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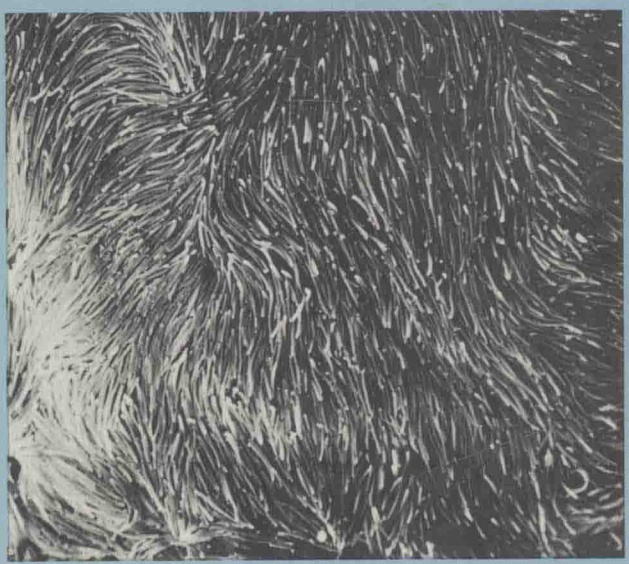
Unusual Microorganisms

Gram-Negative Fastidious Species



edited by
Edward J. Bottone

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Gram-Negative Fastidious Species

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Preface

The microbial species comprising the central theme of this volume are, by virtue of their intrinsic nature, regarded as unusual isolates from human clinical sources. While some species produce organ-specific disease (e.g., endocarditis) others are associated with a broad range of human infections, either causally or reputedly. Each has a special charm which has enticed medical and basic scientists alike to intensive investigation regarding their microbiological, epidemiological, clinical, and therapeutic correlates. Particular emphasis has been placed on their cultural and metabolic attributes leading to isolation and definitive identification. The present volume strives to place into sharper focus contemporary research regarding each of these evolving microbial entities.

Edward J. Bottone

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Eikenella corrodens: A New Perspective

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INTRODUCTION

Eikenella corrodens is a fastidious, facultatively anaerobic gram-negative rod that is part of the normal human oral and intestinal flora. Although "corroding bacilli" were isolated from human sources almost 35 years ago by Henriksen (29), the recognition of *E. corrodens'* clinical importance has been delayed. Because it is a slow-growing microorganism and is usually isolated in mixed culture, *E. corrodens* is easily overgrown and frequently missed on routine culture (6). It may also be missed due to its fastidious nature or as a result of improper selection of media or atmosphere of incubation. Intensive investigation of the cultural attributes and microbiological characteristics of *E. corrodens* has led to its increasing isolation and definitive identification from clinical sources. However, *E. corrodens* may still be overlooked by clinicians. It causes indolent infections from which it is usually isolated in mixed culture and may mistakenly be considered a "contaminant" or "normal flora." Because it is an unusual isolate, its pathogenic potential may be underestimated, and therapy is frequently directed against other isolates, with no

attention being given to the susceptibility of *E. corrodens* until therapeutic failure occurs.

Fortunately, the recovery of *E. corrodens* as the sole isolate in cases of endocarditis, meningitis, osteomyelitis, and other diseases has established *E. corrodens* as a significant pathogen in a wide variety of human infections.

HISTORICAL PERSPECTIVE

Early References: The Corroding Bacilli

In 1948, Sverre D. Henriksen of Oslo University (29) described the isolation of three strains of strikingly peculiar, previously undescribed, "gram-negative, nonspore-forming, anaerobic rods." While each strain produced slightly raised, flat, smooth, or slightly irregular colonies, their most marked peculiarity was colonial variants that "appeared as depressed in the surface" of the agar. He suggested their possible classification as a member of the genus *Bacteroides*.

These strains were isolated from mixed anaerobic cultures of a vaginal specimen, a pulmonary abscess, and a perianal abscess. The colonies were also peculiar because prolonged incubation produced a finely granular surface growth that filled in the depressions and went on to exhibit "continuous spreading growth with concentric zones of spread." Growth was never heavy, but continued for at least 4 weeks. He noted the organisms' preference for blood agar over ascites or lactose agar, and that they were difficult to grow in liquid media.

Two years later, Per Holm from the Statens Seruminstitut of Copenhagen introduced the term "corroding bacillus" (33). While studying the pathogenic importance of the "other microbes" present in the cultures of 785 patients with actinomycosis, he noted the very frequent isolation of "a hitherto undescribed, anaerobic gram-negative bacillus." He wrote, "this microbe is known in the laboratory as the 'corroding bacillus' because its colonies, when seen with the naked eye, resemble small matt, corroded patches on the glistening surface of the blood agar." Although very similar to those studied by Henriksen (29), the organisms described by Holm differed in some important respects.

In 1958, Eiken, also from the Statens Seruminstitut of Copenhagen, reported the isolation of 61 strains of an organism that culturally and in Gram-stained smears corresponded to Holm's "corroding bacillus" (12). He randomly selected 21 strains of these slow-growing gram-negative rods that made "small depressions into the surface of the blood agar plates" for intensive study. Eiken not only described the cultural appearance, morphology, and

growing conditions of these strains, but also their biochemical properties. Eiken's strains did not ferment carbohydrates, liquefy gelatin, or hydrolyze starch, and were in fact biochemically inactive except for the production of H_2S and a positive nitrate reaction. Although he found "a small number of strains," with a yellow pigment, "whose colonies differed slightly, but constantly," Eiken did not biochemically study these variants any further. Nor did he observe spreading growth or filling of the depressions by bacterial growth, even upon continuous cultivation by any of the 21 strains. Believing all the isolates to be a single species of anaerobe, he named them *Bacteroides corrodens*.

However, Eiken did not designate a type strain for the species. Henriksen proposed that strain 333/54-55, a facultatively anaerobic strain from Eiken's collection, be designated as the type strain (30). He deposited it with the National Collection of Type Cultures in London and the Type Culture Collection in Rockville, Maryland (N.C.T.C. 10596, A.T.C.C. 23834).

Bacteroides corrodens

Because most of Eiken's strains were either strictly anaerobic or "adapted to aerobic growth only after a number of subcultures" (37), he placed them in the genus *Bacteroides*, where they remained from 1958 until 1972. While some investigators reported the clinical isolation of *B. corrodens* (45,52), others sought to delineate and end the taxonomically confusing situation of including facultatively anaerobic strains and purely anaerobic strains in the same genus (31,32,36).

Studying the risk of bacteremia due to nonaerobes following dental extraction, Omar Khairat (45) isolated what he initially thought to be a new species of *Fusiformis*. Upon further study, he realized that these organisms which grew "depressed below the level of the agar surface" were similar to those already isolated by Holm (33) and named them *Bacteroides corrodens*. Khairat studied 19 strains, 16 from postextraction bacteremias and 3 from gingival cultures, which were relatively biochemically inactive except for a positive nitrate reaction and the abundant production of H_2S. Interestingly, Khairat's strains "did not grow at all aerobically or in air containing 5% CO_2," yet were nonpigmented, nonmotile, oxidase-negative, and urease-negative. Discrepancies between Khairat's findings and later taxonomic studies on both the strictly anaerobic *B. corrodens* and the facultative *B. corrodens* (*Eikenella corrodens*) have been ascribed to insensitive methodology (36).

Since *B. corrodens* had been isolated exclusively in mixed culture, Henriksen (29) attempted to establish pathogenicity by inoculating *B. corrodens* into mice intraperitoneally but found no reaction. Eiken (12) did

not investigate pathogenicity. Three of Khairat's 19 strains were isolated in pure culture, but from asymptomatic patients (45). Khairat inoculated 30 million viable washed organisms intramuscularly into guinea pigs and intraperitoneally into mice and 70 million intravenously into rabbits and found no effect. Marsden and Hyde (52) began to dispell the idea that *B. corrodens* was relatively harmless when they reported its isolation from six cases of infection in children, including one fatal case of extradural and subdural empyema where "the organism was found in pure culture." All their strains were facultative anaerobes.

The Taxonomic Question

In 1969, Henriksen (30,31), while studying various corroding bacteria isolated from the respiratory tract, noted that a number of *B. corrodens* strains were isolated from aerobic cultures. Previously, all strains of *B. corrodens* had been isolated from anaerobic cultures. Although some of these early strains were subsequently grown aerobically, this was considered an unusual microbiological adaptation. Henriksen obtained six of Eiken's strains and intensively studied them along with fifteen of his own isolates. He found that all strains, except three of Eiken's, grew equally well aerobically and anaerobically. Consequently, he questioned the inclusion of both facultative and strict anaerobic strains in the genus *Bacteroides* which was designated to contain only strict anaerobes. He suggested that the facultatively anaerobic strains be studied further and that they should be reclassified and belong to the family Brucellaceae. In addition, he designated strain 333/54-55 (Eiken) as the type strain for *B. corrodens* even though it was facultative.

One year later, Hill et al. (32), noting Henriksen's study, further described eight clinical isolates of *B. corrodens* and two strains from the National Collection of Type Cultures. They noted that growth was poor in an aerobic environment but better under candle jar and anaerobic conditions. Thus they confirmed the observation that the taxonomic position of *B. corrodens* was uncertain since it included strains that were not strict anaerobes. They also determined the DNA base composition of the facultative *B. corrodens* strains by the melting temperature method and found the range to be 56.2-58.2% guanine plus cytosine (G+C). This was markedly different from the DNA base composition of other *Bacteroides* species.

In 1971, Jackson et al. (36) attempted to assess the taxonomic status of the facultative and strictly anaerobic strains of *B. corrodens*. They found that the facultative strains were oxidase-positive, catalase-negative, indole-negative, nitrate-reducing, lysine decarboxylase-positive, nonfermentative, urease-

negative, gelatinase-negative, casein hydrolysis-negative, nonmotile, and had a G + C content of 57-58%. In contrast, the strictly anaerobic strains were urease-positive, gelatinase-positive, casein hydrolysis-positive, and had a G + C content of 28-29.7%. The anaerobic strains were also nonmotile, had twitching motility, and in electron micrographs had a "convoluted appearance" with polar "processes" (? pili) but no flagellae. Both facultative and strictly anaerobic strains pitted the agar and gave nonpitting variants. Due to the DNA homology studies, they abandoned their tentatively suggested name "*Hemophilus corrodens*" for the facultative strains. Subsequently, Jackson and Goodman (37) proposed "the recognition of a new genus *Eikenella* (M. L. dim. ending-ella; M. L. fem. n. *Eikenella* named for M. Eiken, who first named the type species of this genus)" which was placed in the family Brucellaceae. They designated the transfer of facultative *B. corrodens* strains to *Eikenella corrodens*.

To avoid possible confusion in the literature and to comply with the rules of nomenclature, Jackson and Goodman (38) proposed a new specific name of *Bacteroides ureolyticus* (M. L. noun *urea* urea; Gr. adj. *lyticus* dissolving; M. L. adj. *ureolyticus* urea dissolving) for the strictly anaerobic *B. corrodens*.

HB-1 of King

In 1962, King and Tatum (47) described a "group of small, fastidious, gram-negative rods requiring or preferring carbon dioxide for growth on solid media," which they designated as the HB group. They divided this group into four subgroups, designated as HB-1 through HB-4, and assigned *Hemophilus aphrophilus* to HB-2, and *Actinobacillus actinomycetemcomitans* to HB-3 and HB-4. They referred only passingly to HB-1 as "a group of non-fermenting gram-negative organisms . . . sufficiently different to be considered separately in a future report."

Eleven years later, Bottone et al. (4) reported the isolation of 17 strains of bacillus HB-1 from a variety of human clinical sources. Four of the strains were isolated in pure culture, one from each of the following sources: a blood specimen, a purulent exudate from a frontal sinus, a purulent exudate from a hand wound, and peritoneal fluid. They studied and gave classic descriptions of the morphological, cultural, and biochemical characteristics of this organism. Growth was noted to be enhanced by hemin and CO_2, and pathogenicity could not be demonstrated by mouse inoculation. They agreed with suggestions that HB-1 might be identical to the facultative strains of *B. corrodens*.

In the same year, Kaplan et al. (44) reported the isolation of HB-1 from infections in eight children. These included the isolation of HB-1 in pure culture from a thyroid abscess, pleural fluid, cerebrospinal fluid, and peritoneal fluid. It was isolated in mixed culture from the blood in a septic patient, cases of otogenic brain abscess, osteomyelitis of the mandible, and two skin abscesses. They also acknowledged the similarity of HB-1 to *B. corrodens*.

Earlier that year, Riley and coworkers of the Special Bacteriology Unit at the Center for Disease Control had reported in the *International Journal of Systematic Bacteriology* (57) that "the HB-1 group of organisms and *E. corrodens* are identical." Comparing over 500 strains of HB-1 with the type strain of *E. corrodens*, they reported "no dissimilarity between the organisms was observed either morphologically, biochemically, or serologically."

BACTERIOLOGY

Identification

E. corrodens may be a difficult organism to identify even for the experienced microbiologist. It is a member of the family Brucellaceae, and in laboratory manuals it is usually included as part of the glucose nonfermenting, gram-negative bacteria (61). This latter group is quite heterogenous and includes *Acinetobacter, Alcaligenes, Agrobacterium, Flavobacterium, Kingella, Moraxella*, pseudomonas-like unnamed organisms, as well as *Eikenella*. All these bacteria give no reaction to many of the commonly used biochemical tests. Colonies that "pit" or "corrode" the agar are suggestive of *E. corrodens* (Fig. 1); however, one must remember that only half of *E. corrodens* strains pit the agar and that colonies of other gram-negative rods, including nonfermenters, may also pit the agar (Table 1).

Definitive identification is by a combination of Gram reaction, colonial morphology, growth characteristics, and biochemical reactions. *E. corrodens* has been described as a small (0.2-0.5 μm wide $\times$ 1-4 μm long), gram-negative coccobacillus with regular morphology (Fig. 2). It is nonbranching, noncapsulated, nonsporing, nonflagellated, and occasionally forms filaments; oxidase-positive, catalase-negative, indole-negative, reduces nitrate to nitrite, lysine decarboxylase-positive (±), ornithine decarboxylase-positive (±), nonfermentative, urease-negative, gelatinase-negative, hydrogen sulfide-negative, and esculin-negative. *E. corrodens* may exhibit twitching motility (28).

It requires hemin for growth in an aerobic but not anaerobic environment; growth is enhanced by 5-10% CO_2. Growth is poor to moderate in liquid media. *E. corrodens* grows best on chocolate agar and not at all on

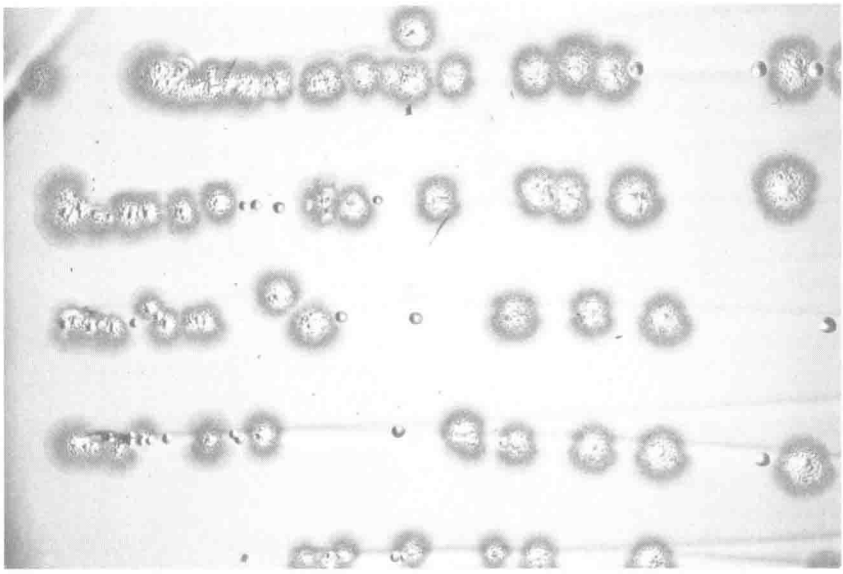

Figure 1a Pitting colonies of *E. corrodens* grown on blood agar after 48 hr of incubation. Some nonpitting variants are also present.

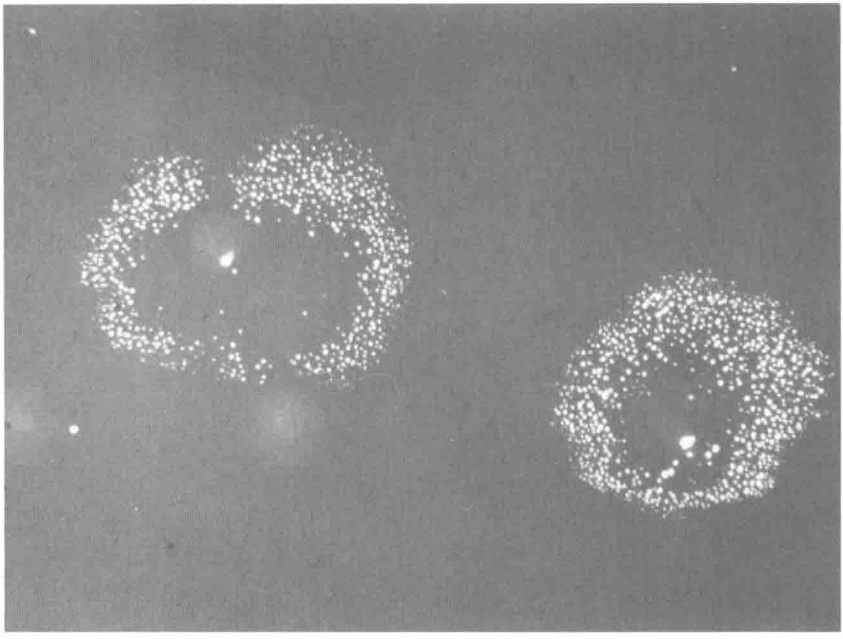

Figure 1b Colonial morphology of pitting strains of *B. corrodens* grown on blood agar after 48 hr of incubation.