is, however, no evidence as yet that OMP composition correlates with pathogenicity (Hampton et al., 1983).

Summary of the roles of these factors. We can now rearrange the established facts and probabilities about H. influenzae virulence factors in accordance with the four stages in the pathogenesis of a bacteraemic infection that were outlined at the beginning of this section.

(i) Colonisation of the nasal mucosa. This is facilitated by PRP, by piliation and perhaps by IgA proteases.

(ii) Penetration through the mucosa into the blood stream. This stages remains to be elucidated. If it is true that the bacteria are carried through to the blood as passengers on host cells, piliation may well be important.

(iii) Survival in the blood stream. This is facilitated by PRP, by phenotypic change, by appropriate LPS composition and possibly by loss of pili.

(iv) Production of local inflammation. This stage also remains to be elucidated.

Localized infections

There is little experimental information about factors that determine local pathogenicity of H. influenzae in the bronchi and elsewhere. Himmelweit (1949) showed that H. influenzae shares with pneumococci, but not with the other respiratory-tract bacteria that he tested, the ability to destroy mucus. He was interested in the possibility that this action made the respiratory-tract mucosa more accessible to viruses; but it is easy to see how it might also relate to the ability of these two species to establish themselves in the bronchi of chronic bronchitics. Similarly, adherence to epithelial cells might facilitate bronchial colonisation and infection. The unexpected findings of Turk and Holdaway (1968), suggesting that capsulated H. influenzae strains differ sharply from non-capsulated strains in playing no part in the infections complicating bronchiectasis, fit rather neatly with more recent evidence that non-capsulated strains of this species adhere to human epithelium far better than do capsulated strains (Lewis and Dajani, 1980; Lampe et al., 1982; Pichichero, 1984).
Use of tracheal organ cultures allowed Denny (1974) to study the action of H. influenzae on live ciliated respiratory-tract epithelium. When he grew strains of this species in cultures containing tracheal sections from human fetuses or from various animal species he observed cessation of ciliary action within a few days. Supernatant fluids from any of his H. influenzae strains, capsulated or non-capsulated, caused ciliostasis, loss of cilia and eventually sloughing of the epithelial cells when applied to rat tracheal sections; similar effects were seen with human fetal trachea, but took longer to appear and were less consistent. The substance responsible for these changes was non-dialysable and was only partly inactivated by boiling for 30 min; a substance with similar actions produced by gonococci has been shown to be LPS (Gregg et al., 1981). Denny's findings were reproduced, and the nature of the epithelial damage was elegantly demonstrated in electromicrographs, by Johnson, Clarke and Osborn (1983). Such damage may be relevant to the role of H. influenzae in chronic bronchial disease.

Readers aware of all that has been written about biotyping of H. influenzae since this system was introduced by Kilian in 1976 may be surprised that it has not been mentioned in this review until now. This is because the apparent relationship between biotype and liability to produce invasive disease is probably no more than a reflection of the unequal distribution of type b strains among the biotypes. However, Long, Teter and Gilligan (1983), in a study confined to children, found an excess of biotype-1 strains among those involved in otitis media and in lower-respiratory-tract infections. Biotyping of strains from adults with chronic bronchial disease might, therefore, show some interesting associations.

**Conclusions**

We have today a fairly clear understanding of what diseases H. influenzae causes, and when, and so of what we want to prevent. The route to such prevention is via a fuller understanding of the pathogenic mechanisms of this species; and the last few years have seen a great deal of new light on this subject, but with areas of darkness still remaining.

**REFERENCES**


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