The Genus *Campylobacter*: a Decade of Progress

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INTRODUCTION

A decade ago, the revelation that campylobacteria were important human pathogens marked the beginning of an explosive burst of interest which has continued unabated to the present and will doubtless continue into the forseeable future. The organisms of the genus have offered many surprises and challenges as they continue to be reluctant to reveal their secrets even under the scrutiny of today’s microbiologist armed with modern technologies. There is wide diversity in the genus. The species are metabolically and genetically different to the extent that one can question whether one genus is adequate to house all of the species.

The genus is complex, but significant taxonomic advances have been made in isolation procedures, taxonomy, pathogenesis, epidemiology, and molecular biology. Rapid progress may be credited in large measure to the biennial international workshops and the published proceedings (123, 129, 130). The workshops attract bacteriologists from many different backgrounds, as well as epidemiologists, veterinarians, geneticists, and molecular biologists, serve as an effective forum for dissemination of information, and provide the setting for organizing cooperative and collaborative studies among different groups.

Timely publications of manuals and reviews also aided in making the medical and veterinary communities aware of the importance of these bacteria. In 1977, enteritis-causing campylobacteria were not isolated, whereas today, with improved media and isolation procedures, the clinical laboratory isolates thermophilic campylobacteria routinely. Information on new developments and progress has been well articulated through a number of review articles and chapters in texts and manuals. Publications appearing early in the last decade that conveyed the importance of thermophilic bacteria in human disease were contributed by Butzler (21), Butzler and Skirrow (24), Karmali and Fleming (76, 77), and Retig (138). Developments up to 1981 were reviewed by Blaser and Reller (10) and Megraud and Latrille (106, 107). A review by Blaser et al. (11) focused on the epidemiology of *Campylobacter* infections, and in another report Blaser et al. (12) described the experiences of clinical laboratories from a collaborating group of institutions. A recent review by Walker et al. (178) appraised the state of understanding of pathogenic mechanisms, host response, genetics, and molecular biology of campylobacteria. Sections in microbiology manuals by Kaplan (75) and Morris and Patton (113) provided information on new and improved procedures for isolation and identification. More complete treatments of the genus are found in separate chapters authored by Blaser (8) and Smibert (158, 159) and in a text on *Campylobacter* spp. edited by Butzler in 1984 (22).

*Campylobacter pylori*, a new species implicated as an agent associated with human gastroduodenitis, has been described within the last decade. It has been the subject of separate reviews by Marshall (97), Goodwin et al. (52), and
McNulty (103) and leading articles by Goodwin and Armstrong (51) and Rathbone et al. (137).

Veterinary microbiologists have been favored with reviews on diseases produced by campylobacteria in animals. A decade ago, Clark (28) and Hoffer (67) reviewed bovine campylobacteriosis (bovine vibriosis) and the agent, *Campylobacter fetus* subsp. *veneraealis*, that causes this disease. A detailed treatment of avian vibrionic hepatitis has been provided by Peckham (131). Comprehensive treatments of campylobacteria from the veterinary point of view are provided by Cottral (29) and Garcia et al. (44). For an appreciation of the taxonomic and nomenclatural changes that the genus has undergone, the reader is referred to descriptions of the campylobacteria by Smibert (157, 160).

As indicated above, a sizable body of literature has accumulated on the genus *Campylobacter* over the last decade. The objective of this article is to review the recent advances with particular emphasis on the new developments in taxonomy and classification that are particularly relevant to the microbiologist in the clinical laboratory.

**GENERAL CHARACTERISTICS OF CAMPYLOBACTERS**

The genus name *Campylobacter* was derived from the Greek word for curved rod. It was proposed by Sebald and Véron to include microaerophilic bacteria that were different from *Vibrio cholerae* and other vibrios in a number of respects (150). Campylobacteria are gram-negative bacteria 0.5 to 8 μm long and 0.2 to 0.5 μm wide with characteristically curved, spiral, or S-shaped cells (150). They generally have a single polar unsheathed flagellum (monotrichous) or a flagellum at each end (amphitrichous). One species, *C. pylori*, has one to six sheathed flagella located at one end of the cell (54, 71). The motility of the bacterium is characteristically rapid and darting in corkscREW fashion, a feature by which their presence among other bacteria can be detected by phase-contrast microscopy (76). Their guanine-plus-cytosine (G+C) content is low, ranging from 28 to 38 mol% (160, 175).

Biochemical reactions by which *Campylobacter* species may be differentiated are relatively few because of their inability to ferment or oxidize the usual carbohydrate substrates available in the diagnostic laboratory. They have a respiratory type of metabolism and use amino acids and intermediates of the tricarboxylic acid cycle. They are oxidase positive and reduce nitrates. The catalase test is one of the few useful tests for differentiating the species.

The genus is currently in a state of flux. New species are being defined at a rapid pace, while at least one species, *C. pylori*, is being considered for reassignment to another genus. Descriptions of new species have been difficult because the number of discriminatory characteristics still remain few. In a number of instances species differ more in the degree to which a characteristic is demonstrable than in number of different characters. As pointed out by Neill et al. (119), with respect to atmospheric conditions, there exists a spectrum for optimal growth extending from the anaerobic requirements of some species to the natural oxygen tolerance of another, *C. cyaerophila*. Most species are microaerophilic, lying between these two extremes. Similarly, there is a wide range in the temperatures for culturing bacteria, extending from 15°C for *C. cyaerophila* to 41 to 42°C for *C. jejuni*, *C. coli*, and *C. lariid*. Moreover, species and individual strains vary in tolerance of growth temperatures from the temperatures considered optimum. While *C. fetus* subsp. *fetus* grows at 25°C and can be cultured at 37°C, some strains have been isolated at 42°C (161). Inhibitory tests introduced to differentiate species have not been spectacularly successful because of the wide tolerance variability among individual strains of the same species. In some cases, species differentiation has been based on the degree of inhibition by the same compound rather than on that by a number of different compounds. As a result, different concentrations of the inhibitory materials are used for characterization of species, making the construction of a classification scheme for the genus as a whole somewhat cumbersome. To illustrate, the range of concentrations of sodium chloride that have been used to test for growth inhibition includes 1.5 (6, 145), 2.0 (39, 90, 119), 3.0 (46), 3.5 (6, 21, 61, 79, 101, 145), and 4.0% (29). Furthermore, the more reliable genetic tests for differentiation cannot be performed in the clinical laboratory.

*Campylobacter* species vary immensely in their habitats. Some (e.g., *C. pylori*) appear to be obligate parasites restricted narrowly to one organ or site in the body, while others (e.g., *C. jejuni*) are widely distributed in nature, evoking the term "ubiquitous" to describe their habitat. In at least one case (*C. fetus* subsp. *veneraealis*), the source of the isolate is as reliable a taxonomic criterion for the bacterium as are the tests for identification that may be performed subsequently in the laboratory. It has been observed in other cases that, soon after a species was described, isolates were found that would be excluded from the species if the criteria used to define the species were rigorously applied. It is not surprising, therefore, that species identification in the genus *Campylobacter* has drawn attention to the importance of the genetic constitution of the strain as the final criterion in taxonomy. The deoxyribonucleic acid (DNA)-DNA hybridization technique can certainly be credited with rescuing the genus from the state of confusion that could be anticipated if taxonomic decisions were based solely on phenotypic traits.

**SPECIES OF THE GENUS CAMPYLOBACTER**

The genus *Campylobacter* currently includes 14 species (listed in Table 1). Some species are in quotation marks, indicating that they were proposed but have not been validated by publication in the *International Journal of Systemic Bacteriology* or by inclusion in the lists of new names published outside that journal. A convenient practice in the past has been to divide the species into two groups based on catalase production. This originated with veterinarians (29) who found that the test permitted differentiation of *C. fetus*, the bovine pathogen of interest, from commensals now classified as *C. sputorum* biovar bubulus. This grouping continued into the last decade (79) but has become less relevant since the newly described nonpathogenic species *C. cyaerophila* and *C. nitrofigilis* are also catalase positive. *C. jejuni* and *C. coli* have been referred to as the thermophilic group of campylobacteria, but *C. lariid* and "*C. upsaliensis*" are also thermophilic. *C. hyointestinalis* may also be considered thermophilic, but perhaps it would be more appropriate to regard this species as thermotolerant because it grows more abundantly at 37°C than at 42°C (38). In contrast, "*C. cinaedi*" and "*C. fennelliae*" grow at 37°C but not at 25 or 42°C (170). The H₂S production based on the use of triple sugar iron agar (TSI) is presented in Table 1 in preference to other methods because the medium has been used to test strains of all species except *C. nitrofigilis* and also because experience
with Enterobacteriaceae has shown that there are fewer contradictory results with TSI than with tests with lead acetate paper strips (5). Edmonds et al. (33) observed positive H$_2$S tests for six species of Campylobacter with lead acetate strips, but only two species were positive when TSI agar was used. Three groups of investigators (79, 113, 160) are in agreement that H$_2$S is not detected in C. jejuni, C. coli, and C. laridis when the TSI medium is used.

Of the armamentarium of biochemical tests available to the clinical microbiologist, the ones for detecting H$_2$S production are perhaps the most notable for causing confusion. A review of the complexities of the reactions involved in H$_2$S production from chemically undefined media and the contradictory results that may arise have been discussed in an elegant review by Barrett and Clark (5). Two enzymes, cysteine desulphhydrase and thiosulfate reductase, produce H$_2$S from cysteine and thiosulfate, respectively. Problems arise because media in diagnostic tests vary in thiosulfate and peptone content. Variation in cysteine content of the peptones can also be expected. Moreover, lead acetate reacts with volatile thiols as well as with H$_2$S, whereas iron used in TSI apparently reacts only with H$_2$S. However, a medium that gives reproducible results and permits the definition of biotypes has been designed for campylobacteria by Skirrow and Benjamin (156). This medium contains ferrous sulfate, sodium metabisulfite, and sodium pyruvate and has been found useful for biotyping thermophilic campylobacteria in at least three laboratories (79, 91, 156). With this medium, C. jejuni biotype 2 and C. laridis strains are H$_2$S positive, whereas C. jejuni biotype 1 and C. coli strains are H$_2$S negative.

A new test included in Table 1 is the hydrolysis of indoxyl acetate as described by Mills and Gherna (110). Although its applicability in the clinical laboratory has not been confirmed by others and not all species have been tested, it nevertheless offers promise as a useful test for differentiation among the species of the thermophilic group and between "C. cinaedi" and "C. fennelliae.

The classification of Roop et al. (145) has been used for C. sputorum and C. mucosalis. The significant changes introduced by these workers include the recognition of C. sputorum subsp. mucosalis as a species and the separation of C. sputorum into three biovars, one of which is biovar fecalis, formerly considered to be a separate species known as "C. fecalis."

Except for the hiruppurate test, reactions or properties distinct for a particular species have not warranted a separate column in Table 1, but some should be mentioned. Yellow-pigmented colonies are a characteristic feature of C. mucosalis. Urease is produced by C. pylori and C. nitrofígilis. C. nitrofígilis also produces nitrogenase and from tryptophan it produces a brown pigment. C. cryaerophila grows at 37°C, but the optimum growth temperature is 30°C and all strains grow at 15°C. C. cryaerophila after isolation will grow anaerobically, in air, or in air containing 10% CO$_2$.

### C. fetus

Strains of C. fetus are divided into two subspecies, C. fetus subsp. fetus and C. fetus subsp. venerealis. This classification stems from the report of Florent (41), who recognized that two different disease entities could be attributed to two varieties of strains. One variety ("Vibrio fetus" var. intestinalis) originates in the intestine and causes sporadic abortion in fertile herds of cattle; the other ("V. fetus" var. venerealis) originating in the prepuce of the asymptomatic bull, causes infertility and only infrequently causes abortion. Although the two groups of bacteria are distinct in their habitats, their biological properties, and the diseases they produce, Véron and Chatelaine (175) did not consider

### Table 1. Differential reactions and characteristics for species of the genus Campylobacter

<table>
<thead>
<tr>
<th>Species</th>
<th>Catalase</th>
<th>Nitrate</th>
<th>H$_2$S (TSI)</th>
<th>Hippurate</th>
<th>Indoxyl acetate</th>
<th>Growth</th>
<th>Susceptibility</th>
<th>G+C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fetus subsp. fetus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ (+)</td>
<td>25°C</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>C. fetus subsp. venerealis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ + (-)</td>
<td>37°C</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>C. hyointestinalis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>42°C</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>C. coli</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>R R</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>C. laridis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>&quot;C. upsaliensis&quot;</td>
<td>(-)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>&quot;C. cinaedi&quot;</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>&quot;C. fennelliae&quot;</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>C. cryaerophila</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>C. nitrofígilis</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>C. sputorum</td>
<td>Biovar sputorum</td>
<td>-</td>
<td>+ (+)</td>
<td>-</td>
<td>-</td>
<td>25°C</td>
<td>+ +</td>
<td>S</td>
</tr>
<tr>
<td>Biovar bubulus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>37°C</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Biovar fecalis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>42°C</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>C. mucosalis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>1</td>
</tr>
<tr>
<td>C. concisus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
<tr>
<td>C. pylori</td>
<td>+</td>
<td>d</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>S</td>
<td>1</td>
</tr>
</tbody>
</table>

*Data were obtained from references 6, 20, 21, 46, 79, 102, 110, 142, 145, 160, and 167.

+ +, Positive reaction; -, negative reaction; ND, no test results found; (+), most strains positive but a low percentage negative; (-), most strains negative but some positive or weakly positive; d, different reactions; R, resistant; S, susceptible; I, intermediate zones of inhibition.

* Susceptibility to antibiotics was determined with 30-µg disks.
them separate species because they do not form separate groups in DNA hybridization tests, they have a similar G+C content, and strains of both subspecies are agglutinable in an antiserum against the same serotype. Later investigations of the relationship between the two subspecies confirmed their close relatedness in DNA-DNA hybridization tests (61, 142). Indeed, Harvey and Greenwood (61) and Romaniuk et al. (141) suggested that there was no genetic basis for the division of the species into subspecies. Harvey and Greenwood (61) referred to their experiences and to observations of others that tolerance to 1% glycine, the test used to differentiate the two subspecies, was quite unreliable. Tolerance to glycine was found to develop in a stepwise fashion by Chang and Ogg (26), leading them to suggest that the glycine tolerance test may not be reliable as a differentiating characteristic. In maintaining the opposite view, Roop et al. (142) noted that changes in tolerance to glycine would not likely occur readily because of the stepwise change and that their experience indicated the test to be reliable. That the habitats of the two species and the diseases they produce are quite distinct were cited as practical reasons for retaining the two-subspecies concept.

Confusion in nomenclature has arisen because of the earlier assignment of the subspecific epithet "fetus" to both subspecies (Table 2). Véron and Chatelaine (175) and Smibert (157) independently designated a strain, unfortunately from different subspecies, as the type strain for the genus. In part, this was because the strains of Smith and Taylor (162) on which the original description of "V. fetus" was based were no longer available for direct comparison. Moreover, publication of the eighth edition of Bergey's Manual of Determinative Bacteriology in 1974 (157) in which Smibert described his nomenclature was delayed. The proposals of Véron and Chatelaine (175) appeared in 1973 and therefore had priority. The validity of the Véron and Chatelaine scheme was recognized in the recent edition of Bergey's Manual of Systematic Bacteriology (160) and in the Approved Lists of Bacterial Names (153), but the use of both nomenclatures in the intervening years caused some confusion (172).

**C. fetus subsp. fetus.** The major habitat of *C. fetus* subsp. *fetus* is the intestine. Isolations from healthy sheep and cattle are not uncommon (44). The genital tract of sheep and cattle and the contents of placentas and stomachs of aborted sheep and cattle fetuses are also sources of this subspecies (160). Other animals and birds have also been implicated but only as secondary reservoirs (44). The prevailing view is that it is only rarely isolated from human intestinal contents (44; S. Lauwers, M. DeBoeck, and J. P. Butzler, Letter, Lancet i:604–605, 1978).

*C. fetus* subsp. *fetus* causes sporadic abortion in cattle and sheep. The infection is believed to arise from bacteria in the intestine acquired through ingestion of food or water contaminated with bacteria from feces, aborted fetuses, or vaginal discharges of the aborting animal. During the course of a bacteremic phase, the organisms, which have a particularly high affinity for placental tissue, invade the uterus and multiply in the immunologically immature fetus. The infected fetuses are generally aborted. If live births occur, the newborn animals may survive for a few hours but not for longer than 4 or 5 days (29). Laboratory diagnosis depends on the isolation and identification of the organism from the placenta or from organs of the fetus in which they occur in large numbers (44).

Human infections caused by *C. fetus* subsp. *fetus* are rare and generally limited to septicemias in patients with predisposing conditions (58, 138, 173, 181). Meningitis is the next most frequently diagnosed disease, but infections at other sites may lead to pericarditis, peritonitis, salpingitis, septic arthritis, and abscesses (138, 173). Among Rettig's survey of early reports, a number of human cases of infections of the fetus were noted (138). Although the incidence of such infections was low and reports of cases are rare in current literature, the tropism exhibited by this organism for fetal tissue can evidently be manifested in the human, and the possibility that human fetal infections can occur with these bacteria should not be overlooked in the clinical laboratory. In this connection it should be noted that *C. fetus* subsp. *fetus* was implicated as a cause of premature labor and neonatal sepsis (25) and that an atypical isolate was recovered from amniotic fluid. Other systemic infections caused by *C. fetus* subsp. *fetus* under unusual circumstances include those acquired by cancer patients undergoing nutritional therapy requiring consumption of raw beef liver subsequently suspected to have been contaminated with this organism (50).

It is currently believed that the subspecies is rarely found in the human intestine and that it is not a cause of human enteritis (21, 24). As pointed out by Harvey and Greenwood (60), this perception may not be accurate. The subspecies, which is susceptible to cephalothin, would not be isolated on medium used for isolating thermophilic campylobacteria if the medium contains this antibiotic. Moreover, the incubation temperature of 42°C would inhibit many strains of this subspecies. The extent to which these conditions affect the isolation rate cannot be estimated, but fecal strains of *C. fetus* subsp. *fetus* associated with gastroenteritis have been isolated by Harvey and Greenwood (60) and Devlin and McIntyre (30). In addition, one of the two strains from stool specimens investigated by Edmonds et al. (32) was from a case of diarrhea. Interestingly, 10 of the 14 atypical *C. fetus* subsp. *fetus* strains of the latter study and all 3 from the study by Harvey and Greenwood (60) were capable of growth at 42°C. If these findings suffice to encourage laboratories to include additional procedures for isolating this subspecies, its incidence in the intestine as well as the percentages of strains capable of growth at 42°C and susceptible to cephalothin could be more accurately estimated.

**C. fetus subsp. venerealis.** In contrast to *C. fetus* subsp. *fetus* which has a tropism for ovine and bovine placental tissue and is recognized as an infrequent cause of systemic infection in humans with underlying conditions, *C. fetus* subsp. *venerealis* has adapted to the bovine genital tract and has not been associated with human infections (160). *C. fetus*

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**TABLE 2. Nomenclatural changes in subspecies of C. fetus**

<table>
<thead>
<tr>
<th>Current classification</th>
<th>Former classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Véron and Chatelaine (175)</td>
<td>Smibert (160)</td>
</tr>
<tr>
<td>Smibert (157)</td>
<td>Florent (41)</td>
</tr>
<tr>
<td>Smith and Taylor (162)</td>
<td></td>
</tr>
</tbody>
</table>

*C. fetus* subsp. *fetus* | "C. fetus" var. *fetus* | "V. fetus" var. *fetus* | "V. fetus" var. *venerealis* | "V. fetus" |
*C. fetus* subsp. *venerealis* | "C. fetus" subsp. *venerealis* | "C. fetus" subsp. *fetus* | "V. fetus" var. *venerealis* | "V. fetus" |
subsp. venerealis is the major cause of bovine genital campylobacteriosis (formerly known as bovine vibriosis), an infectious disease of major concern to the cattle industry. Research on this infectious disease had progressed substantially before the importance of C. jejuni as an infectious agent became generally realized. Investigators had established that the principal habitat of C. fetus subsp. venerealis is the prepuce of the asymptomatic bull and that venereally transmitted bacteria caused a chronic inflammation of the female genital tract and led to infertility (147). Procedures for organism isolation and identification were constantly improved. The use of antibiotic-containing media and membrane filters (Millipore Corp., Bedford, Mass.) for isolation was shown to be practical in 1963 (151). Through animal experimentation, humoral and cell-mediated responses were investigated and developments of vaccines are under way (149). The subject of bovine genital campylobacteriosis has been treated in detail (29) and extensively reviewed (28, 44, 67).

C. hyointestinalis

In 1983, strains isolated from pigs were found biochemically to belong to the genus Campylobacter (47) and a new species, C. hyointestinalis (hyo, hyos, a hog; intestinalis, pertaining to the intestines) was formally proposed in 1985 by Gebhart et al. (46). The organisms were first suspected to be the etiological agents of proliferative enteritis in pigs and possibly in hamsters (46, 47), but recent isolations from patients with proctitis and diarrhea (33, 38) clearly implicate the species as an infrequent pathogen of humans as well. By DNA hybridization, C. hyointestinalis shows a closer relationship to C. fetus than to any other catalase-positive Campylobacter species (142). Like C. fetus, it is resistant to nalidixic acid and susceptible to cephalothin. Of the two subspecies, however, it is closer to C. fetus subsp. fetus in that it tolerates 1% glycine and some strains can grow at both 25 and 42°C. It is differentiated from C. fetus by its production of detectable H₂S in TSI and by its ability to grow anaerobically in 0.1% trimethyamine N-oxide hydrochloride (TMAO).

The procedures in place for isolating fecal campylobacteria in most clinical laboratories are less than ideal for isolating C. hyointestinalis. Fennell et al. (38) found that, although their patient isolate tolerated 42°C, its growth was more abundant at 35°C. Also, the presence of cephalothin in the isolation medium was inhibitory. In addition to the case report of Fennell et al. (38), there have been reports of four other cases (33). In three of these there was strong evidence that the organism caused diarrhea, and the authors concluded that C. hyointestinalis should be regarded as a potential cause of human gastrointestinal disease. It is therefore important that conditions more favorable for the isolation of this species be designed and that prospective studies be undertaken to determine the frequency of occurrence of this organism in patients with enteritis. With such information, a determination of its importance could be made and the need to implement measures in the clinical laboratory for its isolation and identification could be rationally assessed.

C. jejuni and C. coli

Major advances in the identification of new species of Campylobacter have been made in the last decade. In the group known as the thermophilic (thermotolerant) campylobacteria that prefer to grow at 42 to 43°C, two species, C. jejuni and C. coli, were recognized first. Later, a third species, C. laridis, was found to be thermophilic and closely related to C. jejuni and C. coli (see following section). The more important species, C. jejuni, now appreciated as a major infectious agent of humans, has been known as "V. fetus," "C. fetus subsp. jejuni," and as a member of the "related vibrios" (79). Following the discovery of Butzler et al. in 1973 (23) that C. jejuni could be isolated from children with enteritis and its confirmation through a systematic study of 803 patients by Skirrow (154) in 1977, an intense interest in the genus Campylobacter developed. It was soon established that the organisms were indeed a very frequent, if not the most frequent, cause of human diarrhea, particularly among pediatric patients who are more likely to be presented for medical attention (76). In 1977, Skirrow was faced with two classification schemes: one proposed by Véron and Chatelaine (175) and the other proposed by Smibert (157). Both systems recognized the thermophilic nature of the bacteria as a characteristic for differentiating the group in the genus Campylobacter, but both systems lacked clear definitions of the species because other reliable differentiating characteristics were not known. Skirrow referred to the bacteria as the "C. jejuni-C. coli" group, a practice followed by some authors. Others accepted Smibert's classification and used the name C. fetus subsp. jejuni and disregarded the two-species concept. This confusion continued until it was discovered by Harvey (59) that one of the species, C. jejuni, was capable of hydrolyzing hippurate and the other, C. coli, was not.

Development of isolation media. The selective isolation medium developed by Skirrow contained vancomycin, polymyxin B, and trimethoprim in a blood agar base with lyed horse blood (154). The medium is still widely used, but efforts have continued to produce media with greater selectivity and greater ability to suppress other bacterial species. Butzler used thiglycolate agar with 15% sheep blood containing bacitracin, novobiocin, cycloheximide, colistin, and cefazolin (24). Blaser et al. (9) developed a medium referred to as Campy-BAP that consisted of Brucella agar base with sheep erythrocytes and vancomycin, trimethoprim, polymyxin B, amphotericin, and cephalexin. Moskowitz and Chester (114) showed that Pseudomonas and Acidomonas species were most likely to grow as contaminants in the antibiotic media. To increase suppression of Pseudomonas spp., Goossens et al. (55) used a combination of rifampin, colistin, amphotericin B, and cefoperazone, a combination to which Pseudomonas spp. are more susceptible. A study by Gilchrist et al. (49) of the activity of the antibiotics in media revealed that colistin exerted a temperature-dependent inhibiting effect on Campylobacter spp., and in a later modification of the Butzler medium, colistin was omitted (56). With the wider objective of increasing isolation efficiency, not only from human specimens but also from nonhuman sources, Bolton and Robertson (19) designed a medium (Preston medium) that contained polymyxin, rifampin, trimethoprim, and cycloheximide.

The requirement for blood in each of the above media is costly, and furthermore, sterile blood is not readily available in some developing countries. This has stimulated a continuing interest in developing blood-free isolation media; some significant advances have been reported (14). Taking advantage of findings of George et al. (48) and Hoffman et al. (68) that a supplement of sodium metabisulfite, sodium metabisulfite, and sodium pyruvate enhanced growth and aerotolerance of Campylobacter species, Bolton and colleagues attempted to replace blood with this supplement (15, 16, 18). As a result of
their systematic approach, a medium (CCD agar) was developed that incorporated only one antibiotic (cefaclor) and sodium deoxycholate as selective agents in blood-free agar containing charcoal, casein hydrolysates, ferrous sulfate, and sodium pyruvate. Although the CCD medium was found to be less selective than Preston agar, it performed equally well as an isolation medium (18). The incorporation of charcoal and supplements in media as a suitable replacement for blood was confirmed by Karmali et al. (78). Vancomycin, cefoperazone, and cycloheximide were incorporated into this blood-free CSM medium to produce selectivity, and charcoal, hematin, and pyruvate were included as replacements for blood. They found CSM to be more selective than Skirrow’s medium (154) and less inhibiting to antibiotic-susceptible strains of _C. coli_. In a recent comparative study of antibiotic-containing media, one of which was blood-free, Goossens et al. (56) confirmed the findings of Bolton et al. (18) and Karmali et al. (78) that the blood-free medium with charcoal was as effective as blood-containing media for culturing thermophilic campylobacteria but that it tended to suppress contaminating flora less well. Although Karmali et al. (78) and Goossens et al. (56) differed from Bolton et al. (18) in their approach to creating selectivity, blood-free media constitute an acceptable alternative to presently used isolation media that contain blood or blood products. The comparative study of Merino et al. (109) is particularly encouraging as it showed a high isolation efficiency for the Preston blood-free medium which was modified only by the inclusion of cefoperazone.

The use of enrichment media is currently controversial. It is not recommended for routine isolations of thermophilic campylobacteria (113), but the recently developed blood-free enrichment medium is reported to increase the rates of recovery significantly (F. T. H. Chan, A. M. R. MacKenzie, and L. A. Fuite, Abstr. IVth International Workshop on Campylobacter Infections, abstr. no. 34, 1987) and may be more widely used in the future.

Although the use of selective antibiotic media and incubation temperatures of 41 to 43°C has been immensely successful for isolation of _C. jejuni_ and _C. coli_, a total commitment to this one isolation procedure in the clinical laboratory presents some disadvantages. Recently, interest has arisen in the campylobacteria for which these isolation conditions are unfavorable and which are thus not cultured. Presently such “nonculturables” isolates are believed to constitute only a small percentage of fecally derived campylobacteria. However, there is now an increasing awareness that it is important to isolate them to acquire more information on the nature of the organisms themselves, the infections they cause, and their epidemiology. Ng et al. (124) pointed out that some thermophilic campylobacteria are inhibited by most of the antibiotic combinations used in isolation media, and thus isolations of some infectious agents could be missed in the clinical laboratory. Steele and McDermott (164) found that _C. jejuni_ that failed to grow on antibiotic-containing media could be isolated by a filtration technique. By dark-field microscopy, Paisley et al. (126) demonstrated the presence of spiral-shaped bacteria in stools that were culture negative. Tee et al. (169) found that culture incubation at 37°C yielded nine atypical campylobacteria strains, three of which were _C. jejuni_ that grew poorly or not at all at 43°C. By omitting cephalothin from the isolation medium and incubating at 37°C for 2 to 7 days, Fennell et al. (39) isolated campylobacteria with characteristics unlike those of the recognized species. These campylobacterium-like organisms (CLOs) were subsequently shown to represent new species (170) (see following sections). Consideration should be given to adopting additional measures for isolating CLOs and atypical _C. jejuni_. Tee et al. (169) adopted the practice of inoculating two plates for isolation, one for incubation at 43°C and the other for incubation at 37°C. The ingenuity of the clinical microbiologist, well, no doubt, lead to other procedures to increase isolation efficiency once the importance of doing so becomes more fully appreciated.

**Species differentiation and biotyping.** The discovery by Harvey (59) that _C. jejuni_ and _C. coli_ were differentiable by the simple test of Hwang and Ederer (69) for hippuricase hydrolysis was a milestone. To date, no other single biochemical test has had as much impact on clarifying the taxonomy of the thermophilic species (156; G. A. Hébert, D. G. Hollis, and R. E. Weaver, Letter, J. Clin. Microbiol. 22:326, 1985). The hippuricase enzyme hydrolyzes hippurate to produce benzoic acid and glycine, and glycine is detected with a solution of ninhydrin in the tube test used routinely in the clinical laboratory (59, 69). The other end product, benzoic acid, may also be detected by using more complex procedures (4, 81). In the early stages, the test was used to confirm that fecal isolates from patients with diarrhea were thermophilic campylobacteria and not _C. fetus_ subspp. _fetus_ which is negative in this test (59, 95). This differentiation aided in establishing the point that _C. fetus_ subspp. _fetus_ is neither a common human pathogen nor a normal resident of the human intestine. More important, however, was the realization that the test differentiated _C. jejuni_ (hippurate positive) from _C. coli_ (hippurate negative), permitting Skirrow to adopt the test in constructing a simple scheme for identification of intestinal isolates of thermophilic campylobacteria (156). _C. coli_, but not _C. jejuni_, grows on minimum medium developed by Smibert originally for _C. fetus_ (142, 143). Results of differentiating thermophilic campylobacteria with this medium agree with results obtained by DNA hybridization tests, but unfortunately the use of the medium has not been generally adopted by clinical laboratories.

Although the hippurate test is important, its use could lead to misleading results if hippurate-negative _C. jejuni_ and hippurate-positive _C. coli_ are encountered. The extent to which such isolates occur, however, can be reliably determined only by DNA relatedness tests because tests for differentiating the two species are few and can give equivocal results. In most cases, the hippurate-negative isolates described by Luechtefeld and Wang (95) reacted in antisera against serotype reference strains (serostrains) that were also hippurate negative. Harvey and Greenwood (61), in their examination of a few of these isolates, found that four of five hippurate-negative isolates serotyped by antisera against hippurate-negative serostrains were closely related in DNA hybridization tests to _C. coli_ but one isolate (C-92) was clearly related to _C. jejuni_. Leapar and Owen (89) found by DNA hybridization that each of nine hippurate-negative serostrains were related to _C. coli_ and each of eight hippurate-positive serostrains were related to _C. jejuni_. These findings, in addition to the observations that the overwhelming majority of hippurate-positive isolates were serotypable with antisera prepared against hippurate-positive serostrains and that hippurate-negative isolates reacted in antisera against hippurate-negative serostrains, prompted Penner et al. (133) to divide the serotyping scheme into two separate systems for _C. jejuni_ and _C. coli_. It should be noted, however, that a minority of isolates (0.2% hippurate-negative and 4.3% hippurate-negative isolates) did not conform to the pattern exhibited by the majority. A suggestion emerging from these results was the possibility that some _C. jejuni_
strains were hippurate negative and that the hippurate test, while very useful, was not infallible in identifying C. jejuni. Using DNA hybridization tests as the final criterion for identification, Hibbert et al. (62) confirmed that a few strains of C. jejuni were indeed hippurate negative.

The hippurate reactions in all of the above investigations were determined by the method of Harvey (59), and the possibility that the test was insufficiently sensitive for detecting low-level producers of hippuricase was addressed by Morris et al. (112). Using the method of Kodaka et al. (81) to detect benzoic acid by gas-liquid chromatography, they showed that all strains confirmed by DNA hybridization to be C. jejuni were hippurate positive by the gas-liquid chromatography method but three strains gave either negative or variable results in tube tests. Similarly, five isolates negative or untypable by the tube test were all found to be positive when tested for benzoic acid by a modified gas-liquid chromatography method developed by Bär and Frické (4). In another approach, Lin et al. (90) used two-dimensional thin-layer chromatography to detect the glycine end product of hippurate hydrolysis by 21 C. jejuni and 8 C. coli serostrains of the Penner et al. (133) serotyping systems. They observed positive reactions for all C. jejuni strains and negative reactions for C. coli, but some weak reactions in both groups, when the conventional tube test was performed. Although these series of investigations established that the tube test of Harvey (59) was insensitive in some cases, they did not prove or disprove the existence of hippurate-negative C. jejuni or hippurate-positive C. coli. Direct answers to this question have only recently been forthcoming from Totten et al. (171). Taking advantage of genetic classification, hippurate hydrolysis tests by thin-layer chromatography, gas-liquid chromatography, and serotyping, they estimated that 1.6% of their C. jejuni strains were hippurate negative. Remaining to be confirmed in similar fashion is the existence of hippurate-positive C. coli as described by Roop et al. (143) in their biotyping scheme for C. jejuni and C. coli. A negative finding would confirm that hippurate hydrolysis is, in fact, a characteristic exclusive to C. jejuni.

On the basis of a test for H2S production, Skirrow and Benjamin (156) were able to show the occurrence of two biotypes in C. jejuni. With a sensitive test for DNA hydrolysis (92) and a rapid test for H2S, Lior (91) extended the biotyping schemes of C. jejuni and C. coli to include C. laridis. Another approach to defining biotypes within C. jejuni has been reported by Elharrief and Mégraud (35) and McNulty and Dent (104), who noted that C. jejuni biotypes I and II of Skirrow and Benjamin (156) could be separated on the basis of the presence of gamma-glutamyl aminopeptidase activity in strains of biotype II and its absence in strains of biotype I.

Problems related to species identification and biotyping continue to stimulate research to discover additional or alternative tests that may be applicable to routine identification for epidemiology. Bolton et al. (17) described a scheme based on the resistance of strains to 12 reagents. This "resistotyping" scheme enabled them to separate isolates of the same O serotype according to different sources of isolation, but isolates of the same serotype that were from humans and cattle associated with an outbreak were the same resistotype. The scheme was advocated as an alternative method to serotyping for epidemiological studies. Elharrief and Mégraud (34) used carbon utilization tests to characterize C. jejuni, C. coli, and other Campylobacter species and observed that a number of strains in these species could utilize citrate as the sole source of carbon, permitting biotypes to be defined. The utilization of d-malate was associated with positive reactions for hippurate hydrolysis and could be taken as an alternative test for defining C. jejuni in those cases in which strains were hippurate negative or only weakly positive. Karmali et al. (80) showed that testing C. jejuni and C. coli for the presence of d-asparaginase may also be useful in defining biotypes. Using five commercially available lectins, Wong et al. (179) found that they could separate isolates of both species into different types. They observed that the specificities of the reactions were independent of lipopolysaccharide (LPS) antigens and typing with the lectins permitted differentiation of strains belonging to the same O serotype.

**Bacteriophage typing.** A bacteriophage typing system has been assembled by Grajewski et al. (57) and demonstrated to discriminate among isolates of C. jejuni and C. coli. The system has potential use in epidemiological studies but has not yet been adopted by a reference laboratory.

**Serotyping.** Interest in developing serotyping schemes emerged soon after the discovery of the medical importance of C. jejuni. Little information was available on the antigenic structure of the organisms apart from the report of Berg et al. (7) that some thermophilic strains possessed thermostable antigens and thermolabile antigens. In 1980, Abbott et al. (1) demonstrated agglutinating antibodies to heat-labile and heat-stable antigens in rabbit antisera prepared against six strains isolated from patients. Penner and Hennessy (132), however, found that heated cell suspensions gave nonspecific reactions and therefore undertook to use the passive (indirect) hemagglutination technique to detect antigenic specificities because of the reputed superiority of passive hemagglutination over agglutination (37). In 1980 they proposed a scheme based on extracted thermostable antigens that were later proven to be the (LPS) somatic O antigens (72, 94, 111, 134, 136) located in the outer membrane. The O specificities of the isolates are determined by titrating each of the serotyping antisera against antigenic extracts from the isolates by the passive hemagglutination technique. A similar approach for serotyping C. jejuni and C. coli was adopted by Lauerwski et al. (180) and Lauerwski, L. Vlaes, and J. P. Butzler, Letter, Lancet i:158–159, 1981). Some cross-reactions occur between C. jejuni and C. coli O serotypes but, apart from these, each species possesses its own array of O antigens. This, along with other findings (see above), prompted Penner et al. (133) to separate the serotyping scheme originally developed against strains belonging to both C. jejuni and C. coli into separate schemes for each species. Serotyping of C. jejuni is based on 42 antisera and serotyping of C. coli is based on 18 antisera. These antisera serotype the majority of isolates from North America, but greater percentages of isolates from other countries are untypable (74, 84, 108). New serotypes identified among these untypable isolates are being included in extensions of the present schemes (unpublished data). Moreover, the serotyping scheme of Lauerwski et al. (Letter, Lancet i:158–159, 1981) is in the process of being integrated with the systems of Penner et al. (133) to produce single unified systems for serotyping C. jejuni and C. coli on the basis of LPS antigens.

Because antigens must be extracted and passive hemagglutination titrations are necessary, the system of typing has been regarded as labor intensive. Investigations have been undertaken by Wong et al. (180) and Fricker et al. (43) to simplify the procedure by incorporating a slide coagglutination technique, using protein A-bearing staphylococci coated
with antibody from the serotyping antisera and using heated cell suspensions as the antigenic material. Wong et al. (180) found their results to be consistent with those obtained by passive hemagglutination titration when they examined the defined serotype reference strains. However, Fricker et al. (43) typed 74 isolates and found that 5 were untypable and 2 gave results different from those obtained from passive hemagglutination titrations. In a later modification, Illingworth and Fricker (69a) designed a slide coagglutination test in which erythrocytes sensitized with antigenic extracts from the isolates were mixed with antibody-coated Staphylococcus aureus. Increased specificity was noted and not unexpected since the use of the erythrocytes takes advantage of the LPS receptor (163) on the erythrocyte membrane to bind LPS molecules from the extract. In addition, a system of pooled reagents was devised to prescreen isolates. Each “pool” consisted of staphylococci coated with four antisera against four Campylobacter O groups. As this improvement offers both specificity and simplicity, it holds promise as a method that should encourage a wider application of serotyping, particularly in the laboratories in which economical factors are an important consideration. Strains sent for serotyping often lose viability during shipment; to overcome this difficulty, the practice of sending the antigenic extracts for serotyping instead of live cells has been adopted by some laboratories (C. R. Fricker, Letter, Lancet 1:554, 1986; A. J. Lastovic, R. B. Marshall, and J. L. Penner, Letter, Lancet 1:320, 1986). Advantage of this feature may be taken only by laboratories serotyping on the basis of LPS antigens.

Lior et al. (93) and Rogol et al. (140) have described systems for serotyping C. jejuni and C. coli on the basis of thermolabile antigens. Typing antisera are absorbed with heated cell suspensions of the homologous serostain to remove antibodies against the thermostable antigens, leaving antibodies directed against determinants of thermolabile antigens. Further absorptions with cross-reacting strains produce antisera that contain antibodies directed only against thermolabile antigens of the homologous serostain. Serotyping with the absorbed antisera is performed by a slide agglutination method, using live cells as the antigenic material. Cell suspensions that autoagglutinate are treated with deoxyribonuclease to remove extruded DNA, which, according to Ritchie et al. (139), causes cells to adhere to each other. Because of the simplicity of the typing procedure, thermolabile antigen typing has been advocated for use in clinical laboratories, particularly those in underdeveloped countries (74; H. Lior and J. P. Butzler, Letter, Lancet 1:1381-1382, 1986). Serotyping on the basis of thermolabile antigens can be performed by the coagglutination technique instead of slide agglutination and has the advantage of requiring considerably less of the absorbed typing antisera (180). The advantages and disadvantages of serotyping by slide agglutination and passive hemagglutination are described in detail by Patton et al. (128).

Serotyping may also be carried out without the use of absorbed antisera or extracted antigens. Using the slide agglutination technique and suspensions of live cells as the test antigen, Kahlich et al. (73) serotyped isolates on the basis of surface antigens with 13 antisera produced against live cultures. They reported no cross-reactions and a total absence of autoagglutination. The feasibility of serogrouping with fluorescent antibody was demonstrated by Hebert et al. (63). They successfully serogrouped isolates of C. jejuni and C. coli, and their results correlated well with those obtained by serotyping on the basis of thermostable antigens (64). The nature of the antigens identified by the fluorescent-antibody method is presently not known, but LPS as well as proteins are likely to be involved. The involvement of both proteins and LPS was suggested by Hodge et al. (66), who also developed a fluorescent-antibody serotyping scheme, but with antisera prepared according to the same procedures as those for detecting thermolabile antigens by slide agglutination. Their fluorescent-antibody serotyping results were different from the results obtained by slide agglutination. Evidently the antigens involved in the thermolabile antigen typing systems and the surface antigens detectable by fluorescent antibody are not identical. Further information should be forthcoming once methods for the isolation and purification of these antigens are developed and monoclonal or polyclonal antibodies against them can be used in their characterization.

Serotyping of thermophilic campylobacteria has been shown to be of value in numerous epidemiological investigations. The large numbers of thermostable and thermolabile antigens now known to occur in C. jejuni and C. coli preclude serotyping as a service that can be offered by most clinical laboratories and remains primarily a function of reference laboratories. Antisera against the most frequently occurring serotypes of the Lior scheme are commercially available (Sopar-Biochem, Brussels, Belgium).

C. laridis

In 1980, a group of strains isolated predominately from sea gulls was found to be thermophilic, but different from C. jejuni and C. coli by its resistance to nalidixic acid (156). These strains were designated nalidixic acid-resistant thermophilic Campylobacter until 1983, when the species name, C. laridis (laridis, of a sea bird), was proposed (6). Resistance to nalidixic acid may also be encountered in strains of C. jejuni (174, 177) and C. coli (91), creating a potential problem in identification. The resistance may be acquired by previous exposure of patients to norfloxacin (2). Anaerobic growth in the presence of 0.1% TMAO was suggested as a possible feature unique to C. laridis (6), but as indicated in Table 1, it has subsequently been shown to occur in four other species (142). However, all strains shown by DNA hybridization to be C. laridis did grow in TMAO, thereby lending confidence to the test as a definitive characteristic of the species. Roop et al. (142) showed that strains of C. laridis did not grow on minimal medium developed in their laboratory whereas strains of C. coli did. More strains need to be examined to determine whether growth on this medium offers a simple means of reliably differentiating C. coli and C. laridis.

A consistent feature of C. laridis appears to be the production of H2S, but sensitive methods are required for its detection. Lead acetate paper may be used as the indicator (6, 142), but according to Lior (91) H2S is consistently and reproducibly detectable when the ferrous sulfate, sodium metabisulfite, and sodium pyruvate medium is used.

Tauxe et al. (168) reported on six human infections with C. laridis, one of which was a bacteremia in an elderly man with multiple myeloma previously described in detail by Nachamkin et al. (115). In the five other cases, symptoms mimicked those generally described for patients with C. jejuni enteritis. In this study all isolates were confirmed as C. laridis through DNA relatedness tests. In another study an isolate of C. laridis was implicated as the cause of self-limiting diarrhea in a 32-year-old male (152). Only when more laboratories undertake the complete species identification of thermophilic campylobacteria will greater insight be gained on the significance of C. laridis in human infections.
"C. upsaliensis"

Concern about the occurrence of pathogenic campylobacteria in diarrhoeic dogs and their transmission to humans prompted Sandstedt et al. (148) to undertake a study of Campylobacter spp. in dogs. As expected, C. jejuni and C. coli were isolated, but an unusual group of hipurate-negative strains was isolated that grew at 42°C and gave either negative or weak catalase reactions. These catalase-negative or weak strains possessed phenotypic characteristics which excluded them from C. spatorium, C. mucosalis, and C. concisus, the catalase-positive species. DNA hybridization tests have shown that they represent a new species (145, 148). Since then, catalase-negative or weak strains have been recovered from both healthy and diarrhoeic dogs, from cats, and from feaces of children (105, 165), but an etiological role in infectious diarrhea remains to be established (174). In this connection, it is interesting to note that catalase-negative or weak strains adhered to cells in an endothelial cell monolayer in the same manner as strains of other Campylobacter species, suggesting a mechanism of pathogenesis (F. Megraud, F. Camborde, F. Bonnet, and A. M. Gavinet, Abstr. XIV Int. Cong. Microbiol., abstr. no. P. B8–19, 1986). The name "C. upsaliensis" has been proposed for the catalase-negative or weak group of campylobacteria (K. Sandstedt and J. Ursing, Abstr. XIV Int. Cong. Microbiol., abstr. no. P. B8–17, 1986), but at the time of writing it has not been formally published or validated.

"C. cinaedi" and "C. fennelliae"

The inability of the selective isolation media to recover all campylobacteria capable of causing enteritis has been suspected for some time (105, 125, 126, 165, 169). The importance of this premise was brought to light in a recent study by Fennell et al. (38), who were examining the causes of enteritis in homosexual males in Seattle, Wash. In addition to isolations of C. jejuni and C. fetus subsp. fetus, they recovered strains with patterns of behavior quite distinct from described Campylobacter species. These were referred to CLOs and divided into three groups (CLO-1, CLO-2, and CLO-3). The CLO-1 and CLO-2 strains were recognized as two new species and have been described, the former as "C. cinaedi" (cinaedi, of a homosexual) and the latter as "C. fennelliae" (fennelliae, feminine form of the name of the technologist, C. L. Fennell, who first isolated the organisms) (170). The taxonomic status of the CLO-3 strain has not been resolved. Although it is morphologically and biochemically similar to other Campylobacter species, its G+C content of 45 mol% is considerably greater than that of other members of the genus (170). "C. cinaedi" and "C. fennelliae" grow microaerobically at 37°C, but not at 25 or 42°C, and they do not grow anaerobically or aerobically. The CLO-3 strain grows microaerobically at both 37 and 42°C. Conditions for isolation of thermophilic bacteria are not optimal for isolating these species. A modified medium consisting of Brucella agar with 10% sheep blood and containing vancomycin, polymyxin, trimethoprim, and amphotericin is recommended (170). Incubation at 37°C in a reduced oxygen atmosphere for 7 days for isolation and 3 days for subculture is required. Phenotypic characteristics by which the two species may be identified and differentiated are few in number. "C. cinaedi" reduces nitrate and shows intermediate zones of inhibition around a 30-μg cephalothin disk, whereas "C. fennelliae" does not reduce nitrate and is susceptible to cephalothin. A distinguishing feature of "C. fennelliae" is the odor of hypochlorite produced by the cultures. The indoxyl acetate test listed in Table 1 shows promise as a means of separating the two species, but only seven strains were examined (110). More strains should be tested before it is accepted as a definitive characteristic for the species. In the investigations of these two species and other CLOs, a qualitative genotypic test was developed in which bacterial inocula on nitrocellulose were probed with 32P-labeled DNA from strains of each of the CLO groups (39, 170). With this test the CLO-1 strains could be separated into two groups (CLO-1A and CLO-1B) that could not be differentiated by biochemical tests. This "taxonomic spot blot test" permitted rapid screening and classification and represents an innovation to circumvent problems in identifying and classifying bacteria which possess only a small number of phenotypic characteristics useful in differentiation.

The susceptibility of "C. cinaedi" and "C. fennelliae" to antimicrobial agents is somewhat different from C. jejuni in that a higher percentage are resistant to erythromycin, but all are susceptible to tetracycline and doxycycline, indicating a different choice of drug in the treatment of infections caused by these species (42). "C. cinaedi" of the group designated CLO-1A occur in asymptomatic homosexuals but significantly less frequently than in symptomatic homosexual patients (170). Bacteremias caused by both species have been reported (27, 125). At this point, isolates have not been obtained from heterosexual males or females, and epidemiological studies to determine the sources and routes of transmission of these agents have not been undertaken. Such information is necessary before it can be recommended that clinical laboratories introduce procedures for isolating "C. cinaedi," "C. fennelliae," and CLO-3 strains routinely. However, it would appear prudent to undertake such measures for the special case of the homosexual male patient suffering with enteritis.

C. cryaerophila

The medical importance of thermophilic campylobactera encouraged studies on the taxonomy and nomenclature of other campylobacteria. A curiosity among spiral organisms was a group of strains that were recoverable on semisolid Leptospira isolation media incubated at 30°C under microaerophilic conditions. Subculturing could be carried out in a variety of media and, once established, the bacteria could be cultured on blood agar under aerobic, microaerophilic, or anaerobic conditions at 30°C. (36). The organisms were isolated from internal organs of aborted bovine fetuses, from the placenta and amniotic fluid of normal fetuses (36), and from aborted porcine fetuses (65; R. Higgins and R. Degre, Letter, Vet. Rec. 104:559, 1979), but the bacteria were not pathogenic in laboratory animals. In a series of studies by researchers in Belfast, Northern Ireland, an improved two-stage procedure was developed for isolation (122). The bacteria were characterized biochemically and compared with other Campylobacter species (120); evidence was obtained by G+C content determination and electron microscopy to justify their inclusion in the genus Campylobacter (121). The group has been shown to constitute a separate species by DNA relatedness tests (142). In an elegantly designed comprehensive study, Neill et al. (119) provided a thoroughly documented description of a new Campylobacter species, C. cryaerophila (cry, cold; aero, air; philo, fond of) to include these bacteria.

These organisms are associated with bovine and porcine fetal tissue and have been isolated from animal feces and
milk from cows with mastitis, but a role in pathogenesis has not been determined. There is, to my knowledge, no record of an isolate from a human. Moreover, if such strains do occur in humans, they would be missed in culture because the two-stage method of isolation (120) is not used in the clinical laboratory. The presence of campylobacteria in culture-negative human feces has been reported. They are detectable with dark-field microscopy by their darting motility. Could some of these be C. cyaerophilica? Although this question is highly speculative, it should be noted that, although their optimum temperature of growth is 30°C, they do grow at 37°C (although more poorly [36]) and they have also been isolated from animal feces (119).

C. nitrofigilis

*C. nitrofigilis* (*nitrum*, salt; *figo*, to fix; *ilis*, ability) is found associated with roots of plants in salt marshes (101, 102). It is catalase positive and microaerophilic. A major difference from other *Campylobacter* species is in its ability to produce nitorgenase, pigment from tryptophan, and a number of other biochemical traits. It has no known significance with respect to disease production in humans or animals and is unlikely to be isolated in the clinical laboratory in which isolation medium is incubated at 41 to 43°C.

C. sputorum

Bacteria now classified as *C. sputorum* have not been the subject of the extensive research that has been directed at other species mostly because they have not been found pathogenic for humans and animals. A significant recent development, however, is the clarification of their taxonomy and nomenclature (146). In a comprehensive DNA relatedness study, Roop et al. (145) found that strains previously known as *C. sputorum* subsp. *mucosalis* did not belong to *C. sputorum*, but the poorly defined catalase-positive species "*C. fecalis*" did belong. They elevated the former to the rank of species as *C. mucosalis*. "*C. fecalis*" was reassigned to *C. sputorum*. Their DNA hybridization studies showed that the strains formerly known as *C. sputorum* subsp. *sputorum*, *C. sputorum* subsp. *bubulus*, and "*C. feca lls" were in a single DNA relatedness group. Because of these data and because the organism has no clinical significance, the authors thought there was no justification for maintaining their subspecies status and elected to separate them only at the rank of biovar (biogroup or biotype) under their originally designated epithets: *C. sputorum* biovar *sputorum*, *C. sputorum* biovar *bubulus*, and *C. sputorum* biovar *fecalis*. The three biovars can be differentiated on the basis of their habitats and by simple tests.

Strains of the three biovars are oxidative positive, all grow in 1% glycerine, and all grow at 42°C but not at 25°C. Except for the occasional strain, *C. sputorum* produces H₂S in TSI slants. *C. sputorum* biovar *fecalis* is catalase positive; strains of the other two biovars are catalase negative. Other characteristics by which the biovars may be differentiated include the inability of *C. sputorum* biovar *sputorum* to grow in 3.5% NaCl or in 0.1% TMAO and its ability to grow in 1% bile. *C. sputorum* biovar *bubulus* and *C. sputorum* biovar *fecalis* tolerate 3.5% NaCl and 0.1% TMAO but not 1% bile.

*C. sputorum* biovar *sputorum*. *C. sputorum* biovar *sputorum* is not known to be pathogenic for humans, although Roop et al. (145) reported a human isolate from a leg abscess and another that was from feces of an infant with diarrhea. The significance of human isolates is not known. The biovar is thought to be a commensal of the human oral cavity, but unpublished data of Skirrow and Benjamin (reported by Karmali and Skirrow [79]) acknowledged that this organism was isolated from 2% of fecal samples of healthy people through the use of a filtration method of isolation.

*C. sputorum* biovar *bubulus*. Organisms of *C. sputorum* biovar *bubulus* are commensals found in healthy cattle and have never been implicated as agents of disease in cattle or in humans. They are normally found in the preputial cavity of the male and in the genital tract of the female.

*C. sputorum* biovar *fecalis*. Bacteria belonging to *C. sputorum* biovar *fecalis* were isolated by Firehammer in 1965 (40) from sheep feces. Prior to their transfer by Roop et al. (145) to *C. sputorum*, they were known as "*V. feca lls*" and "*C. feca lls*". Although the organisms are catalase positive, it was suspected by Harvey and Greenwood (61) that they were more closely related to *C. sputorum* than to other catalase-positive species. Strains of the biovar are prolific producers of H₂S, and in this respect they resemble more the catalase-negative than the catalase-positive species. The organism has been isolated from bovine semen and from the vagina, but no evidence has been forthcoming to suggest a pathogenic role for this species in humans or animals.

C. mucosalis

One of the more significant developments within the last few years has been the clarification of the taxonomy of *C. mucosalis*. These oxidase-positive, catalase-negative bacteria were found to constitute a separate DNA relatedness group and were therefore removed as a subspecies of *C. sputorum* and given status as a separate species (145). The characteristics of *C. mucosalis* and its close relationship to *C. concisus* have been investigated in detail by Roop et al. (144, 145) and Tanner et al. (166, 167). *C. mucosalis* requires hydrogen or formate (from which it produces hydrogen) as an electron donor for growth. Under microaerophilic conditions the organism uses oxygen as the electron acceptor, but fumarate is required to serve as the terminal electron acceptor for anaerobic growth. In this requirement for hydrogen (or formate) the organism is unique among the present list of *Campylobacter* species, except *C. concisus*. *C. mucosalis* strains can be differentiated from *C. concisus* in their susceptibility to cephaloridine grown at 25°C, and production of "dirty yellow" colonies (85, 145).

*C. mucosalis* has been of particular interest to veterinarians since it can be isolated in large numbers from the mucosa of pigs with porcine intestinal adenomatosis (PIA), but not from healthy mucosa of pigs without that condition (86). Other conditions of the porcine alimentary tract from which the organisms may be isolated include necrotic enteritis, regional ileitis, and proliferative hemorrhagic enteropathy. *C. mucosalis* can also be isolated from the oral cavity of the pig. Experimental infections, however, rarely produce PIA and direct evidence to implicate *C. mucosalis* as the sole etiological agent of PIA has not been obtained (87). Indirect evidence is being sought through the use of cell cultures to determine invasiveness and intracellular multiplication of *C. mucosalis* isolates and by examining lesions for the presence of specific *C. mucosalis* antigen (70). A serotyping scheme with multiply absorbed antisera to produce single-factor reagents against surface antigens has been developed by Lawson et al. (88). In the application of this scheme to strains isolated from various disease conditions, and to strains from different geographical areas, the investigators observed no distinctive antigenic patterns that could be
associated with particular disease conditions or sources. However, the production of the disease may be much more complex than was originally envisioned. Two or more species may be involved. Gebhart et al. (46, 47) have recently isolated an organism, C. hyointestinalis, that occurs with higher frequency in the intestines of animals with disease than does C. mucosalis and propose that C. hyointestinalis appears to be involved in the pathogenesis of the disease. This indicates that considerably more work will be required to clarify the individual roles of these two participants.

C. concisus

Interest in understanding the role of anaerobic gram-negative bacteria in periodontal disease has stimulated research to characterize this group of bacteria more completely. An outcome has been the recognition of C. concisus by Tanner et al. (167). This organism is catalase negative and requires hydrogen or formate as a source of energy. C. concisus organisms, but neither C. fetus subsp. fetus nor C. jejuni, are attracted to formate. This chemotactic response is believed to be a factor in facilitating colonization and attachment to oral surfaces (127). In DNA hybridization tests, strains of C. concisus form a separate relatedness group (145). In its requirement for hydrogen or formate the species resembles C. mucosalis but can be differentiated from it by the absence of pigmented colonies, inability to grow at 25°C, and resistance to cephalothin (145). Isolates of C. concisus have been obtained from cases of human periodontal disease, but there are currently no reports of isolations from other human or animal sources. A proven role in pathogenesis has not been reported.

C. concisus and Wolinella species are present in large numbers in periodontal pockets of patients with periodontal disease. The characterization and differentiation of these species are matters of prime importance if one is to determine their role, if any, in disease. Tanner et al. (167) showed, by DNA hybridization, that C. concisus is in a separate genus from Wolinella and that it could be phenotypically separated from Wolinella on the basis of tests that could be performed in the clinical laboratory. Except for one cross-reaction, antisera against strains of one species did not react with strains of other species and C. concisus and Wolinella recta formed serologically distinct groups (3). C. concisus was also differentiable from W. curva and W. recta by protein profiles obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of sonicated cells (166). However, differentiation of C. concisus from some Wolinella spp. is most readily accomplished by applying the test for oxidase; the former species, like all members of the genus Campylobacter, is positive and W. succinogenes and W. recta are negative. Separation of C. concisus from C. sputorum biovar sputorum, a commensal in the oral cavity, is accomplished readily by the test for susceptibility to cephalothin. Further details on the characteristics of C. concisus are provided in the papers by Roop et al. (145) and Tanner et al. (167).

C. pylori

The discovery or rediscovery of a group of bacteria resembling campylobacteria that are hypothesized to have an important role in the etiology of human gastritis has been one of the most exciting developments in microbiology in the past decade. Interest in these bacteria increased immensely after reports appeared that they could be isolated from the stomachs of patients with gastritis and peptic ulcerations (96, 100; J. R. Warren, Letter, Lancet i:1273–1275, 1983). These findings were soon confirmed by others for adult populations in developed countries (13, 70, 83, 176) and in developing countries (45) and more recently for pediatric patients (31). The name given to the species, C. pyloridis, was later corrected to C. pylori (of the pylorus) in accordance with the International Code of Nomenclature of Bacteria (99). However, unsettling problems regarding its taxonomy and nomenclature remain in light of mounting evidence that the organisms may not belong in the genus. Romaniuk et al. (141) reported that C. pylori is genetically more closely related to W. succinogenes than it is to other species of Campylobacter. C. pylori has unusual fatty acids that are not found in other campylobacteria. The major fatty acids of C. pylori are tetradecanoic acid (C_{14:0}) and cis-9,10-methyleneoctadecanoic acid (C_{19:0}). Only a small amount of hexadecanoic acid (C_{16:0}) is present, whereas in other campylobacteria the major fatty acids are hexadecanoic (C_{16:0}), octadecenoic (C_{18:1}), and hexadecenoic (C_{16:1}) acids (54). Considerable differences in the ultrastructure between C. pylori and other campylobacteria also exist. The cell surface of C. pylori is smooth, whereas it is wrinkled in C. jejuni (54). The ends of the cells are curved in C. pylori and tapered in C. jejuni (71). C. pylori possesses one to six sheathed flagella rather than the single unsheathed flagellum characteristic of other campylobacteria (54, 71). Other structural differences exist as well, but the presence of sheathed flagella, the unusual fatty acids, and its relatedness to W. succinogenes strongly indicate that the species does not belong in the genus Campylobacter.

Unlike other Campylobacter species such as C. fetus and C. jejuni, the role of C. pylori as a human pathogen has not been firmly established, but evidence in support of a pathogenic role in accumulating. An experiment with a human volunteer was successful in showing that consumption of a suspension of C. pylori was followed by dyspepsia and histologically confirmed gastritis (98). Animal models have not been available, but the gnotobiotic piglet is now being evaluated as an experimental model (82). The bulk of the present evidence for a pathogenic role is therefore largely circumstantial. Goodwin et al. (53), using the enzyme-linked immunosorbent assay technique and an acid glycine extract as the test antigen, in preference to sonicated cell suspensions, found high levels of antibody in sera from patients with active chronic gastritis in contrast to low levels in sera from individuals in control groups. The documentation of effective therapy with the use of antibacterial agents in double-blind studies would represent another line of evidence. Preliminary reports on studies of this kind are considered encouraging (103) and the final outcome will be awaited eagerly. Epidemiological studies that would shed light on the role of these organisms as infectious agents are presently not feasible as simple systems for biotyping and serotyping have not been developed. Biotyping with preformed enzymes was shown not to be feasible as all isolates gave the same reactions (104). Development of a simple serotyping scheme on the basis of LPS antigens appears feasible because these antigens, some with long O side chains, have been shown to be present in C. pylori (135). Restriction endonuclease DNA analysis has been used in the laboratory and reported to be an effective epidemiological tool (83).

C. pylori has selected the gastric mucosa as its habitat and has never been cultured from saliva, feces, or other sources. Indeed, organisms administered orally to gnotobiotic piglets
cannot be recovered from the ileum, the colon, or the feces of the animals (82). The organisms grow under microaerophilic conditions in an atmosphere of 10% CO2-5% O2-85% N2 or in standard CO2 incubators with CO2 concentrations of 10%. They are generally regarded as incapable of growth under anaerobic conditions; however, Buck et al. (20) reported good anaerobic growth for a small percentage of the strains but poor growth or no growth for others. The temperature for isolation and routine culturing is generally 37°C, although most strains will grow at 42°C. High humidity produces better growth and the period of incubation is 3 to 4 days, although a period as long as 7 days may be required (103). The bacteria are sensitive to atmospheric oxygen, and plates left on the bench for more than 2 h in air may not be successfully subcultured.

C. pylori can be cultured on media generally used for campylobacteria that contains whole or lysed blood and antibiotics. However, Buck et al. (20) found that C. pylori could be cultured on gonococcal agar or chocolate agar without supplements. Colonies are grey, translucent, and small (0.5 to 1 mm in diameter). They can be identified as C. pylori by the Gram stain and by positive tests for urease, oxidase, and catalase.

With the continuing interest in these bacteria it is certain that clinical laboratories will be expected to accommodate increasing demands to culture endoscopic specimens. Only in the future will it become known whether these organisms will remain in the genus Campylobacter and whether they are as severe as enemy as some suggest, or whether they will be found to be innocuous bystanders whose major impact will have been to stimulate microbiologists and gastroenterologists into interesting debates.

CONCLUDING REMARKS

It is evident that the final chapter on the taxonomy of the genus Campylobacter remains to be written. The genus is in a state of flux and may be cited as an example to illustrate that taxonomy is a dynamic science. There is relatively little phenotypic information that can be used for classifying these spiral-shaped organisms in the classical manner. Two criteria that have been given much weight in justifying the inclusion of a species in the genus are cell morphology and the G+C content of DNA. As a result, the genus now includes species that are genetically and metabolically very different. The wide diversity leads one to question whether one genus is adequate to house all 14 species. Recently, comparisons of 16S ribosomal ribonucleic acid sequences from C. jejuni, C. coli, C. lariis, C. fetus, C. sporum, and C. pylori indicated that C. pylori should be excluded from the genus (141). The other five species showed high interspecies homology and constituted a distinct phylogenetic group. Studies of this nature need to be carried out with the other eight species. Such studies would be expected to stimulate interest in other genera of spiral-shaped organisms, and it is therefore most appropriate that a resurgence of interest in developing media for isolating "unusual campylobacteria" is under way in several laboratories and that there are continuing efforts to define additional phenotypic characteristics that may be exploited for identification and classification in the clinical laboratory. It is not inconceivable that more "nonculturable" spiral organisms will be identified during the next decade and be added to an expanding list of species. It can be confidently predicted that campylobacteria and other spiral-shaped organisms will continue to challenge and excite microbiologists for many more decades.

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