Taxonomic Study of the Genus *Campylobacter* Sebald and Véron and Designation of the Neotype Strain for the Type Species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron

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A critical study of the present state of the classification of vibrio-like, curved, microaerophilic bacteria was made. The species originally described under the names *Vibrio coli* Doyle, *V. jejuni* Jones et al., *V. sputorum* Prévot, and *V. bubulus* Florent are transferred to the genus *Campylobacter* Sebald and Véron 1963. The authors suggest that the type species of this genus, *C. fetus*, be divided into two subspecies: *C. fetus* subsp. *fetus* (Smith and Taylor) comb. nov. (syn. *V. fetus* subsp. *intestinalis* Florent), which contains the neotype strain of the species, and *C. fetus* subsp. *venerealis* (Florent) comb. nov. The previously described subspecies *V. fetus* subsp. *intermedius* Elazhari is regarded as an infrasubspecific taxon with the name *C. fetus* subsp. *venerealis* biotype *intermedius*. CIP 5396 (= ATCC 27374 = NCTC 10842) is proposed as the neotype strain of *C. fetus* subsp. *fetus*. This strain, then, is also the neotype strain of *C. fetus* (Smith and Taylor) Sebald and Véron.

In 1913, McFadyean and Stockman (34) discovered a "vibrio" which seemed to be responsible for abortion of pregnant ewes; they obtained an experimental abortion in pregnant cows by inoculation with this organism. Subsequently, Smith (52) recovered a microaerophilic "spirillum" from aborted calves. Smith (52) suspected that this organism was identical to that described by McFadyean and Stockman (34). It was designated *Vibrio fetus* by Smith and Taylor (53), the generic attribution being based on the fact that comma-shaped cells predominate over spirilloid ones, particularly in young cultures.

Nevertheless, assignment of this organism to the genus *Vibrio* is unsatisfactory: *V. fetus* differs greatly in phenotypic respects from the type species of the genus *Vibrio*, *V. cholerae* Pacini 1854 (14, 24, 55). Moreover, the G + C content of the deoxyribonucleic acid (DNA) of *V. fetus* is very far from that characteristic of *V. cholerae* and related *Vibrio* species (47, 55).

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The only shared property—curved cells—is now recognized to be a characteristic of dubious taxonomic value (10).

For these reasons, Sebald and Véron (47) proposed a new genus, *Campylobacter*, with *C. fetus* (Smith and Taylor) Sebald and Véron as the type species. The genus *Campylobacter* is defined (47, 54) as comprising gram-negative, slender, and curved bacteria which are motile by means of a single, polar flagellum, microaerophilic with a strictly respiratory metabolism, produce no acid in media with carbohydrates, and which have DNA with a G + C content between 29 and 36 mol %. The genus *Vibrio*, on the other hand, comprises bacteria which ferment glucose (24, 25, 54) and contain DNA with a G + C content between 40 and 53%, (J. M. Shewan and M. Véron, *Bergey's Manual*, 8th ed., in press), the value for *V. cholerae* being about 47 mol % (54).

Though the taxonomic notion of the family among the bacteria is difficult, or even impossible, to justify by means of genetic arguments, this taxon is useful for purposes of classification. Thus we have proposed (54, 55) to

include the genus *Campylobacter* in the family *Spirillaceae* because there is much morphological and physiological likeness between the genera *Campylobacter* and *Spirillum*. In agreement with this view, striking analogies were observed (13, 45) between the anatomical features of strains belonging to both of these genera, particularly on account of the presence of: (i) an outer wavy membrane, or "integument," easily separated from the inner membrane with a large vacant space between both membranes; (ii) a "complex cytoplasmic membrane" consisting of a triple-layered membrane associated with short bar-like elaborations making up centripetally oriented compartments; and (iii) individual flagellar basal granules.

Since Smith and Taylor's description of *V. fetus* (53), there have appeared descriptions of several other species or subspecies of microaerophilic curved bacteria which should also be included in the genus *Campylobacter*. These taxa are the following:

(i) *Vibrio fetus* subsp. *intestinalis* Florent 1959 (20, p. 7), the strains of which occur principally in the intestinal tracts of sheep, cattle, swine, birds, and humans, where it is usually commensal. It has also been isolated occasionally from the genital tract, the viscera, or the blood. All strains can cause sporadic abortion (principally in cows and sheep) (1, 20) because they are able to multiply in the placenta after passage from the intestine into the viscera. Human infection is very often due to this subspecies. Some strains are able to be transferred by venereal contact and to survive in genital tracts of cattle (13, 21, 39).

(ii) *V. fetus* subsp. *venerealis* Florent 1959 (20, p. 7) seems strictly adapted to the bovine genital tract (preputial and vaginal cavities) and is unable to survive in the intestinal tract (8). It causes enzootic venereal sterility in cows (49) and, contingently, abortion in pregnant cows (20).

(iii) *V. fetus* subsp. *intermedius* Elazhary 1968 (13, p. 17), first described by Florent (21, p. 1072) as "intermediate group," is adapted in cattle to the genital tract as well as to the intestines (21). Strains of this subspecies could be transferred by venereal processes (39).

(iv) *V. coli* Doyle 1948 (11, p. 51) is a normal inhabitant of the intestines of swine, poultry, and (occasionally) man, but it is absent in sheep and cattle (13). It can cause dysentery in swine, hepatitis in birds, and bloody diarrhoea in man.

(v) *V. jejuni* Jones et al. 1931 (26, p. 861) frequently occurs in the intestinal tracts of cattle and sheep (13), in which it can cause

mild dysentery.

(vi) *V. sputorum* Prévot 1940 (43, p. 123) and *V. bubulus* Florent 1953 (18, p. 2066) have very similar characteristics; their consolidation as two separate subspecies in a single species, as proposed by Loesche et al. (33), appears desirable. On the basis of priority, the specific epithet to be used in the name of the species is *sputorum*. *V. sputorum* subsp. *sputorum* Prévot is an occasionally pathogenic human parasite (33, p. 1109); it has been principally recovered from the oral cavity in gingivitis or from sputum in bronchitis. *V. sputorum* subsp. *bubulus* (Florent) Loesche et al. 1965 (33, p. 1109) appears to be nonpathogenic; it has been isolated from vaginal and preputial secretions, sperm, fetuses, and fetal membranes, and from stools of sheep, horses, and cattle.

At times, another species, *Vibrio metschnikovii* Gamaleia 1888 (23, p. 485), has been improperly included in this group of bacteria because it was recovered from the intestinal tract of poultry suffering from dysentery. In fact, the reference strains of this species possess a G + C content of DNA of about 46 mol % (47) and a fermentative metabolism of glucose like that of *V. cholerae* (55). Consequently, this species belongs to the genus *Vibrio* and not to the genus *Campylobacter*.

Finally, the genus *Campylobacter*, at present, appears to comprise four species: *C. fetus* (Smith and Taylor) Sebald and Véron 1963 (47, p. 907), which contains several subspecies, *C. coli* (Doyle) comb. nov., *C. jejuni* (Jones et al.) comb. nov., *C. sputorum* subsp. *sputorum* (Prévot) comb. nov., and *C. sputorum* subsp. *bubulus* (Florent) comb. nov. The inclusion of these last subspecies in the genus *Campylobacter* was previously suggested by Loesche et al. (33).

In the course of our work on this genus, we examined the properties of 40 *Campylobacter* strains, and we shall describe here the characteristics that permit the recognition or differentiation of species and subspecies.

When they proposed the creation of the genus *Campylobacter*, Sebald and Véron (47) selected *C. fetus* (Smith and Taylor) Sebald and Véron as the type species, but failed to indicate a type strain. No type strain was proposed by Smith and Taylor (53), who originally described the species, and none of their strains is now in culture. We have therefore selected a neotype strain, the characteristics of which are in full conformity with the rather fragmentary original description of the species (53) and of which a complete description follows.

MATERIALS AND METHODS

Bacterial strains. Forty strains of *Campylobacter* species were examined, the designations and origins of which are indicated in Table 1. Strain CIP (Collection of the Pasteur Institute, Paris) 5396 was isolated in 1952 by Doctor Vincent (Le Havre), who referred to it as "souche mouton 1." It was found in the brain of a sheep fetus belonging to a flock in which repeated abortions occurred; this enzootic was described by Vincent and Alloy (56). This strain appears to be the first found in France from cases of sporadic cattle abortion. Since its isolation it has been preserved freeze-dried in the Collection of the Pasteur Institute in Paris, and it appears to have remained stable upon subculture. It has been deposited in the American Type Culture Collection, Rockville, Maryland (ATCC 27374) and the National Collection of Type Cultures, London, England (NCTC 10842).

Cultivation procedures. Agar plates and slants were incubated in a gaseous atmosphere of 5% O₂, 10% CO₂, and 85% N₂, as recommended by Kiggins and Plastringe (27). All liquid and semi-solid media were incubated in air. Except where otherwise stated, the incubation temperature was 37 C.

Media used. (i) Nutrient agar: Lab Lemco beef extract (Oxoid Ltd., London), 5 g; bacteriological peptone N° 2 (Industrie Biologique Française, 92-Gennevilliers, France), 10 g; NaCl, 5 g; Oxoid agar N° 3, 12 g; water, 1,000 ml; pH 7.5.

(ii) Blood agar: nutrient agar with 10% defibrinated horse blood.

(iii) Thiol agar: thiol broth (Difco Laboratories Inc., Detroit, Mich.) with 1.2% Oxoid agar N° 3.

(iv) Yeast extract agar: Difco yeast extract, 7 g; NaCl, 5 g; Oxoid agar N° 3, 12 g; water, 1,000 ml.

(v) Deep nutrient gelatin agar (or "géluse-gélatine" of Legroux; formula quoted in reference 38): semi-solid nutrient gelatin agar with glucose (1%) and KNO₃ (0.05%), dispensed in 15-ml amounts in test tubes (16 by 160 mm), sterilized by autoclaving, and inoculated through the mass, after melting and cooling at 45 C.

(vi) Fluid Albimi broth: brucella broth (Albimi Laboratories, Flushing, N. Y.) with 0.12% Oxoid agar N° 3.

(vii) Defined medium of Fletcher and Plastringe (17): L-aspartic acid, 2 g; L-cysteine, 0.5 g; L-glutamic acid, 3 g; sodium acetate, 5 g; MgCl₂·6H₂O, 1 g; NaCl, 5 g; K₂HPO₄, 0.05 g; nicotinic acid, 0.1 mg; Oxoid agar N° 3, 12 g; demineralized water, 1,000 ml.

Assessment of relative growth rate on different media and at different temperature. For a comparative study of growth on a defined medium and on complex media, the method of Fletcher and Plastringe (17) was employed for CIP 5396. The complex media used were nutrient agar, blood agar, thiol agar, and yeast extract agar. The defined medium was that of Fletcher and Plastringe.

Each medium was dispensed in 7-ml amounts in test tubes (16 by 160 mm), autoclaved at 115 C, and solidified in the same slanted position so that they each had the same surface area. Each slant was inoculated with 0.05 ml of a saline (0.85% NaCl) suspension containing approximately 10⁸ cells/ml. The cultures were incubated for 3 days, whereupon

the cells from each medium were suspended in 8 ml of 0.3% formalinized saline. The optical density (OD) was determined in a Meunier photometer at 550 nm.

A comparison of growth was made in different atmospheres (air; 10% CO₂ in air; 5% O₂, plus 10% CO₂, in nitrogen) and at various temperatures (25, 37, and 45 C) in fluid Albimi broth.

Microscope investigations. The cells were examined after 40 h of growth on blood agar and nutrient agar. Motility was determined in hanging-drop preparations, and flagella were stained by Rhodes' method (44). CIP 5396 was examined in the electron microscope by metallic shadowing with palladium.

Biochemical tests. The production of catalase was determined by a slide test with 3% H₂O₂, and the production of oxidase with Kovacs' method (30) using 1% *para*-amino-dimethyl-aniline oxalate as reagent. To detect nitrate reductase, a fluid Albimi broth supplemented with 0.1% KNO₃ was inoculated and incubated for 48 h, and then was tested for the presence of nitrites. The effect of chlorate on anaerobic growth was investigated by the method of Pichinoty and Piéchaud (41). Ability to produce H₂S was determined according to the method of Florent (20), as modified by Elazhary (13), by suspending a lead acetate paper strip set above two different media, either a "standard medium" which was a fluid Albimi broth or a "sensitive medium" consisting of the same medium supplemented with 0.02% cystine-HCl; reading of blackening of paper was done after 4 and 6 days. The effect of the bacteria on glucose was detected in the medium of Hugh and Leifson (25) after 2, 4, and 8 days. Gelatin hydrolysis (22) was determined after 4 days, and the production of indole after 2 and 4 days in peptone water (1% bacteriological peptone IBF N° 2) containing 0.5% NaCl.

Tests of tolerance. Glycine tolerance (32) was determined according to Florent's technique (20) in fluid Albimi broth containing 1% glycine, and readings were made after 6 days. The effects of bile (46), NaCl (31), and glucose (31) on growth were tested for after 6 days in fluid Albimi broth supplemented with 1 or 10% ox bile, 3.5% NaCl, or 8% glucose, respectively. Tolerance towards and reduction of sodium selenite (5) was determined after 6 days on brucella agar (Albimi) with 0.1% sodium selenite. The ability to grow on Brilliant Green agar was estimated by a modification of the technique of Florent (19), using a blood agar supplemented with 1/33,000 Brilliant Green (Difco), and read after 3 days. Tolerance towards nalidixic acid and towards 2,3,5-triphenyl-tetrazolium chloride (TTC) (Thibault and Florent, personal communication) was determined by the ability to grow after 3 days on blood agar containing either 40 µg of nalidixic acid per ml or 1 mg of TTC per ml. The test of Shewan et al. (48) was performed on blood agar with disks impregnated by an acetone solution of 1% vibriostatic compound 0/129 (2,4-diamino-6,7-di-isopropylpteridine) and was read for growth after 3 days.

G + C content of DNA. In this study, as in a previous work (47), the extraction of bacterial DNA was done by the method of Bøvre and Henriksen (4). The ribonucleic acid (RNA) was hydrolyzed overnight in 1 N NaOH at 37 C, after which the DNA was

TABLE 1. *Designations and origins of the 40 strains of Campylobacter examined*^a

Reference in CIP	Supplied by	Strain designation	Source
<i>C. fetus</i> subsp. <i>fetus</i>			
A168	J. G.	Leg.	Human blood
A169	R. V.	Mar.	Human blood
5392	R. V.	VB	Animal
5393	A. F.	661	Sheep
5395	Curator of NCTC	NCTC 5850	Sheep, contagious abortion
5396	R.V.	Mouton 1	Sheep fetus
5411	P. T.	R. Br.	Human blood
5515	P. T.	Gal.	Human blood
571	P. T.	Lix.	Human blood
5721	P. T.	Via.	Human blood
5867	P. T.	Leb.	Human blood
688	A. F.	7572	Bull prepuce
6823	W. W.	Pb 819	Unknown
6824	W. W.	Ab 71	Unknown
6943	A. F.	922	Bull prepuce
6944	A. F.	2137/2	Bull prepuce
7084	P. T.	Dur.	Human blood
7085	P. T.	Tho.	Human blood
<i>C. fetus</i> subsp. <i>venerealis</i>			
53105	A. F.	483	Cow
5419	A. F.	995	Cow
5427	A. F.	S	Cow
6822	W. W.	Pb 1225	Unknown
6829	Curator of NCTC	NCTC 10354	Vaginal mucus of heifer
6945	A. F.	16977	Bull prepuce
<i>C. fetus</i> subsp. <i>venerealis</i> biotype <i>intermedius</i>			
5399	R. V.	Ville.	Human blood
5443	E. O.	1134	Human blood
<i>C. coli</i>			
6825	W. W.	A4	Unknown
7054	A. F.	3949	Pig feces
7077	A. F.	P2	Pig feces
7078	A. F.	P3	Pig feces
7079	A. F.	P6	Pig feces
7080	A. F.	1407	Pig feces
7081	A. F.	P9	Pig feces
7086	P. T.	Tan.	Human blood
715	A. F.	P875	Pig feces
<i>C. jejuni</i>			
702	A. F.	Uccle 91 F.B.	Bovine feces
<i>C. sputorum</i> subsp. <i>bubulus</i>			
53103	A. F.	Wat.	Bull sperm
5420	A. F.	Ato.	Bull sperm
5421	A. F.	Imp.	Bull sperm
5422	A. F.	Mix.	Bull sperm

^a Abbreviations: CIP, Collection of Pasteur Institute, Paris, France; NCTC, National Collection of Type Cultures, London, England. J. G., J. Gaillard, Paris, France; R. V., R. Vincent, Le Havre, France; A. F., A. Florent, Bruxelles, Belgium; P. T., P. Thibault, Paris, France; W. W., W. Winkenwerder, Hanover, Germany; E. O., E. O. King, Atlanta, Georgia, U.S.A.

hydrolyzed with 12 N HClO₄ at 100 C for 1 h. The free bases were separated by paper chromatography by Wyatt's method (57) by using a mixture of isopropanol-HCl-water (68:18:14, vol/vol/vol). The four bases were eluted in 0.1 N HCl and measured with a Beckman DB spectrophotometer. The results are expressed as moles percent of guanine + cytosine in the DNA.

Serological typing. Typing was performed by tube agglutination using heated bacterial suspensions and dilutions at 1/20 or higher of O:1 and O:2 antisera. The bacterial suspensions for agglutination reactions and immunizations were prepared from smooth cultures incubated for 40 h on the surface of nutrient agar; the cells were suspended in 0.3% formalinized saline, then heated at 100 C for 2 h, and diluted to provide an optical density of 0.31 at 550 nm. Antisera were prepared in rabbits, using strains Pb 1225 and Pb 819 of *C. fetus* received from W. Winkenwerder as immunizing antigens. These strains belong to serotypes O:1 and O:2, respectively, of Mitscherlich and Liess (35), which correspond to serotypes A and B, respectively, of Morgan (37) and Berg et al. (3).

RESULTS

Previous publications (1, 3, 7, 13, 20, 21, 39, 42, 50) have revealed three basic criteria which permit specific differentiation of campylobacters. These are: production of catalase (6); growth in a medium containing glycine (32) at 1% concentration (20); and production of H₂S determined in the "standard medium" (13) described above. Our own experience, based on a study of more than 40 strains, has confirmed the usefulness of these three criteria, supplemented by certain other tests shown in Tables 2 and 3, for the differentiation of species and subspecies of the genus *Campylobacter*. All strains studied here gave similar results for the tests reported in Tables 2 and 3.

The serotypes of 26 strains of *C. fetus* were determined with O:1 and O:2 antisera. For *V. fetus* subsp. *venerealis*, 3 strains belonged to serotype O:1, and 1 strain (CIP 6829) was not agglutinable. Both strains of *V. fetus* subsp. *intermedius* also belonged to serotype O:1. For *V. fetus* subsp. *intestinalis*, 5 strains belonged to serotype O:1, 8 strains to serotype O:2, and 1 strain (CIP 5721) was not agglutinable. Determinations of the heat-stable serotypes of the strains of *V. coli*, *V. jejuni*, and *V. sputorum* subsp. *bubulus* were not made.

Strain CIP 5396, designated as the neotype of *C. fetus*, is described below. Strains CIP 7080, CIP 702, and CIP 53103 are proposed, respectively, as reference strains for *C. coli*, *C. jejuni*, and *C. sputorum* subsp. *bubulus*.

DISCUSSION

On the basis of the results presented here and those previously published, *Campylobacter* strains can be grouped as follows:

1. Strains catalase positive and H₂S negative. These strains, which are moderately microaerophilic, constitute the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. This species can be split into three subspecies (or varieties) on cultural, biochemical, and ecological grounds; it can also be divided into several serotypes.

The clear differentiation of serotypes among strains of *C. fetus* was possible only after the demonstration of specificity of somatic heat-stable antigens by means of agglutination tests (3, 13, 37) as well as complement fixation tests (13, 35). According to the results of these studies, three main heat-stable serotypes, designated A, B, and C by Berg et al. (3), could be distinguished in *C. fetus*. Rare strains may simultaneously possess antigens A and B, or A, B, and C (3).

The subspecies of *C. fetus* are as follows:

(a) *C. fetus* subsp. *fetus* (Smith and Taylor) comb. nov. (synonym: *Vibrio fetus* subsp. *intestinalis* Florent). This subspecies has a fairly fast growth rate, colonies being well developed after 48 h. The chief features for the diagnosis of this subspecies are given in Table 2. *C. fetus* subsp. *fetus* corresponds to: group III strains of Akkermans et al. (1); some "related vibrios" of King (28, 29); type II strains of Mohanty et al. (36); type 2 strains of Bryner et al. (7); group II organisms of Plastring et al. (42); the majority of biotype 2 strains of Smibert (51); and organisms of groups A-2 and B of Berg et al. (3).

(b) *C. fetus* subsp. *venerealis* (Florent) comb. nov. (synonym: *Vibrio fetus* subsp. *venerealis* Florent). This subspecies grows somewhat more slowly than the preceding subspecies; the colonies are well developed only after three days. Growth is inhibited in a 1% glycine medium (see Table 2). This subspecies is probably identical to type I strains of Akkermans et al. (1), type I strains of Mohanty et al. (36), type 1 strains of Bryner et al. (7), type A strains of Park et al. (39), group I organisms of Plastring et al. (42), biotype 1 strains of Smibert (51), and group A-1 strains of Berg et al. (3). The majority of strains in this subspecies belong to serotype A (3).

V. fetus subsp. *intermedius* Elazhary is very similar in its biochemical characteristics (Table 2) to *C. fetus* subsp. *venerealis* except that it produces H₂S in the "sensitive medium" described above. In a 1% glycine medium, growth does not occur or is very slow. Reduction of 0.1% Na selenite is weak or absent. This subspecies corresponds closely to type III strains of Mohanty et al. (36), sub-type strains 1 of Bryner et al. (7), biotype 1A strains

TABLE 2. *Differentiation of catalase-positive strains of Campylobacter*

Species and subspecies	No. of strains	Characteristics ^a										
		BG1	BG3	GLY	GLU	NA	TTC	25C	42C	SN	H ₂ S	H ₂ S*
<i>C. fetus</i> subsp. <i>fetus</i>	18	+	+	+	+	+	—	+	—	+	—	+
<i>C. fetus</i> subsp. <i>venerealis</i>	6	+	+	—	+	+	—	+	—	—	—	—
<i>C. fetus</i> subsp. <i>venerealis</i> biotype <i>intermedius</i>	2	+	+	—	+	+	—	+	—	—	—	+
<i>C. coli</i>	9	+	—	+	+	—	+	—	+	+	d	+
<i>C. jejuni</i>	1	—	—	+	—	—	w	—	+	w	w	+

^a Abbreviations and symbols: BG1, growth in Brilliant Green 1/100,000; BG3, growth in Brilliant Green 1/33,000; GLY, growth in 1% glycine; GLU, growth in 8% glucose; NA, growth in 40 μ g of nalidixic acid per ml; TTC, growth in 1 mg of 2,3,5-triphenyltetrazolium chloride per ml; 25C, growth at 25 C; 42C, growth at 42 C; SN, reduction of Na selenite; H₂S, production of H₂S in "standard medium;" H₂S*, production of H₂S in "sensitive medium;" —, no growth or negative reaction; w, weakly positive reaction; +, growth or positive reaction; d, various results.

TABLE 3. *Differentiation of catalase-negative strains of Campylobacter*

Species and subspecies	No. of strains	Characteristics ^a						
		CAT	H ₂ S	GLY	Bile	NaCl	45C	CO ₂
<i>C. sputorum</i> subsp. <i>sputorum</i>	0 ^b	—	+	w	+	—	—	—
<i>C. sputorum</i> subsp. <i>bubulus</i>	4	—	+	+	—	+	—	—
Catalase-positive strains	36	+	d	d	d	—	d	d

^a Abbreviations and symbols: CAT, production of catalase; H₂S, production of H₂S in "standard medium;" GLY, growth in glycine 1%; Bile, growth in 1% ox bile; NaCl, growth in 3.5% NaCl; 45C, growth at 45 C; CO₂, growth in air with 10% CO₂; —, no growth or negative reaction; +, growth or positive reaction; w, weak growth; d, various results.

^b Characteristics cited from Loesche et al. (33).

of Smibert (51), and group A-sub 1 strains of Berg et al. (3). In general, these strains belong to serotype A (3).

2. Strains catalase positive and H₂S positive. These strains are characterized by an abundant production of H₂S in "sensitive media." The majority of them also produce copious H₂S in "standard media," that is to say in media in which a relatively insensitive method of detection is used (20). Two species are recognized:

(a) *C. coli* (Doyle) comb. nov. (synonym: *Vibrio coli* Doyle). This organism is the most aerotolerant and has the fastest growth rate of all the campylobacters, colonies usually appearing after 24 h. According to our results, all strains reduce nitrates to nitrites; this characteristic is contrary to the original report of Doyle

(11) but is in agreement with more recent publications (13, 29). Furthermore, the reduction of nitrates to nitrites is a common property among the campylobacters. Of the characters that are of value for the differentiation of this species (see Table 2), two recently described by P. Thibault and A. Florent (personal communication) greatly facilitate recognition of this species: the absence of growth on blood agar containing 40 μ g of nalidixic acid per ml; and good growth on blood agar containing 1 mg of triphenyltetrazolium chloride per ml (TTC). Some strains from human sources described as "related vibrios" by King (28, 29), some chicken strains investigated by Peckham (40), and the strains of biotype 3 of Smibert (51) probably belong to this species. The last author (51) reports that the majority

of *C. coli* strains (his biotype 3) are ribonuclease (RNase) or deoxyribonuclease (DNase) positive, or both, contrary to *C. fetus* strains (his biotypes 1 and 2). Elazhary (13) has demonstrated at least three specific O antigenic groups in this species.

(b) *C. jejuni* (Jones et al.) comb. nov. (synonym: *Vibrio jejuni* Jones et al.). Classically, this is a rather rare and poorly defined organism. We have been able to examine only one strain of *C. jejuni*, a rather short, very motile, vibrio-shaped organism which is distinctly microaerophilic, very sensitive to Brilliant Green, does not grow in an 8% glucose medium, and weakly produces H_2S in the "sensitive medium." These characteristics are in agreement with the observations of Elazhary (13). According to Florent (personal communication), strains of *C. jejuni* do not grow in the nalidixic medium and irregularly grow in the presence of 1 mg of TTC per ml. There are at least two specific O antigenic groups in this species (13).

3. **Catalase-negative strains.** *C. sputorum* (Prévot) comb. nov. (synonym: *Vibrio sputorum* Prévot). This species is much less aerotolerant than the catalase-positive species, and it was originally considered to be a strict anaerobe. However, Loesche et al. (33) have shown that both subspecies of this species are microaerophilic and cannot grow without oxygen. The properties of our strains of *C. sputorum* subsp. *bubulus* (Florent) comb. nov. (synonym: *vibrio bubulus* Florent) are in full agreement with those of the strains described in the literature (18, 33). This subspecies agrees with type II strains of Akkermans et al. (1), type IV strains of Mohanty et al. (36), and group IV strains of Plastring et al. (42).

The validity of including the four species cited above in the genus *Campylobacter* is supported by: (i) genetic data, as the average G + C contents of the DNA of these four species are 34.6, 32.7, 31.5, and 30.1 mol %, respectively (see Table 4); and (ii) ultrastructure evidence, as the membrane structure of *C. fetus* described by Ritchie and Keeler (45) was also observed in *C. coli* and *C. jejuni* by Elazhary (13).

If the differentiation of *C. fetus* subsp. *venerealis*, *C. sputorum* subsp. *sputorum* (Prévot) comb. nov. (synonym: *Vibrio sputorum* subsp. *sputorum* Prévot), and *C. sputorum* subsp. *bubulus* presents no real difficulty, it is not the same for the campylobacters that are catalase positive and H_2S positive. These strains frequently are misidentified, which is easily understood because the principal character in

their differentiation is the quantity of H_2S produced in more or less sensitive media.

It appears that the features used in differentiating *V. fetus* subsp. *intermedius* from the other two subspecies of *C. fetus* are not very numerous (see Table 2). Consequently, because *V. fetus* subsp. *intermedius* and *C. fetus* subsp. *venerealis* often have the same ecological niche, it is reasonable to consider the former as an H_2S -positive biotype (in a "sensitive medium") of the latter, the name of the latter having priority. Now, recognizing that a more extensive study is necessary to clarify the classification of the so-called *C. jejuni* group, the greatest taxonomic difficulty that remains with the campylobacters is differentiating between *V. fetus* subsp. *intestinalis* and *C. coli*.

A differentiation of these two taxa could actually be made on the basis of the following five characteristics showed by all of our strains of *C. coli* but not by strains of *V. fetus* subsp. *intestinalis* (see Table 2): (i) no growth occurs in the medium containing 40 μ g of nalidixic acid per ml; (ii) there is growth in blood agar containing 1 mg of TTC per ml; (iii) growth is possible at 42 C; (iv) there is no growth at 25 C; and (v) cultures in fluid Albimi broth at 37 C do not remain viable for more than 8 to 10 days. The temperature criteria for growth were proposed by King (28, 29) to differentiate her "related vibrio" from other *V. fetus* strains. On account of the last three characteristics, it is possible that the serotype C strains of Berg et al. (3), designated by them as *Vibrio marshi*, belong therefore to *C. coli*. In 1965, Firehammer (15) described under the name *Vibrio faecalis* strains that not only produced as much H_2S as *C. sputorum* subsp. *bubulus* but also produced catalase in abundance. We have not encountered strains of this sort, but it is not impossible that at least some strains identified as *V. faecalis* actually belong to *C. coli*.

The biochemical and ecological characteristics of *C. fetus* subsp. *venerealis* are so different from those of *V. fetus* subsp. *intestinalis* that these two subspecies should be maintained. However, recognition of these taxa as two distinct species cannot be justified because: (i) the G + C contents of their DNA are similar (see Table 4); (ii) hybridizations up to 100% were obtained between the glycine-positive and glycine-negative biotypes of *C. fetus* (2); (iii) there is a close genetic relationship between *C. fetus* subsp. *venerealis* and *V. fetus* subsp. *intestinalis* concerning the specific structures of phage adsorption (9, 16); (iv) almost all strains of *C. fetus* subsp. *venerealis* and *V. fetus* subsp. *intestinalis* contain the same carbohydrates in

TABLE 4. *G + C contents of DNA of 15 strains of Campylobacter species*^a

Strains identified as ^b	Ref.	Method	G + C mean value (%)	
			Strains	Taxa ^c
1. <i>C. fetus</i> subsp. <i>fetus</i>				
CIP 5395	47	C	35.4	GC _i = 34.4
CIP 5396	47	C	33.1	
Ovine 751	2	T	34.7	
2. <i>C. fetus</i> subsp. <i>venerealis</i>				
CIP 5443	47	C	34.4	GC _v = 34.4
CIP 6829	^c	C	35.3	
CIP 6829	^e	D	35.7	
Bovine R9	2	T	33.4	
3. <i>C. coli</i>				
Uccle 1408	^f	T	33.1	GC _c = 32.7
Human 662	2	T	33.3	
Avian 1802 GB	2	T	32.1	
Avian 1803 C	2	T	32.4	
4. <i>C. jejuni</i>				
Uccle 91	^f	T	31.5	
5. <i>C. sputorum</i> subsp. <i>bubulus</i>				
CIP 5420	47	C	30.9	GC _b = 30.1
CIP 5421	47	C	29.7	
CIP 5422	47	C	30.4	
CIP 53103	47	C	29.5	

^a Abbreviations Ref., references; C, chromatography; T, melting temperature analysis; D, buoyant density in CsCl.

^b For strains studied by Basden et al. (2), the identification was made on the basis of the published characters (especially growth in glycine, growth at 42 C, and H₂S production).

^c The four observed averages are significantly different ($P < 0.001$). The differences are significant between GC_i and GC_c ($0.02 < P < 0.05$) and between GC_c and GC_b ($0.001 < P < 0.01$).

^d Personal, unpublished results.

^e M. Mandel, personal communication.

^f A. Florent, personal communication.

their cell walls (51); and (v) probably all strains of *C. fetus* subsp. *venerealis* and some of the strains of *V. fetus* subsp. *intestinalis* belong to the same serotype, O:1 (35) or A (3, 38).

From the above it is clear that the previously described subspecies of *V. fetus* (synonym: *C. fetus*) do belong to the same species. One of these subspecies must be designated as the type subspecies of the species. The type subspecies is that which contains the type strain of the species. Since no type strain exists for *C. fetus*, a neotype strain must be designated, and the subspecies which contains the neotype becomes the type subspecies. In accordance with Rule 21 of the International Code of Nomenclature of Bacteria (12), the subspecific epithet in the name of the type subspecies must be the same as that for the species. Thus, whichever subspecies of *C. fetus* contains the neotype strain, the subspecific epithet in its name must be changed to *fetus*.

The choice of *V. fetus* subsp. *intestinalis* as the type subspecies seems better to us for the following reasons:

(a) *C. fetus* was stated by the original observers of this microorganism (34, 53) to be the agent responsible for infectious abortion of cattle and sheep but not for enzootic venereal sterility in cattle; this latter disease was recognized later by Sjollem et al. (49), and its etiological agent was proposed as a new subspecies by Akkermans et al. (1). The first strains isolated by Smith (52) were recovered from fetuses and placentas of aborting cows, and thus the specific epithet "*fetus*" was used in the name of this bacterial species (53). At the present time, it is known that the *C. fetus* strains responsible for infectious abortion have the characteristics of *V. fetus* subsp. *intestinalis*.

(b) *C. fetus* subsp. *venerealis* is adapted to a very restricted ecological niche (genital tract of cattle) owing to its high degree of parasitism; its pathogenicity is unvaried. Therefore, it is possible to consider *C. fetus* subsp. *venerealis* as a "defective mutant" of *V. fetus* subsp. *intestinalis*. The latter subspecies can be considered a "wild type," more versatile with regard to its ecological niche and pathogenicity. Thus,

it better represents the species from an evolutionary point of view.

Consequently, we designate strain CIP 5396 (= ATCC 27374 = NCTC 10842), which has the characteristics of *V. fetus* subsp. *intestinalis*, as the neotype strain of *Campylobacter fetus*. This strain, described below, is also the neotype strain of the type subspecies of *C. fetus*.

Description of strain CIP 5396, the designated neotype strain for *C. fetus* and its type subspecies. **Morphology.** The cells are gram negative, curved, comma-like, slender rods, 0.3 to 0.4 by 1.5 to 2.5 μm (see Fig. 1 and 2). They often occur in the shape of an "S" or in chains

with three to five spirals. No spherical forms have been observed in young cultures. Capsules are not produced.

The comma forms are actively motile by means of a single, polar flagellum showing two to four undulations (see Fig. 3 and 4). The "S"-shaped and helicoidal forms usually possess a single flagellum at each pole.

G + C content. The guanine + cytosine content of the DNA is 33.1 mol % (Table 4).

Growth characteristics. After 24 h of incubation, the colonies on nutrient agar, blood agar, and thiol agar are small, being scarcely perceptible to the naked eye. After 48 h the colonies

Fig. 1 and 2. Micrographs of gram-stained cells of *C. fetus* subsp. *fetus* CIP 5396. Magnification bars represent 5 μm .

Fig. 3 and 4. Electron micrographs of *C. fetus subsp. fetus* CIP 5396 shadowed with palladium at an angle of 10 degrees. Scale marker, 1 μ m.

attain a diameter of 1 mm; they are round, raised, and translucent with a regular edge and a smooth, glistening surface.

In nutrient gelatin agar stabs, colonies extend throughout the medium, except in a surface zone of 2 mm, after 48 h of incubation; at this time they have the aspect of bubbles centered on a point. On further incubation, colonies also develop in the surface zone.

In fluid Albimi broth, growth first occurs in a disk-shaped zone 2 to 3 mm beneath the surface and then after 48 h it becomes abundant in the upper portion of the medium.

The growth yields on solid media, calculated according to the method of Fletcher and Plastringe (17) and expressed in optical densities, are: nutrient agar, 0.37; blood agar, 0.50; thiol agar, 0.66; yeast extract agar, 0.40; and defined medium, 0.08. Thus, this strain is not particularly exacting with respect to its nutritional requirements since the growth rate is good on all complex media used; however, its

growth rate on the defined medium is weaker than that of the majority of strains studied by us and by Fletcher and Plastringe (17).

In an atmosphere of 5% O₂, 10% CO₂, and 85% N₂, growth is a little faster and more abundant than in an atmosphere of 90% air and 10% CO₂. In air, even with a heavy inoculum, only a few colonies grow after 3 or 4 days.

Good growth occurs at 25 C, but there is no growth at 42 C.

Biochemical properties. CIP 5396 is catalase positive, oxidase positive, and reduces nitrates to nitrites. In deep nutrient gelatin agar, anaerobic growth is not inhibited by chlorate; the nitrate reductase of this strain must therefore be of type B (41).

H₂S is not produced in fluid Albimi broth without cystine but is produced in fluid Albimi broth supplemented with 0.02% cystine-HCl.

This strain does not produce acid from carbohydrates in Hugh and Leifson's medium, does not liquefy gelatin, does not grow on

Simmons' citrate, and does not produce indole.

Tolerance. Good growth occurs in media containing 1% glycine, 10% ox bile, 8% glucose, 0.1% sodium selenite, 1/33,000 Brilliant Green, or 40 µg of nalidixic acid per ml. The growth on selenite produces a reduction of this compound.

There is no growth in media containing 3.5% NaCl or 1 mg of TTC per ml. The strain is insensitive to vibriostatic compound 0/129.

Serological typing. The cells of this strain were agglutinated by O:2 antiserum with a titer of 1/320 but not by O:1 antiserum. Therefore, this strain belongs to serotype O:2 (or B).

Because strain CIP 5396 has been designated herein as the neotype strain of *Campylobacter fetus* and because this strain belongs to *V. fetus* subsp. *intestinalis*, this subspecies is the type subspecies of the species. According to Rules 7 and 21 of the International Code of Nomenclature of Bacteria (12) and as pointed out earlier in this paper, the subspecific epithet in the name of the type subspecies must be the same as the specific epithet. Thus the correct name of the type subspecies of *C. fetus* is *C. fetus* subsp. *fetus*, of which *V. fetus* subsp. *intestinalis* Florent 1959 is an earlier, but illegitimate, objective synonym.

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REPRINT REQUESTS

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