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Biology of *Haemophilus ducreyi*

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INTRODUCTION

In 1889, Auguste Ducrey published his classic study (36) on chancroid and established the etiological agent of this infectious disease as a gram-negative bacillus now known as *Haemophilus ducreyi*. It seems appropriate after 100 years to review our current knowledge of the organism from an historical perspective.

Also, over the past 15 years, the rate and number of reported cases of chancroid have increased significantly (Table 1). Over 2,000 annual cases have been reported in the United States since 1985. Such numbers have not been reported in the United States since 1956. A rising incidence of disease observed in Europe (90) and in armed forces in Asia in the 1970s (50), with urban epidemics reported in North America (15, 47, 121) prior to the sustained increase in disease observed in the 1980s, have resulted in an expanded literature on the disease chancroid and the microbiology of its etiological agent.

This review is based largely on English language publications related to the microbiology of *H. ducreyi* published or cited in *Index Medicus* prior to 1 July 1989 and does not cover an extensive body of literature related to the epidemiology and clinical management of the disease chancroid (88).

EARLY DESCRIPTIONS OF THE ORGANISM

Chancroid, or soft chancre (ulcus molle), had been differentiated clinically from syphilis, or hard chancre, by the mid-1850s. Credit is generally given to Bassereau, a pupil of Ricord, and his 1852 treatise (9) is cited, although some degree of clinical differentiation of genital ulcers was recognized much earlier (66). It was not until 40 or 50 years later, in 1889, however, that the presumptive etiological agent was seen in pure form by Auguste Ducrey at the University of Naples when he reported his findings with the technique of repeated autoinoculation of the skin of the forearm of patients with purulent material from their own genital ulcers (36). This observation alone supported different etiological agents for syphilis and chancroid since repeated autoinoculations were not observed for syphilis. Ducrey described the rod-shaped morphology and negative staining by Gram's method. His observations were supported by the work of Krefting (77) and Unna (142), who found similar organisms in tissue sections from chancres and the associated inguinal buboe. The morphological characteristics of the organisms seen in the deeper tissue and their lack of association with leukocytes when compared with the common intraphagocytic location in surface ulcers led Unna originally to question the identity of the two organisms. Many other investigators confirmed these original observations, but the inability to grow the organism on artificial media substantially hindered study of the organism. These early observations were summarized by Pusey (109) in 1893 and Cheinisse (24) and Petersen (106) in 1894. Both Cheinisse and Petersen added original contributions. It is quite clear from the literature to this date that the organism had not been isolated on artificial media.

It is apparent that, between 1895 and 1900, several investigators succeeded in isolating the causative agent of chancroid on artificial media. The first convincing isolations have been credited by Himmel (56), Davis (27) and Ritchie (114) to Istamanoff (Istamanov) and Akspianz (Akopiantz) in 1897, who reported cultures in a medium of macerated human skin and agar. However, we have been unable to locate the original publication communicated to the Medical Society of Tiflis (*Comptes Rendus de la Societé Médicale de Tiflis*,

TABLE 1. Fifteen-year historical summary of reported cases of chancroid in the United States and *Index Medicus* citations of reports on chancroid or *H. ducreyi*

Yr	No. of citations in *Index Medicus*		Cases[a]	Rate[a]
	Chancroid	*H. ducreyi*		
1974	3	1	945	
1975	3	0	700	
1976	1	0	628	
1977	3	1	455	
1978	3	3	521	0.24
1979	6	3	840	0.38
1980	8	4	788	0.35
1981	7	2	850	0.37
1982	16	10	1,392	0.60
1983	18	16	847	0.36
1984	12	15	665	0.28
1985	10	7	2,067	0.87
1986	13	8	3,756	1.57
1987	14	8	4,998	2.07
1988	12	6		

[a] Reported cases of chancroid and rate per 100,000 population (23).

1897) or the published summary (S. S. Istamanoff and G. Akspianz, "Zur Bakteriologie des Weichen Schankers," *Jahresbericht über Pathologische Mikroorganismen*, volume 14, 1898) to verify the credit. Lenglet (79) reported isolations in 1898 of organisms that appear to be *H. ducreyi* by using similar human skin agar with blood. Other reported isolations in the same year by Maréchal (84) and Jullien (65) appear doubtful based on the descriptive characteristics of the organism. Bezancon et al. (13) are frequently credited with the first isolation of *H. ducreyi* in 1900. This work was especially significant since the isolations were on blood agar alone and the organisms after serial passage were able to produce soft chancres when reinoculated into humans. This work was confirmed by Tomasczewski in 1903 (139). These observations on the initial isolation of *H. ducreyi* were summarized by Himmel (56), Davis (27), and Tomasczewski (139), all of whom also contributed original observations.

It is somewhat surprising that this elegant body of work clearly established in textbooks of the early 1900s was not accepted by the British Medical Research Committee in 1918, who "found no sufficient evidence that what is clinically known as 'soft chancre' or 'soft sore' is a specific disease induced by a single species of microorganism" (cited by Pijper [107]). Thus, at the time of establishment of the genus *Haemophilus* with the report of the Society of American Bacteriologists on characterization and classification of bacterial types in 1920, which included the bacillus of Ducrey (147), there was controversy regarding both the organism and the disease.

LATER DESCRIPTIONS OF THE ORGANISM

Despite the controversy developing after the early descriptions of the organism, significant work continued through the 1920s and 1930s. Teague and Deibert (137, 138) continued to refine the cultural methods and emphasized the importance of isolation in the diagnosis of chancroid. Saelhof (116) reported isolation rates in 1924 of 65% and discussed the effects of media, temperature, and moisture on *H. ducreyi* viability. Because of the continuing difficulty in isolation and subsequent identification of *H. ducreyi*, extensive effort was directed towards the development of immunological diagnostic methods and the production of skin test antigens (40, 55, 129). Nevertheless, in 1935 the U.S. Public Health Service, like the British Medical Research Committee two decades earlier, found that chancroid "is a local disease of the external generative organs in which a sore develops. The cause of this sore is believed to be an infection with a germ, although some physicians question the part which this germ plays" (cited by Greenblatt and Sanderson [41]). Many clinical laboratories gave up attempts to isolate the organism and, with the exception of the determination of hemin requirement by Lwoff and Pirosky in 1937 (81), it was not until chancroid reemerged as an important clinical disease in the military forces in the 1940s that significant new work was published (118, 134). Two series published in 1946 and 1956 are particularly notable in reestablishing the earlier work. Sheldon, Heyman, and Beeson (10, 11, 54, 55, 123) published a series of articles in 1946 which established the efficiency of the cultural method as compared with biopsy, smears, skin tests, and autoinoculation and discussed the in vitro growth requirements and inhibition of the organism by antibiotics. The second series (5, 29, 30, 67, 68), published as the "V.D.R.L. Chancroid Studies" in 1956, furthered the nutritional studies, especially comparing virulent and avirulent strains, and studies in the rabbit, an animal model developed by Reenstierna (110, 111) in the 1920s. In addition to these two series, Mortara and Feiner (39) published several papers in the mid-1940s confirming earlier studies in the rabbit model.

During the later 1960s and early 1970s, interest in the disease chancroid and the organism *H. ducreyi* again waned (Table 1). It was not until the later 1970s and early 1980s, when isolates became available from several urban outbreaks of chancroid in North America and sporadic cases in Europe, Asia, and Africa associated with an apparent increased global recognition of disease, that there was renewed interest in characterizing the organism (15, 47, 82, 90, 121).

STRUCTURE

Colony Morphology

Various solid-agar-based formulations have been reported for the primary isolation and maintenance of *H. ducreyi* (37, 45, 48, 96, 97, 128). Small, nonmucoid, yellow-grey, semiopaque, adherent colonies are characteristic on most solid media, with occasional translucent colonies observed (Fig. 1). Polymorphic colonial morphology may be observed under aerobic growth conditions (132) and gives the appearance of mixed flora from pure cultures. Colonies may be pushed intact across the agar surface and are not associated with surface pitting, but may be associated with zones of alpha-hemolysis on some blood agars, especially in areas of subsurface inoculation. Aggregation of starch on some clear agars has also been reported (43). Adherence of cells within the colony and substantial loss of cell viability within the colony make single-cell colony isolations extremely difficult and have hindered the development of quantitative genetic studies. Colonies examined with the scanning electron microscope suggested that the coherent colony was due to some type of bonding due to the presence of an intercellular matrix (86). Recent studies with low-cohesion variants of other members of the family *Pasteurellaceae* would suggest that a variable low-molecular-weight protein is involved in colony cohesiveness and is recognized by the host immune system (148).

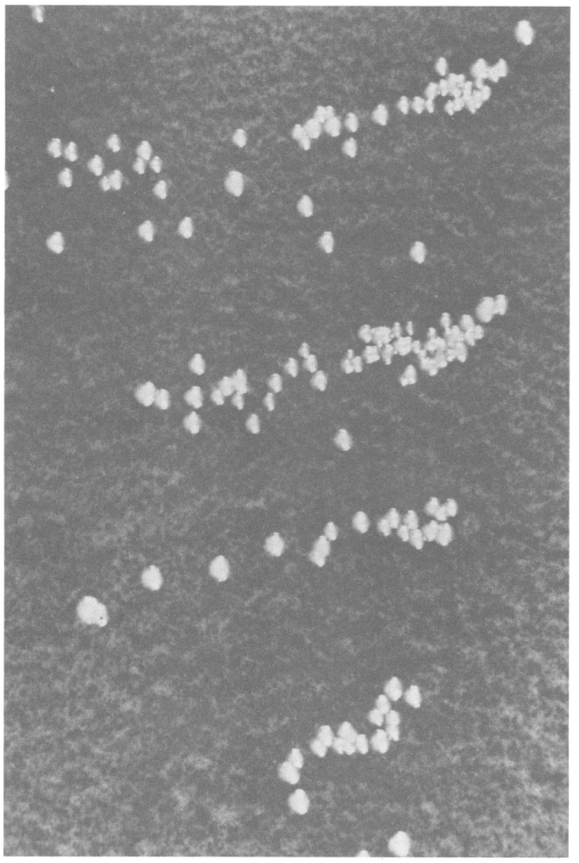

FIG. 1. Colonial morphology of *H. ducreyi*. Reprinted from reference 108 with the permission of the American Public Health Association.

Gram Stain

From the original observations of Ducrey, the Gram staining characteristics and morphology of *H. ducreyi* have been the most important structural features of the organism. Although occasionally somewhat pleomorphic, the average bacillus has a length of 1.2 to 1.5 μm and is approximately 0.5 μm in width with rounded ends. The streptobacillary form and the characteristic parallel chains described as "railroad tracks" are seen in liquid culture or tissue. The more complicated "schools of fish" and whorls described as "fingerprints" (Fig. 2) are more commonly seen on solid agar. Some authors have suggested that a presumptive microscopic identification of *H. ducreyi* could be made based on these morphological criteria alone (16), although the sensitivity and specificity have not been shown to be acceptable for routine use (95, 131). Most studies have regularly reported gram-negative staining, but several studies have reported variable gram-positive characteristics. Nicolau and Banciu (92) reported gram-negative staining on initial isolation, with gram-positive staining after subculture. deAssis (31) reported variable staining based on the method of cell fixation. Deacon et al. (28) also reported the isolation of a gram-positive smooth phase. Confirmation of the identify of the reported isolates is rarely possible, but the isolation and identification of *H. ducreyi* are sufficiently difficult that many reported studies are suspect. The series of studies by Reymann (112, 113) have been questioned by Kilian (71), and the reported isolates by Deacon et al. (28) were subsequently identified as *Corynebacterium acnes* (29).

Electron Microscopy

Kilian and Theilade (72) reported the first English description of the cell wall ultrastructure of *H. ducreyi* by electron microscopy. Their only authentic strain of *H. ducreyi*, CIP542^T, demonstrated typical gram-negative features. Marsch et al. (85) extended earlier cited work by Cazarre and Barreto (22) and Ovchinnikov et al. (102) and suggested that electron microscopic examination of tissue biopsies could be used to establish a presumptive diagnosis of chancroid. As with the Gram stain, the sensitivity and specificity have not been demonstrated to be acceptable for routine use. Bertram (M.Sc. thesis, University of Manitoba, Winnipeg, 1980) undertook an ultrastructural study of a number of *H. ducreyi* isolates and described the presence of antibody-stabilized extracellular capsular material by ruthenium red staining as well as the regular gram-negative characteristic of the cell wall (Fig. 3 and 4). The use of ruthenium red or Alcian blue in normal fixation procedures revealed the presence of a discontinuous distribution of exocellular material which could be stabilized as a continuous layer by polyvalent rabbit antiserum, although lacking the fine structure detail of antibody-stabilized, ruthenium red-stained capsular material of *H. influenzae* type b (115) (Fig. 5). Similar results were seen with Alcian blue for the *H. ducreyi* type strain, CIP542^T, as well as recent clinical isolates. Studies with other organisms suggest that ruthenium red and Alcian blue stain acidic polysaccharides (18, 69). Recent studies by Johnson et al. (64) failed to demonstrate surface appendages such as pili or flagella and showed no evidence of an extracellular capsule. These mixed results are similar to earlier reports with the gonococcus. Discontinuous antibody-stabilized capsules have been demonstrated for *Neisseria gonorrhoeae* with both ruthenium red and Alcian blue in broth-grown cultures as well as in cultures grown in guinea pig subcutaneous chambers, but results were variable (32, 52). Intercellular stranding of the exocellular material was observed for both the gonococcus and *H. ducreyi*. Bertram was unable to demonstrate differences in exocellular material in virulent and avirulent strains, but biochemical characterization of the exocellular, ruthenium red-staining material was not undertaken.

Cell Surface Composition

Several studies have used indirect immunofluorescence techniques to demonstrate *H. ducreyi*-specific and cross-reacting surface antigens (33, 125). Cross-reactions were most notable with other species of *Haemophilus* and members of the related genera *Pasteurella* and *Actinobacillus*. The nature of the surface antigens was not described in these studies.

Outer membrane protein (OMP) profiles have demonstrated sufficient heterogeneity to provide a basis for epidemiological studies (98, 136). In vitro radio-iodination studies have been reported for *H. ducreyi* (1) demonstrating a variety of labeled proteins. Similar studies with *H. influenzae*, however, have shown that cytoplasmic membrane proteins as well as OMP are labeled by this procedure (80). Western blot (immunoblot) analysis of antigens detected by polyvalent rabbit (119) and mouse (3) antisera raised against whole organisms have been reported. Major antigens detected were among the proteinase K-sensitive OMP. Cross-reactions were most notable with *H. influenzae* and *H. parainfluenzae*. Similar techniques have been used to characterize monoclonal antibodies produced against *H. ducreyi*

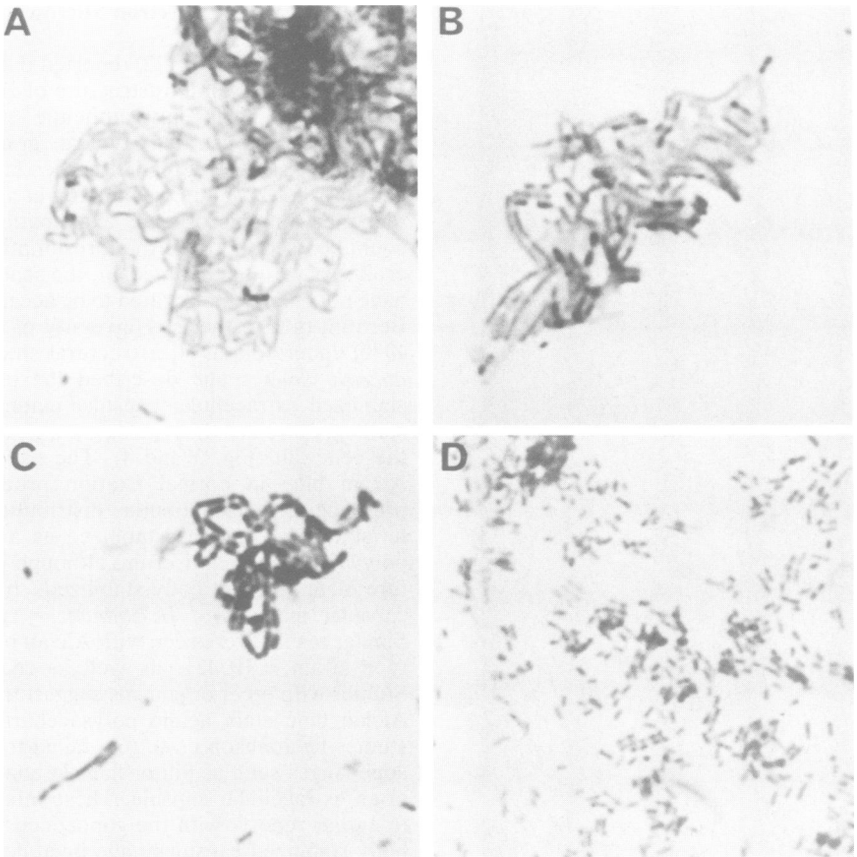

FIG. 2. Gram stain characteristics of *H. ducreyi*. Complex whorls (A); parallel chains (B); streptobacillary chains (C); short chains and individual bacilli (D). Kindly provided by D. Hardy.

(49, 120). These studies demonstrated monoclonal antibodies which recognized a common epitope of an OMP of *H. ducreyi*, *H. influenzae*, and *H. parainfluenzae*. Specific monoclonal antibodies were also found which recognized all *H. ducreyi* strains and subsets of strains. These studies clearly demonstrate that the OMPs of *H. ducreyi* are antigenically variable structures recognized by the immune system. Their role in the pathogenesis of infection has not been determined. Similar studies with the penicillin-binding proteins (PBPs) have not demonstrated cross-reactions between monoclonal antibodies raised against epitopes of *H. influenzae* PBP and those of *H. ducreyi* (122).

Structure-function studies of the PBPs of *H. ducreyi* demonstrated only two detectable PBPs compared with three to eight for most eubacteria and a dual function of PBP1 of *H. ducreyi* similar to PBP1 and PBP3 of *Escherichia coli* (78).

In addition to the ruthenium red-staining exocellular material observed in some electron micrographs, surface carbohydrates have been demonstrated for a number of strains by lectin binding (76). Cell agglutination by lectin binding demonstrated a variety of surface-exposed carbohydrates. All strains were agglutinated by the lectin of *Phaseolus vulgaris*, indicating the presence of *N*-acetylgalactosamine. Every strain reacted with at least 2 lectins from a panel of 14 and at least one strain reacted with each of the lectins. Many combinations were observed, indicating a variety of surface carbohydrates, and it was suggested that lectin binding patterns might be useful for epidemiological studies. The chemical nature of these surface-exposed carbohydrates, however, was not determined.

The lipopolysaccharide (LPS) structure of *H. ducreyi* has been determined by several investigators (4, 101). O-carbohydrate side chains were not found, and the LPS of *H. ducreyi* appears to resemble the rough LPS of other organisms. C_{14}, C_{16}, C_{18}, and C_{20} fatty acids were found in all strains as well as a highly substituted keto-deoxyoctulosonic acid, as reported by others for *H. influenzae* (51, 104). Rhamnose and mannose, characteristic of O carbohydrates of other species, were not found. Electrophoretic variation between virulent and avirulent strains was observed, however, suggesting that core LPS is more highly substituted in virulent strains of *H. ducreyi*. This observation is supported by further studies on the role of LPS in complement-mediated phagocytosis and serum killing of *H. ducreyi* (100, 101). Cultural conditions have been shown to affect LPS profiles (2, 60), however, and additional studies are needed to determine the biochemical structure of the LPS from *H. ducreyi*.

Cell wall fatty acid composition studies have shown small but reproducible quantitative differences between *H. ducreyi* and related bacteria (61, 62). *H. ducreyi* differed slightly from other hemin-requiring species of *Haemophilus* by a higher concentration of 14:0 and lower concentration of 16:0 fatty acids. The simple fatty acid profile of *H. ducreyi* (Table 2) is shared by *H. influenzae* and other members of the family *Pasteurellaceae*. Studies in other groups have not

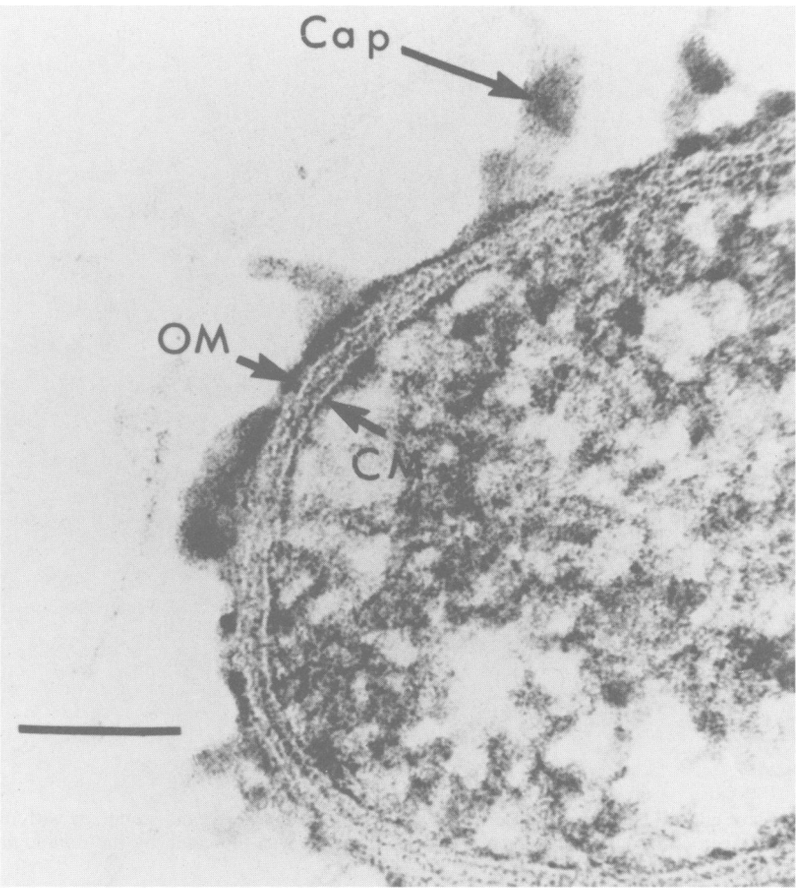

FIG. 3. Electron micrograph of clinical isolate of *H. ducreyi* stained with ruthenium red and demonstrating discontinuous capsular-like (Cap) material and the typical outer membrane (OM) and cytoplasmic membrane (CM) of the gram-negative cell wall. Bar, 0.1 μm. Kindly provided by P. Bertram.

demonstrated much differential power in fatty acid composition analysis below the genus level.

Clearly, additional structural and compositional studies are needed. The cell surface is almost certainly responsible for the cohesive colonial structure and the inability to grow the organism in liquid culture without substantial autoagglutination. The role of surface structures in adherence and virulence requires further study, and the role of the host response to surface components in limiting spread of the organism and protection from infection are needed if vaccines are to be developed.

METABOLISM

Growth Requirements

Media. The fastidious nature of *H. ducreyi* has been recognized since the first in vitro studies of the organism. Bezancon et al. (13, 14) were apparently the first to isolate and passage strains on a defined blood agar medium. Lwoff and Pirosky (81) determined the requirement for hemin, but it was not until recently that significant progress has been made toward a chemically defined medium similar to that described for *H. influenzae* (53, 75). A number of studies have reported the comparative efficacy of various media in the primary isolation of *H. ducreyi* from clinical chancroid (37, 96). All media, for this purpose, are nutritionally complex, and only a few studies have looked at requirements for individual components. Hemin requirement was confirmed by demonstrating the absence of enzymes involved in the conversion of δ-aminolevulinic acid to protoporphyrin (44). Quantitative hemin requirements of *H. ducreyi* are considerably higher than those reported for other hemin-requiring *Haemophilus* species (6, 44). Free hemin is not required and *H. ducreyi* seems to be able to utilize hemoglobin, myoglobin, and certain other heme proteins, such as catalase, as a source of hemin. Albumin has been shown to be an essential serum component for growth. It has not, however, been established whether albumin serves as a nutritional source, absorbs toxic metabolic products (17, 103), or provides a source for bound trace components or elements. One study has shown a requirement for selenium and L-glutamine for optimum growth (143). Thus, a base medium containing acid-hydrolyzed protein, such as Mueller-Hinton agar, or enzymatically hydrolyzed protein, such as GC medium base, supplemented with hemin, albumin, selenium, and L-glutamine seems to provide growth requirements similar to complex media such as chocolatized blood agar with IsoVitaleX.

Environment. Few studies have systematically evaluated environmental conditions for optimum growth of *H. ducreyi*. One recent study (132), which avoids some concerns of earlier studies regarding the identification of the organisms

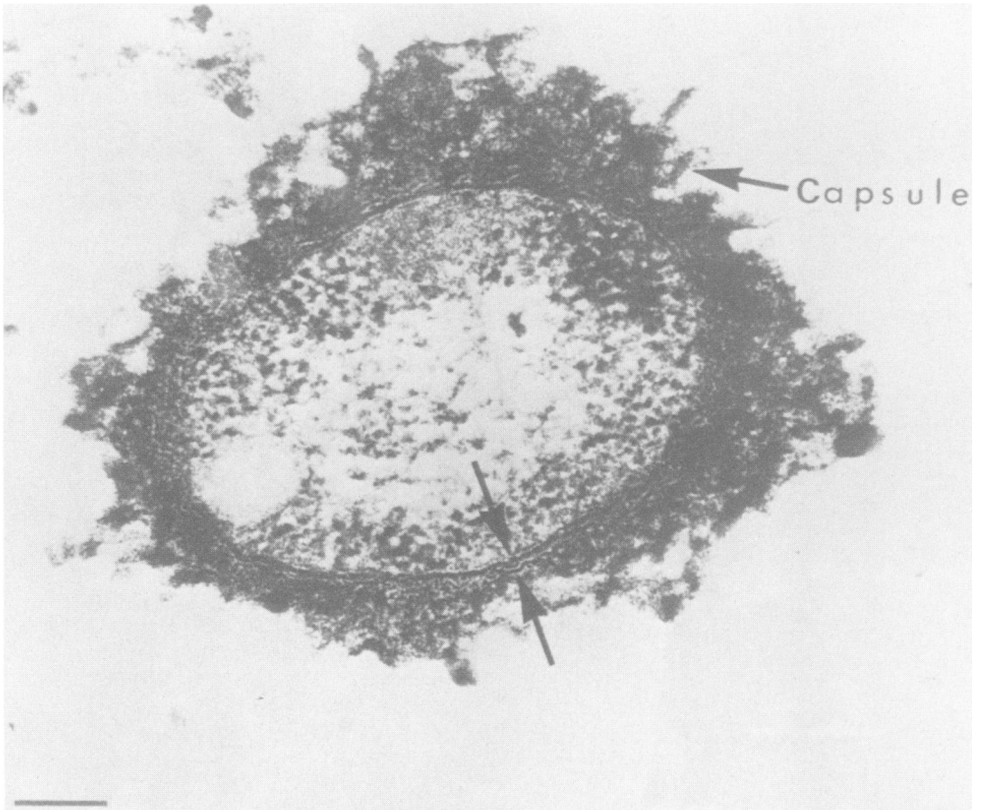

FIG. 4. Electron micrograph of clinical isolate of *H. ducreyi* stained with ruthenium red and stabilized with rabbit antiserum. Continuous exocellular material (capsule) is seen external to the typical gram-negative cell wall indicated by the double arrows. Bar, 0.1 μm. Kindly provided by P. Bertram.

used for the study, clearly demonstrated a water-saturated atmosphere with increased CO_2 optimum for most strains but demonstrated that this was likely a strain and passage variable. In their hands, the best growth was obtained under microaerophilic conditions obtained in a closed anaerobic jar without a catalyst but with two CO_2- and H_2-generating envelopes, so-called *Campylobacter* growth conditions. All strains also grew under strict anaerobic conditions. An optimum pH of 6.5 to 7.0 and a temperature of 28 to 35°C were also observed. Similar findings of optimum growth temperature below 37°C have been made by others.

Electron Transport

Systematic studies of the central cyclic pathways of *H. ducreyi* have not been reported. Preliminary studies with other *Haemophilus* species (141) have demonstrated a partial tricarboxylic acid cycle and increased malate dehydrogenase as being characteristic of hemophili. Production of acid from glucose has been reported for *H. ducreyi*, but acid end products have not been characterized and acid is frequently produced from the peptone broth used for these studies without glucose. Most strains of *H. ducreyi* are positive in the Voges-Proskauer reaction. The specific enzymes involved in the conversion of pyruvate to α-acetolactate and the decarboxylation of α-acetolactate to acetoin have not been described, however (38). Studies on the production of 2,3-butanediol and diacetyl are needed to establish the importance of this metabolic pathway in *H. ducreyi*.

Carlone et al. (19) demonstrated both demethylmenaquinone (DMK), with a six-unit unsaturated isoprene side chain, and menaquinone, with a seven-unit unsaturated isoprene side chain, in 11 strains of *H. ducreyi*. A single strain reported by Hollander et al. (58) produced only DMK. The presence of menaquinone had been described previously in only two members of the *Pasteurellaceae*, *Actinobacillus actinoides* and *Pasteurella anatipestifer*, both species of questionable taxonomic status (58, 83). Previous studies with other species of *Haemophilus* have demonstrated the presence of DMK, ubiquinone, or both (59). The most typical species of *Haemophilus* (*H. influenzae*, *H. parainfluenzae*, *H. aegyptius*, *H. paraphrophilus*, *H. parahaemolyticus*, and *H. paraphrohaemolyticus*) produced DMK only and showed increased growth anaerobically in the presence of fumarate. Both DMK and menaquinone are capable of mediating electron transfer from reduced nicotinamide adenine dinucleotide to fumarate and oxygen, but DMK is required for electron transfer to succinate (57). Anaerobic electron acceptors have not been described for *H. ducreyi*, but nitrate reductase is found in most strains.

Metabolic Pathways

Nothing is known of the organization and control of metabolic pathways in *H. ducreyi*. Various enzymatic activities have been reported, largely related to differential characteristics used for identification. Cumulative results with several hundred strains reported in the literature suggest reasonably consistent patterns of selected enzyme activity,

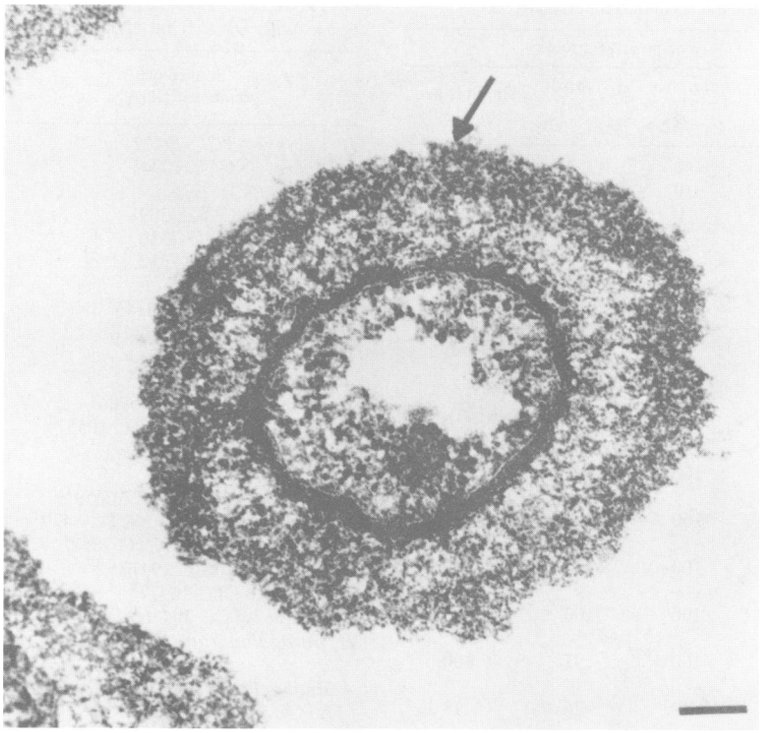

FIG. 5. Electron micrograph of clinical isolate of *H. influenzae*, type b, stained with ruthenium red and stabilized with type-specific antiserum. Arrow indicates the exocelluar polysaccharide. Bar, 0.1 μm. Kindly provided by P. Bertram.

but notable discrepancies exist, possibly related to methods and media (Table 3). All strains reported to date have a broad range of phosphatase activity, including alkaline phosphatase, acid phosphatase, and phosphoamidase. Alkaline phosphatase is used as a differential character for identification. Catalase activity has been uniformly negative when tested by dropping hydrogen peroxide on surface colonies but was recently reported as positive when a tube test was used (133). Oxidase activity is generally negative when *N,N*-dimethyl-*p*-phenylenediamine oxalate is used and positive when *N,N,N,N*-tetramethyl-*p*-phenylenediamine dihydrochloride is used (94), but in one series it was reported positive in 18 of 29 strains tested with the dimethyl compound (133). Indole production and urease activity have not been convincingly demonstrated, although one report (128) described three strains with weak urease activity. Lysine decarboxylase, ornithine decarboxylase, and arginine dihydrolase have not been described, raising questions about polyamine synthesis in *H. ducreyi*. The most striking observation is the uniform absence of glycohydrolase activity and the widespread aminopeptidase activity (Table 4). Low levels of esterase activity have also been reported (21, 133, 144). Trypsin or chymotrypsin-like activity has not been described.

It is not clear why *H. ducreyi* has such an unusual metabolic profile, and careful studies of selected metabolic pathways are needed to establish the relationships of this species with other eubacteria in general and the *Pasteurellaceae* specifically (63).

TABLE 2. Percent cellular fatty acid composition of *H. ducreyi* compared with that of *H. influenzae*

Species	Fatty acid						
	14:0[a]	3-OH-14:0[b]	16:1	16:0	18:2	18:1	18:0
H. influenzae	12.7	11.6	31.4	40.6	0.2	0.5	2.4
H. ducreyi	25.1	10.5	34.7	25.5	0.3	0.9	0.8

[a] The number preceding the colon indicates the number of carbon atoms in the chain; the number following the colon indicates the number of double bonds.

[b] "3-OH" indicates a hydroxyl group and its position (modified from reference 61).

GENETICS

Chromosome

The guanine-plus-cytosine content of *H. ducreyi* has been shown to be 38 to 39 mol% (20, 71). Deoxyribonucleic acid

TABLE 3. Major biochemical characteristics of *H. ducreyi*

Characteristic	Reaction[a]
Porphyrin biosynthesis	−
Nitrate reduction	+[v]
Catalase	−[v]
Oxidase	+[v]
Voges-Proskauer	+[v]
Indole production	−
Urease	−[v]
Ornithine or lysine decarboxylase	−
Arginine dihydrolase	−
Alkaline or acid phosphatase	+
H_2S production	−
Deoxyribonuclease	−

[a] From references 21, 26, 48, 94, 97, 127, 128, 130, 133, and 144. See text for discussion of reported variable (v) reactions.

TABLE 4. *H. ducreyi* hydrolase activity

Enzyme	% of positive strains			
	Reference 133 (n = 29)	Reference 21 (n = 32)	Reference 144 (n = 200)	Unpublished[a] (n = 30)
α-Glucosidase	0	0	0	0
β-Glucosidase	0	0	0	0
α-Galactosidase	0	0	0	0
β-Galactosidase	0	0	0	0
β-Glucuronidase	0	0	0	0
N-Acetyl-β-glucosaminidase	—[b]	0	0	0
α-Mannosidase	—	0	0	0
α-Fucosidase	0	0	0	0
β-Xylosidase	0	0	0	—
α-Arabinosidase	—	0	0	—
L-Arginine aminopeptidase	100	100	100	100
L-Alanyl aminopeptidase	—	100	100	100
L-Lysine aminopeptidase	—	100	100	100
Glycine aminopeptidase	90	100	100	100
Glycyl-glycine aminopeptidase	75	100	100	100
L-Serine aminopeptidase	59	100	97	100
L-Proline aminopeptidase	—	9	26	13
L-Hydroxylproline aminopeptidase	—	9	42	13
L-Ornithine aminopeptidase	—	—	60	47
L-Glutamine aminopeptidase	—	—	97	83

[a] W. L. Albritton, W. E. DeWitt, M. L. Thomas, and F. Sottnek, unpublished observations.
[b] —, Not reported.

TABLE 5. Hybridization of DNA from *H. ducreyi* CIP542^T with DNA from species of the family *Pasteurellaceae*

Source of unlabeled DNA[a]	Relative binding ratio (%)	
	55°C[b]	60°C[c]
H. ducreyi ATCC 27722	94	—[d]
H. ducreyi NCTC 10945	90	—
H. ducreyi CIP A76	100	—
H. ducreyi CCUG 7309	—	99
H. ducreyi CCUG 7310	—	93
H. ducreyi CCUG 7312	—	98
H. influenzae NCTC 8143^T	13	4
H. aegyptius ATCC 11116^T	16	2
H. haemoglobinophilus NCTC 1659^T	19	3
H. aphrophilus NCTC 5906^T	17	1
H. paraphrophilus NCTC 10557^T	—	1
H. paraphrohaemolyticus NCTC 10670^T	—	1
H. haemolyticus NCTC 10659^T	16	1
H. parahaemolyticus NCTC 8479^T	—	4
H. parainfluenzae NCTC 7857^T	16	4
H. parasuis ATCC 19417^T	—	1
H. segnis NCTC 10977^T	12	—
H. avium ATCC 29456^T	16	1
H. paragallinarum ATCC 29545^T	—	3
Actinobacillus pleuropneumoniae ATCC 27088^T	31 (9 at 70°C)	6
A. equuli NCTC 8529	33	—
A. actinomycetemcomitans ATCC 29239	6	—
Pasteurella multocida NCTC 10322^T	16	—
P. pneumotropica NCTC 8141^T	19	—
P. ureae NCTC 10219^T	27	—

[a] CIP, Collection of the Institute Pasteur; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures; CCUG, Culture Collection University of Goteborg. Superscript T indicates type strain of species.
[b] Hydroxylapatite method (8; Albritton et al., unpublished observations).
[c] S1 nuclease method (20).
[d] —, Not determined.

(DNA) hybridization studies have shown *H. ducreyi* strains to belong to a highly homogeneous group with homology values of 85 to 100% and thermal denaturation changes of <1°C (20). Our own results with clinical isolates and the culture collection strains *H. ducreyi* ATCC 27722, NCTC 10945, and CIP A76 gave similar results (Table 5). The type strain of *H. ducreyi*, CIP542^T, however, appears unrelated to any of the current species of *Haemophilus* or several members of the genera *Actinobacillus* and *Pasteurella*. The S1 nuclease method gives values similar to the hydroxylapatite method when strains are highly related but gives lower values for distantly related strains (42). In addition, Casin et al. (20) have shown no hybridization of DNA from *H. ducreyi* CIP542^T and the related organisms *H. agni*, *H. somnus*, *H. equigenitalis*, and *H. piscium*, which historically were included in the genus *Haemophilus* but are not closely related to the type species *H. influenzae* or other members of the genus *Haemophilus*.

Three fragments of *H. ducreyi* chromosomal DNA coding for proteins expressed in *E. coli* and reactive with polyvalent rabbit antiserum have been isolated from a lambda gt11 library and shown to be specific for *H. ducreyi* (105). It was suggested that amplification by limited growth or adaptation of the polymerase chain reaction could improve sensitivity. As well as being useful for detection and identification, this approach should be useful in developing probes for epidemiological studies by restriction fragment length polymorphism analysis and searching for species-specific repetitive elements described for other bacteria.

In genetic transformation studies to determine relatedness within the family *Pasteurellaceae*, we found that *H. ducreyi*, unlike some members of the family, showed no competition for homospecific transformation of *H. influenzae* (7, 8). Transfer of chromosomal genes by transformation has not been demonstrated for *H. ducreyi*. All things considered, *H. ducreyi* does not appear to be genetically related to members of the genus *Haemophilus* or the family *Pasteurellaceae* despite requiring hemin for growth and sharing common surface antigens.

Plasmids

Although apparently unrelated at the level of the chromosome, *H. ducreyi* shares a significant gene pool with members of the *Pasteurellaceae* and the *Enterobacteriaceae* families. The core plasmid for the several plasmids conferring ampicillin resistance in *H. ducreyi* is found in other species of *Haemophilus* and *Neisseria* (87). Sequence analyses of the ampicillin resistance transposon (Tn*A*) in plasmids from *H. ducreyi* and *N. gonorrhoeae* support a model

of transposition of the resistance determinant (Tn*A*) from the *Enterobacteriaceae* to an indigenous *Haemophilus* plasmid with mutation to create a more efficient *Haemophilus* promoter before transfer of the entire plasmid to *Neisseria*, accompanied by deletion of a portion of the resistance transposon (25). Studies of other *H. ducreyi* plasmids shared by other genera showed substantial homology in regions coding for antibiotic resistance and replicative function, but also showed sequence divergence (145). These studies suggest the presence of consensus or polypromoters as well as consensus or polyreplication sequences in these shared minireplicons. Few studies have reported susceptibilities to agents other than antibiotics (89, 126), and no information is available regarding the genetics of susceptibility to nonantibiotic chemicals, including heavy metals.

Transfer of conjugative plasmids and mobilization of nonconjugative plasmids have been demonstrated for *H. ducreyi* (88), although transfer of chromosomal genes by conjugation has not been demonstrated.

Bacteriophage have never been demonstrated in *H. ducreyi*, and neither *H. influenzae* nor *Enterobacteriaceae* phage have been demonstrated to replicate in *H. ducreyi*.

The significance of such observations to the taxonomy of *H. ducreyi* remains to be determined.

PATHOGENESIS

Very little is known about the pathogenesis of infection due to *H. ducreyi*. To date, the organism has not been isolated from nonhuman sources, and avirulent strains have not been reported on primary isolation. Avirulent strains, defined in the animal model (35), have been isolated by repeated passage in vitro, although Nicolle (93) was able to passage a virulent strain 50 cycles over 3 months and retain virulence in the monkey. Some avirulent strains showed alterations in OMP profiles and susceptibility to polymyxin (46, 99). Subsequent studies showed no correlation of this phenotype with virulence (100, 101). These studies demonstrated variation in electrophoretic mobility of LPS between virulent and avirulent strains and correlated virulence in isogenic virulent/avirulent strains with resistance to complement-mediated bactericidal activity of human and rabbit sera.

Considerable controversy exists regarding an asymptomatic carrier state. Studies as early as 1925 (117) have suggested the possibility of asymptomatic carriage, but, as with many studies of chancroid, sufficient criteria were not reported to be confident the organism was the same as currently identified. Recent studies in the United Kingdom (73, 74) have reported isolation of *H. ducreyi* from herpetic lesions after healing and from asymptomatic men, but other studies have been unable to reproduce these results (35). Isolation of *H. ducreyi* from asymptomatic females has been reported more often (70), but unsuspected genital ulcers are common in sexual contacts of men with chancroid and transient physical carriage without replication is always difficult to exclude in females. *H. ducreyi* has occasionally been isolated from other skin or mucosal lesions presumably following autoinoculation from a genital source. Clearly, additional studies are needed to establish a significant carrier state.

The organism is thought to penetrate the normal skin through minor abrasions, but essential factors for adherence and the mechanisms by which the organism produces tissue necrosis are unknown. Both humans and other animals mount a humoral immune response to infection with *H. ducreyi*, but this response is apparently nonprotective since reinfection is common and repeated autoinoculations were observed in earlier human studies of chancroid. A cellular immune response to infection with *H. ducreyi* has not been reported.

The clinical disease is classically that of a genital ulcer with associated inguinal bubo. Systemic infection or isolation from visceral organs has never been reported. Animal models of the genital ulcer, consisting of the development of a papule and ulcer following cutaneous inoculation, have replaced autoinoculation as a test of virulence. The rabbit model, introduced by Reenstierna (110, 111) in the early 1920s, remains the standard animal model. Recently, a mouse model has been introduced which should facilitate studies of virulence (140).

Early histological studies of the human ulcer demonstrated a primary pyogenic response and have been reproduced in the animal model. It is interesting that phagocytosis of organisms was seen only in the superficial layers with less inflammatory infiltration of the deeper tissue. Organisms are rarely isolated from the inguinal bubo, and the pathogenesis of this lesion is unclear. No animal model of the inguinal bubo has been described.

CLASSIFICATION AND NOMENCLATURE

The taxonomy of *H. ducreyi* should include aspects of nomenclature, identification, and classification. This review does not cover the primary isolation and identification of *H. ducreyi*. Readers are referred to any of several sources dealing with the diagnosis of chancroid for this information (45, 95, 97, 108, 128, 131) and especially to the recent review by Morse (88).

The nomenclature of *H. ducreyi* has historically followed the tenets of the *Bacteriological Code*. The genus *Haemophilus* was established with the preliminary report of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types (146) and the bacillus of Ducrey was included in this genus, although not given a specific name (147). *Hemophilus ducreyii* was the species designation in the first edition of *Bergey's Manual of Determinative Bacteriology* (12), but the species designation *Coccobacillus ducreyi* had been used earlier by Neveu-Lemaire (91). Spelling of the genus has varied. *Hemophilus* was retained through the 6th edition of *Bergey's Manual* (1948) and indexed under both spellings in that edition. *Haemophilus* was adopted in the 7th edition published in 1957 and has always been the preferred spelling outside the United States. With the acceptance of the approved lists of bacterial names in 1980 (124), however, the only acceptable spelling of the genus name became *Haemophilus*.

The current classification of *H. ducreyi* would appear to be incertae sedis, although it is listed as a true *Haemophilus* species in *Bergey's Manual of Systematic Bacteriology*. Requirement for hemin or nicotinamide adenine dinucleotide or both is no longer restricted to members of the genus *Haemophilus*; however, these growth factor requirements have not been demonstrated for species outside the *Pasteurellaceae*. Unlike other species of *Haemophilus*, *H. ducreyi* has not been demonstrated convincingly to attack carbohydrates fermentatively. The presence of menaquinones is also unusual for the *Pasteurellaceae*. All things considered, *H. ducreyi* would appear to be a monospecific genus genetically distant from members of the family *Pasteurellaceae* but sharing many morphological, structural,

and metabolic features with members of the *Pasteurellaceae*, including the type species, *H. influenzae*. Additional studies are required to determine relationships with minor species of the family.

IMPORTANT UNANSWERED QUESTIONS

There are a number of unanswered important questions regarding the microbiology of *H. ducreyi*. The genetic relatedness of *H. ducreyi* to other eubacteria in general, and to members of the *Pasteurellaceae* specifically, seems to be the most important at this time. Studies are needed to determine the ribosomal ribonucleic acid oligonucleotide catalogs or sequences to determine the relatedness of *H. ducreyi* to organisms with similar phenotypes but DNA-DNA homology of <20%. Considerable information is needed regarding the central metabolic pathways, electron transport, role of menaquinone, and requirement for hemin to understand the unusual biochemical profile. No information is available yet regarding genome organization and metabolic regulation. The presence or absence of exocellular material should be confirmed, and its structure should be determined. Explanations are needed for the similarities of some surface proteins as evidenced by the sharing of OMP epitopes among the *Pasteurellaceae*, while demonstrating such divergence of structure among the PBPs. A clear understanding of the essential virulence factors and the molecular basis of pathogenesis is needed. Many of these studies will require the development of as yet unavailable dispersed liquid cultures and chemically defined growth media.

As we enter the second century of study of this organism, it is apparent that there is much to be learned.

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