

## Differentiation of *Campylobacter* and *Campylobacter*-Like Organisms by Cellular Fatty Acid Composition

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The cellular fatty acid compositions of 368 strains of *Campylobacter* species or *Campylobacter*-like organisms were determined by gas-liquid chromatography. Most of the strains (339) were placed in one of three groups based on differences in fatty acid profiles. Group A contained *Campylobacter jejuni* (97%) and most *C. coli* (83%) strains and was characterized by the presence of a 19-carbon cyclopropane fatty acid (19:0 cyc) and 3-hydroxytetradecanoic acid (3-OH-14:0). Group B included all *C. laridis* and some *C. coli* (17%) strains; its profile was similar to that of group A, except that 19:0 cyc was absent. Group C contained *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* and was characterized by the presence of 3-OH-14:0 and 3-hydroxyhexadecanoic acid (3-OH-16:0) and the absence of 19:0 cyc. Twenty-nine isolates were placed in four additional groups. Group D included the type strain of "*C. cinaedi*" and 14 other isolates, which were differentiated by the presence of dodecanoic acid (12:0), 3-hydroxydodecanoic acid (3-OH-12:0), and 3-OH-16:0 and the absence of hexadecenoic acid (16:1) and 3-OH-14:0. Group E contained the type strain of "*C. fennelliae*" and two additional isolates, which were differentiated by the presence of a 16-carbon aldehyde and a 16-carbon dimethylacetyl and the absence of 16:1. Group F included the type strain and one reference strain of *C. cryaerophila* and six human isolates whose phenotypic characteristics were similar to those of this species; this group was distinguished by the presence of two isomers of 16:1, tetradecenoic acid (14:1), and 3-OH-14:0. Group G included three strains of *C. pyloridis* and was characterized by the presence of 19:0 cyc, 3-OH-16:0, and 3-hydroxyoctadecanoic acid (3-OH-18:0) and by the absence of 16:1 and 3-OH-14:0.

Since 1980, the biochemistry laboratory of the Division of Bacterial Diseases at the Centers for Disease Control (CDC) has used gas-liquid chromatography (GLC) to determine the cellular fatty acid compositions of reference strains and clinical isolates of *Campylobacter* species (2, 19) and *Campylobacter*-like organisms (CLO). In an earlier study (19), reference strains of nine *Campylobacter* species or subspecies were differentiated into at least four GLC groups based on differences in their cellular fatty acids. These results have been used as a basis for characterizing many clinical isolates of campylobacters sent to CDC for identification.

During the past several years, techniques for the isolation of campylobacters have improved, and more species of these bacteria are being detected in clinical sources (4, 15, 21, 23, 24). *Campylobacter*s are difficult to identify to species level by conventional methods because they are asaccharolytic and inactive in most biochemical tests and may have atypical or inconclusive biochemical reactions (4, 17, 24). Since campylobacters cannot always be identified to species level with confidence, many types of serologic (7, 14), biochemical (1, 9, 16, 17, 21), and chemical (2, 3, 9, 12, 16, 19, 21) tests have been used to differentiate them. This report describes our laboratory's experience with cellular fatty acid analysis and its usefulness in differentiating *Campylobacter* species or CLO.

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### MATERIALS AND METHODS

**Cultures.** The 368 *Campylobacter* or CLO isolates that were examined for cellular fatty acids were received from the

*Campylobacter* Reference and Meningitis and Special Pathogens laboratories at CDC. The majority of the strains tested (75%) were human clinical isolates which had been referred to CDC from other laboratories for identification. Most were isolated from fecal samples or blood, but some (approximately 30 strains) were isolated from other body sites and fluids. The remaining 25% of the strains were animal isolates, reference strains, or type strains. Strains were cultured on blood agar plates and incubated at 35 to 37°C for 24 h in an atmosphere of approximately 5% O<sub>2</sub>-10% CO<sub>2</sub>-85% N<sub>2</sub>. Strains of three species ("*Campylobacter cinaedi*," "*C. fennelliae*," and *C. hyointestinalis*) that grew slowly were incubated for 48 to 96 h in 5% O<sub>2</sub>-10% CO<sub>2</sub>-10% H<sub>2</sub>-75% N<sub>2</sub> because this atmosphere enhanced their growth. The phenotypic characteristics were established as described previously (1, 17; R. J. Owen, S. R. Martin, and P. Borman, Letter, Lancet i:111, 1985); DNA hybridization studies (4), antimicrobial susceptibility testing (17), and fluorescent-antibody testing (7) were done on some of the strains. The identities of most strains were not known until the GLC analyses had been completed.

**Preparation of cellular FAME.** Cells were removed from the plate with 1 ml of sterile distilled water, placed in a screw-cap tube fitted with a Teflon-lined cap, and saponified with 2 to 4 ml of 15% NaOH in 50% aqueous methanol for 30 min at 100°C. The fatty acids were methylated and extracted as described previously (19), except that approximately one-half the amount of cells and reagents was used for some of the analyses. The methanolic aqueous layer which remained after the fatty acid methyl esters (FAME) were extracted was mixed with 1 to 2 ml of concentrated HCl and heated at 85°C for 16 h to liberate and methylate any bound or amide-linked fatty acids which were not released by alkaline hydrolysis (saponification). The methyl esters of the amide-linked fatty acids were extracted and combined with

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the saponified FAME, and the mixture was washed with 0.3 M phosphate buffer (10, 19). The organic layer containing the FAME was concentrated to 0.2 to 0.5 ml, and a 1- $\mu$ l sample was analyzed by GLC.

**GLC.** The FAME samples were analyzed on a fused-silica capillary column (50 m by 0.2 mm) with cross-linked methyl silicone (OV-101) as the stationary phase (Hewlett-Packard Co., Avondale, Pa.). The column was installed in a Hewlett-Packard 5790A gas chromatograph equipped with a flame ionization detector and coupled with a Hewlett-Packard 3390 reporting integrator. Samples were also analyzed with a Hewlett-Packard 5880 level 4 gas chromatograph and data system. The instrument conditions, column temperatures, and other analytical conditions have been described previously (19). The FAME peaks were identified by comparing retention times with those of methyl ester standards (Supelco, Bellefonte, Pa.; Alltech Associates, Inc., Applied Science Div., State College, Pa.; Laradon Fine Chemicals, Malmö, Sweden). The identities of the acids were confirmed by hydrogenation of unsaturated acids (10, 18), trifluoroacetylation of hydroxy acids (10, 18), and mass spectrometry (8, 18).

## RESULTS

All of the *Campylobacter* strains and CLO tested contained tetradecanoic acid (14:0), hexadecanoic acid (16:0), octadecanoic acid (18:1  $\omega$ 7-cis), and octadecanoic acid (18:0). However, they could be differentiated into seven GLC groups based on differences in cellular fatty acids. The largest group, designated group A, was characterized by the presence of 1% or greater amounts of a 19-carbon cyclopropane fatty acid (19:0 cyc) tentatively identified as *cis*-11,12-methylene octadecanoic acid, the presence of 3-hydroxytetradecanoic acid (3-OH-14:0), and a ratio of 16:0 to hexadecanoic acid (16:1  $\omega$ 7-cis) of  $\geq 4$  (Fig. 1A). The major fatty acid in most strains was either 16:0 or 18:1  $\omega$ 7-cis. The peaks labeled "Art" in the chromatogram are artifacts from the partial degradation of 19:0 cyc during the methylation step (11, 13); these artifacts can be prevented if methylation is done at 85°C for 10 min (11). Group A included 139 of the 143 *C. jejuni* strains and 50 of the 60 *C. coli* strains examined. The type strains of both *C. jejuni* and *C. coli* were assigned to group A; these two species can be differentiated biochemically because *C. jejuni* usually hydrolyzes sodium hippurate and *C. coli* does not (17, 22). With four exceptions, all *C. jejuni* strains contained 19:0 cyc ( $\geq 1\%$ ) and were assigned to group A; these four strains (D131, D1420, D1934, and D1935) were regrown and retested, and the positive hippurate reactions and absence of 19:0 cyc were confirmed. The identity of two of these strains was confirmed by DNA hybridization to be *C. jejuni*. Two *C. jejuni* strains (D424 and D1887) were both hippurate and 19:0 cyc positive but were resistant to nalidixic acid; their identity was questioned because most strains of *C. jejuni* are susceptible to this antimicrobial agent (17, 22). Subsequent DNA hybridization of strain D424 confirmed that its initial identification as *C. jejuni* by biochemical and GLC testing was correct.

The next group of isolates, designated group B, had a fatty acid profile similar to that of group A, except that 19:0 cyc was absent and the relative concentration of 18:1  $\omega$ 7-cis was greater than that found in group A strains (Fig. 1B). Group B included all 20 *C. laridis*, 10 *C. coli*, and 4 *C. jejuni* strains. One of the *C. laridis* strains (D382) was oxidase negative, and its identity was confirmed by DNA hybridization (23).

The identity of four *C. coli* strains was confirmed by DNA hybridization, and the other six had phenotypic characteristics which were typical for this species. Although 17% of the *C. coli* strains and 3% of the *C. jejuni* strains could not be differentiated from *C. laridis* by fatty acid analysis, these three thermophilic species can be differentiated by the hippurate reaction, susceptibility to nalidixic acid, and anaerobic growth in trimethylamine-*N*-oxide hydrochloride (1, 17, 22).

Twenty-five strains with one or more atypical phenotypic characteristics were also included in group B. Eighteen of the strains were catalase negative or very weakly positive; their identities and those of seven catalase-positive CLO are still under study. Almost all of the strains assigned to group B contained 2% or less linoleic acid (18:2  $\omega$ 6-cis,  $\omega$ 9-cis) and oleic acid (18:1  $\omega$ 9-cis); however, eight strains contained slightly higher percentages (4 to 6%) of these two acids. Four of these strains were identified by DNA hybridization and one was identified by phenotypic characteristics as *C. hyointestinalis* (5); the identities of three strains are still undetermined.

Group C included 4 reference strains of *C. fetus* subsp. *fetus*, 4 reference strains of *C. fetus* subsp. *venerealis*, and 75 clinical isolates. This group was characterized by the presence of 3-hydroxypalmitic acid (3-OH-16:0) and 3-OH-14:0, the absence of 19:0 cyc, and a lower ratio of 16:0 to 16:1  $\omega$ 7-cis ( $\leq 2$ ) than was found in either group A or group B (Fig. 1C). Fourteen of the isolates assigned to group C were difficult to identify by conventional testing because one or more phenotypic characteristics were atypical as compared with those of the type strain of *C. fetus* subsp. *fetus*. These included growth at 42°C, negative H<sub>2</sub>S reaction, resistance to cephalothin, and nonmotility. However, DNA studies confirmed that these 14 isolates were *C. fetus* subsp. *fetus* (4).

A total of 339 (92%) of the *Campylobacter* strains or CLO tested could be placed in group A, B, or C, but there were 29 strains whose profiles were different. They were assigned to four additional groups on the basis of their fatty acid compositions. Of the 29 strains, 15 were designated group D; they were differentiated from groups A, B, and C because 3-OH-14:0 and 16:1  $\omega$ 7-cis were either absent or present in only trace concentrations (<1%) to 1% concentrations (Fig. 1D). These 15 strains contained dodecanoic acid (12:0), 3-hydroxydodecanoic acid (3-OH-12:0), and 3-OH-16:0. Included in group D were the type strain of "*C. cinaedi*" (24), the strain designated CLO-1B by Totten and co-workers (24), and two isolates (E8774 and F2682) which had been tested for cellular fatty acids in 1980 and 1982, respectively. At the time the initial GLC analyses were done on E8774 and F2682, we reported that the fatty acids of these two strains did not match those of any known *Campylobacter* species or other genera we had tested. Because of the phenotypic characteristics of these strains, the CDC reference laboratory reported E8774 and F2682 as atypical *Campylobacter* species. Strain E8774 has now been confirmed as genetically homologous to "*C. cinaedi*" (24). DNA hybridization studies are currently being done on most of the other strains included in group D to determine whether they are also "*C. cinaedi*."

Of the 29 strains, 3 were designated group E; this group was characterized by the presence of 3-OH-14:0 and 3-OH-16:0 and the absence of 16:1  $\omega$ 7-cis (Fig. 1E). The peaks which eluted before pentadecanoic acid (15:0) and heptadecanoic acid (17:0) were identified by mass spectrometry as a 16-carbon aldehyde and a 16-carbon dimeth-

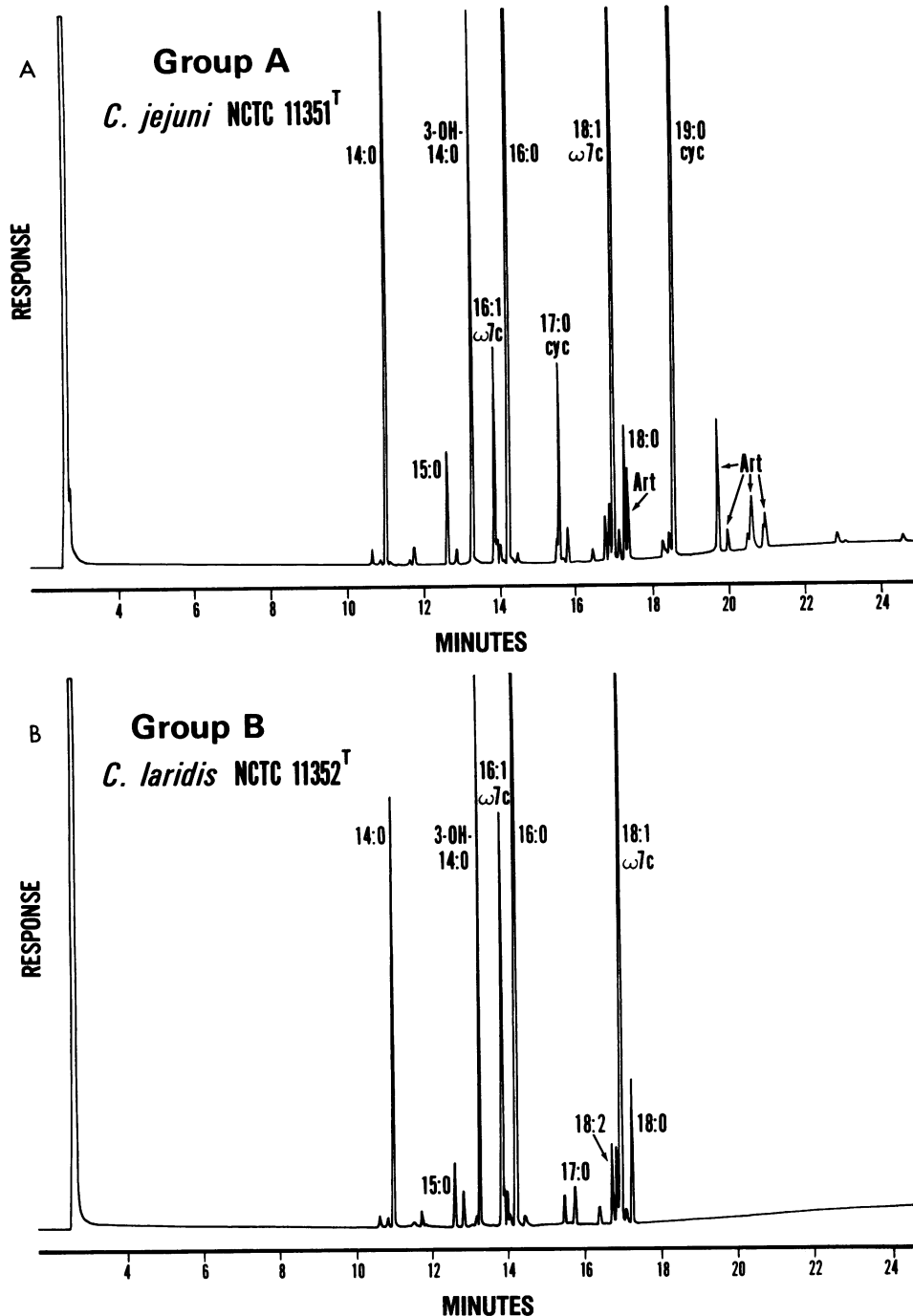


FIG. 1. Gas chromatograms of methylated fatty acids of *Campylobacter* species analyzed on an OV-101 fused-silica capillary column (50 m by 0.2 mm). See the text and footnote *d* of Table 1 for explanations of compound abbreviations and designations. Art, Artifacts from the partial degradation of 19:0 cyc; Un, unidentified.

ylacetyl, respectively. Group E included the type strain of "*C. fennelliae*," another reference isolate of this species (isolate 528, supplied by Cynthia L. Fennell), and the strain designated CLO-3 by Totten and co-workers (24).

Of the 29 strains, 8 were designated group F. The fatty acid profile of this group was quite different from those of groups A to E because tetradecenoic acid (14:1) and two isomers of 16:1 were present (Fig. 1F). The first isomer was identified as 16:1  $\omega$ 7-cis, and the second isomer [16:1 (x)]

was tentatively identified as 16:1  $\omega$ 5-cis; however, a standard was not available to confirm the position of the double bond. The strains included in group F also contained 12:0 and 3-OH-14:0; several of the strains contained two isomers of heptadecenoic acid (17:1). One of the eight strains was the type strain of *C. cryaerophila* (21), one was a reference strain of this species, and six were human clinical isolates which had been sent to the CDC reference laboratory from 1983 to 1985. They were from six different states and had

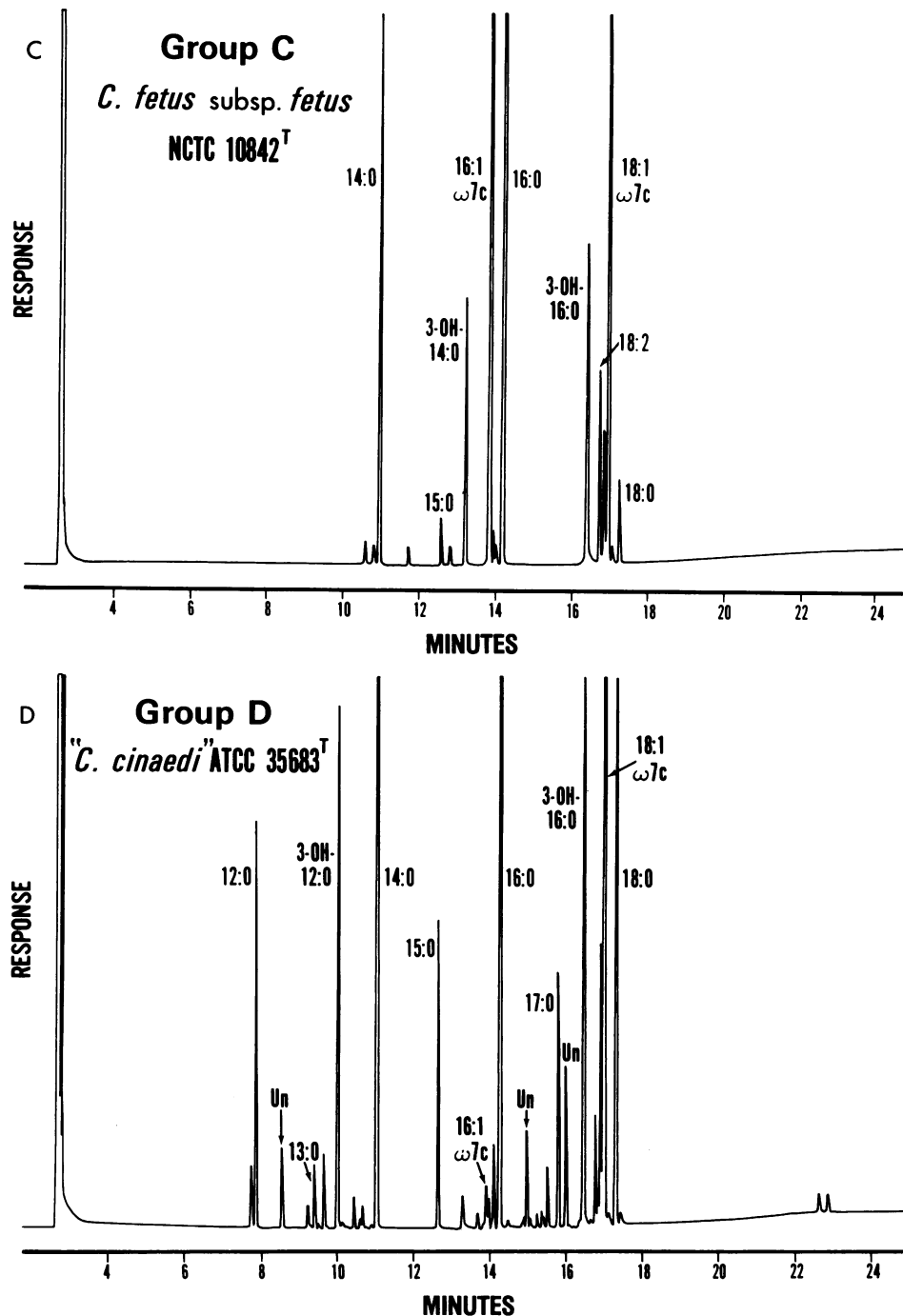


FIG. 1—Continued.

been isolated from various sites, including a mitral valve, stool, abdominal contents, and blood. CDC reported these six isolates as "unidentified" or possible *Pseudomonas* species because their biochemical reactions had some similarities to those of *Pseudomonas alcaligenes* (Dannie Hollis, personal communication). However, the cellular fatty acid profiles did not match that of the type strain of *P. alcaligenes* (20). Since these six strains grew aerobically, their initial identification excluded the genus *Campylobacter*. Several phenotypic characteristics of the clinical isolates were similar to those of *C. cryaerophila*, the species recently de-

scribed by Neill and co-workers (21). In addition, the cellular fatty acid profiles of the type and reference *C. cryaerophila* strains and the six clinical isolates were essentially identical. DNA hybridization studies are currently being done to determine whether these are strains of *C. cryaerophila*.

Group G included three strains of *C. pyloridis*, a recently described species associated with human gastritis and peptic ulcers (6, 15, 16). This group was characterized by relatively large amounts of 14:0 and 19:0 cyc, smaller amounts of 3-hydroxyoctadecanoic acid (3-OH-18:0), 3-OH-16:0, and 16:0, and the absence of 3-OH-14:0 and 16:1  $\omega$ -cis (Fig. 1G).

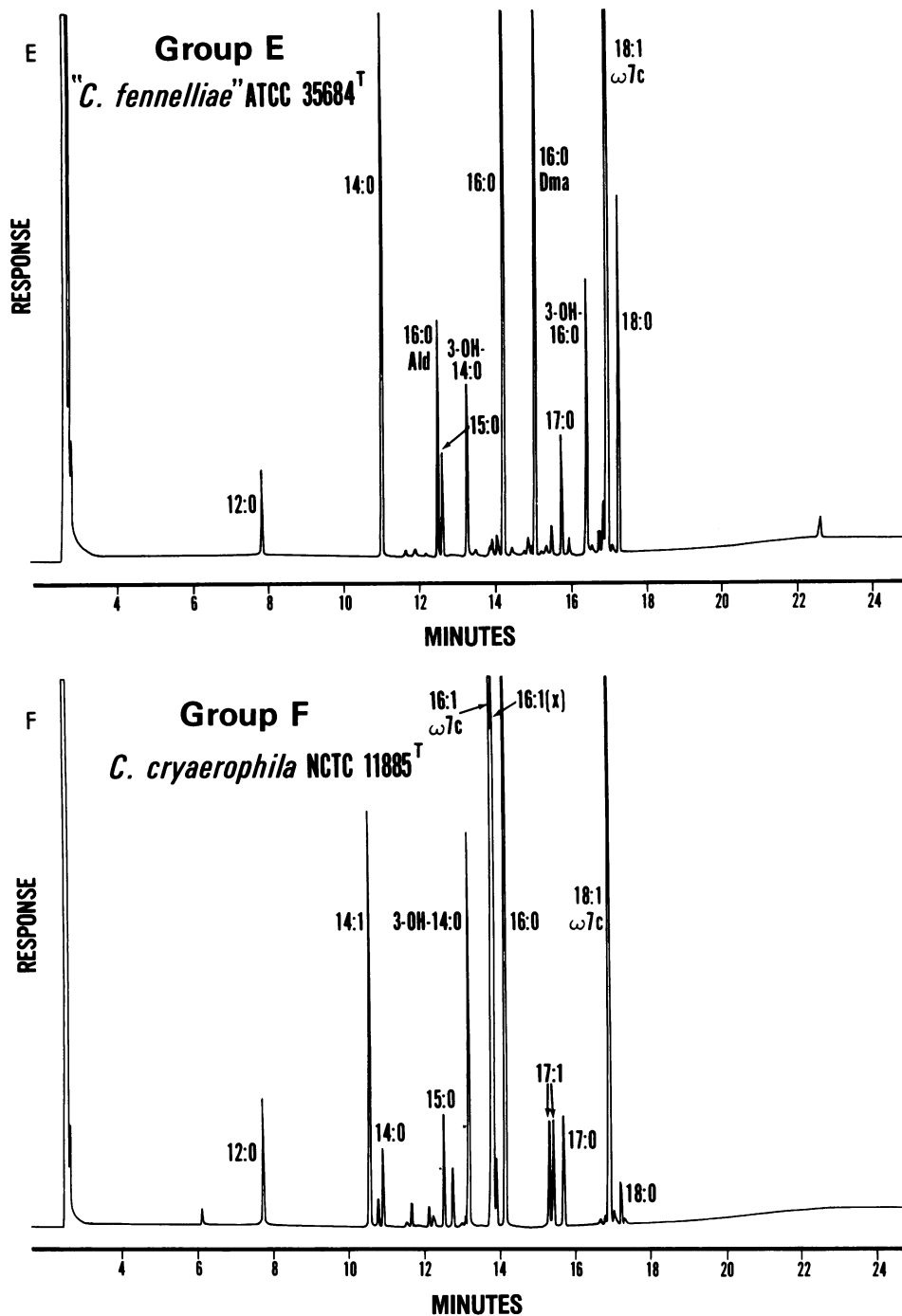


FIG. 1—Continued.

The relative amounts of 12:0, tridecanoic acid (13:0), 14:1, heptadecanoic acid (17:0), and nonadecanoic acid (19:0) and of the unidentified peaks varied from trace to 3% in the three strains tested.

The relative percentages of the cellular fatty acids and other compounds which are useful in differentiating the eight *Campylobacter* species into seven GLC groups are summarized in Table 1. The values for *C. jejuni*, *C. coli*, *C. laridis*, and *C. fetus* subsp. *fetus* include those for the type strain and 14 reference strains of each of these four species (Table 1). With the exception of *C. laridis*, the identity of all 14

reference strains was confirmed by DNA hybridization; the identity of 6 of the *C. laridis* strains was confirmed by DNA hybridization, and the biochemical reactions of the other 8 strains were typical for this species. Data for four *C. coli* strains without 19:0 cyc but identified by DNA hybridization are also included to show that their fatty acid compositions were almost identical to those of the *C. laridis* strains. A total of 92% ( $n = 311$ ) of the *Campylobacter* isolates assigned to groups A, B, and C were identified by biochemical or genetic testing as *C. jejuni*, *C. coli*, *C. laridis*, *C. hyointestinalis*, or *C. fetus* subsp. *fetus*. If the conventional

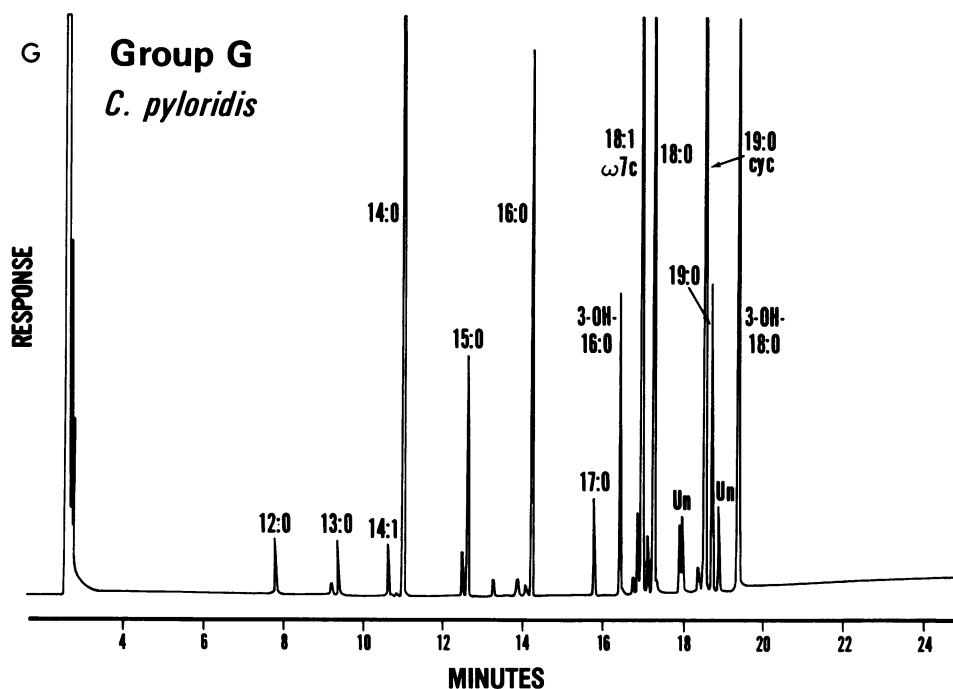


FIG. 1—Continued.

test results matched those described for a certain species (1, 17, 22; Owen et al., Letter), the fatty acid profile was the same as that found for the type or reference strain of that species (19); the only exceptions were the 4 strains of *C. jejuni* and the 10 strains of *C. coli* which did not contain 19:0 cyc.

Fatty acid data from only the type strains of "*C. cinaedi*" (group D), "*C. fennelliae*" (group E), and *C. cryaerophila* (group F) are given in Table 1 because the identities of most of the clinical isolates in groups D, E, and F have not been confirmed by DNA hybridization. The percentages for the fatty acids do not always total 100% because small amounts ( $\leq 3\%$ ) of several acids (13:0, 15:0, 17:1, a 17-carbon cyclopropane fatty acid [17:0 cyc], 17:0, 18:2, 18:1  $\omega$ -7-cis, and 19:0) and unidentified compounds which are not useful in differentiating the *Campylobacter* species are not included.

## DISCUSSION

These results show that five of eight *Campylobacter* species could be differentiated by cellular fatty acid composition. These five species included *C. fetus*, "*C. cinaedi*," "*C. fennelliae*," *C. cryaerophila*, and *C. pyloridis*. *C. coli* could not be distinguished from *C. jejuni* or *C. laridis* by fatty acid composition because *C. coli* strains had two different profiles. However, by integrating the results of selected biochemical tests (1, 17, 22) with the fatty acid results, identification of *C. jejuni*, *C. coli*, and *C. laridis* could be made to the species level.

The group B strains were a diverse group because they represented at least four different species (*C. laridis*, *C. coli*, *C. jejuni*, and *C. hyointestinalis*), which were all confirmed by DNA hybridization. In addition, the identities of 25 strains assigned to group B are still undetermined, but preliminary results of phenotypic and DNA hybridization studies indicate that they may represent two additional

*Campylobacter* species. The higher concentrations of linoleic and oleic acids found in five of the group B strains identified as *C. hyointestinalis* appear to be representative of this species. However, additional testing is needed to determine whether these higher concentrations of unsaturated fatty acids are a stable characteristic of this species or whether they are a result of the physiological age of the cells (13).

All 75 of the clinical isolates included in group C were catalase positive and were identified by conventional testing or DNA hybridization as *C. fetus* subsp. *fetus*. A previous study showed that the fatty acid composition of this species was similar to those of *C. fetus* subsp. *venerealis*, "*C. fecalis*," *C. sputorum* subsp. *sputorum*, and *C. sputorum* subsp. *bubulus* (19). However, *C. fetus* subsp. *fetus* can be distinguished from the two catalase-positive species which are not associated with human disease, *C. fetus* subsp. *venerealis* and "*C. fecalis*," and from the catalase-negative *C. sputorum* subspecies by biochemical tests (4, 17).

The four recently described *Campylobacter* species, "*C. cinaedi*," "*C. fennelliae*," *C. cryaerophila*, and *C. pyloridis*, had fatty acid profiles distinct from one another and from those of all other *Campylobacter* species previously described (2, 3, 6, 12, 19). The profiles of "*C. cinaedi*" and *C. pyloridis* were unique because of the absence or presence of only trace amounts of 3-OH-14:0 and 16:1  $\omega$ -7-cis. Both of these acids were detected in  $>1\%$  concentrations in nine *Campylobacter* species or subspecies examined in earlier studies (2, 3, 19). In addition, "*C. cinaedi*" contained 3-OH-12:0, and *C. pyloridis* contained 3-OH-18:0, two acids which have not been previously reported in *Campylobacter* species (2, 3, 6, 12, 19). With one exception, the relative amounts of these two acids and the other hydroxy acids (3-OH-14:0 and 3-OH-16:0) found in the *Campylobacter* species were increased 1.5 to 3 times by the acid hydrolysis step. This increase indicates that the acids were bound to the cell structure through an amide linkage, a characteristic

TABLE 1. Differentiation of *Campylobacter* species by cellular fatty acids

Species <sup>a</sup>	No. of strains <sup>b</sup>	GLC group	Amt <sup>c</sup> of indicated compound <sup>d</sup>														Ratio 16:0/16:1 <sup>e</sup>	
			12:0	14:1	14:0	16:1 ω7c	16:1 (x)	16:0	18:1 ω7c	18:0	19:0 cyc	3-OH-12:0	3-OH-14:0	3-OH-16:0	3-OH-18:0	16:0 Ald		16:0 Dma
<i>C. jejuni</i>	15	A	—	—	9	4	—	38	24	1	15	—	5	—	—	—	—	≥4
<i>C. coli</i>	15	A	—	—	7	4	—	42	29	1	10	—	5	—	—	—	—	≥4
<i>C. coli</i>	4	B <sup>f</sup>	—	—	7	5	—	38	42	2	—	—	4	—	—	—	—	≥4
<i>C. laridis</i>	15	B	—	—	4	6	—	36	45	1	—	—	6	—	—	—	—	≥4
<i>C. fetus</i> subsp. <i>fetus</i>	15	C <sup>g</sup>	—	—	8	19	—	33	29	1	—	—	3	4	—	—	—	≤2
" <i>C. cinaedi</i> " ATCC 35683 <sup>T</sup>	1	D	2	—	15	—	—	35	27	5	—	3	—	6	—	—	—	NA <sup>h</sup>
" <i>C. fennelliae</i> " ATCC 35684 <sup>T</sup>	1	E	1	—	7	—	—	32	37	4	—	—	3	4	—	3	6	NA
<i>C. cryaerophila</i> NCTC 11885 <sup>T</sup>	1	F	1	4	1	29	15	20	21	—	—	—	4	—	—	—	—	<1 <sup>i</sup>
<i>C. pyloridis</i>	3	G	—	—	31	—	—	4	11	12	23	—	—	3	8	1	—	NA

<sup>a</sup> ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.

<sup>b</sup> Includes the type strain of each species, except for *C. pyloridis*.

<sup>c</sup> Values are percentages of total fatty acids and are arithmetic means; —, not detected or less than 0.8%.

<sup>d</sup> Number before the colon = number of carbon atoms; number after the colon = number of double bonds; ω7c = double-bond position from hydrocarbon end of cis isomer; (x) = double-bond position unknown; cyc = cyclopropane fatty acid; 3-OH = hydroxy group at carbon 3; Ald = aldehyde; Dma = dimethylacetyl.

<sup>e</sup> Ratio of the relative percentages of 16:0 and 16:1 ω7c; calculated for all strains of each species tested.

<sup>f</sup> *C. hyointestinalis* and the catalase-negative or very weakly positive strains are also in GLC group B.

<sup>g</sup> *C. fetus* subsp. *venerealis*, *C. sputorum* subsp. *bubulus*, *C. sputorum* subsp. *sputorum*, and "*C. fecalis*" are also in GLC group C, but the 16:0/16:1 ratio is >2 for the *C. sputorum* subspecies and "*C. fecalis*" (19).

<sup>h</sup> NA, Not applicable.

<sup>i</sup> Ratio of 16:0 to both isomers of 16:1.

associated with most gram-negative bacteria (11, 13). In "*C. cinaedi*," the relative concentrations of 3-OH-12:0 and 3-OH:16:0 increased from 1 and 2% in the saponified FAME samples to 3 and 6%, respectively, when the acid-hydrolyzed portion was combined with the saponified samples and reanalyzed. In *C. pyloridis*, the relative concentration of 3-OH-18:0 increased from 5 to 8%; the relative concentration of 3-OH-16:0 did not increase. The profile shown in Fig. 1G for *C. pyloridis* is essentially identical to the one reported by Goodwin et al. (6) for this species, except that these workers did not report the presence of 3-OH-18:0. The use of a two-step hydrolysis procedure and analysis of the FAME on a capillary column instead of a packed column may account for the discrepancy between our results and those reported by Goodwin and co-workers (6). Although *C. pyloridis* contains 19:0 cyc, it can be differentiated from *C. jejuni* and most *C. coli* strains because it does not contain 16:1 ω7-cis or 3-OH-14:0 (Table 1). *C. pyloridis* can be differentiated from the other five *Campylobacter* species by the presence of 19:0 cyc and 3-OH-18:0 and its relatively low concentration (4%) of 16:0. This last acid is a major component (18 to 49%) in all other *Campylobacter* species (2, 3, 12, 19).

The two strains of "*C. fennelliae*" and the other strain (CLO-3) assigned to group E were different from all the other *Campylobacter* species examined because they contained two compounds which are associated with the plasmalogen moiety found in some anaerobic bacteria (8, 13). Additional studies are needed to determine the significance of the 16-carbon aldehyde and 16-carbon demethylacetyl (Table 1 and Fig. 1E) found in these *Campylobacter* strains. There were some quantitative differences in the relative amounts of several of the compounds found in the three group E strains, but their qualitative profiles were similar. One of the strains (CLO-3) is different genetically from "*C. fennelliae*" (24),

and this may account for the quantitative differences in their profiles. These initial findings will be confirmed when additional isolates of "*C. fennelliae*" and CLO-3 are available for testing.

The fatty acid profiles of *C. cryaerophila* and the other strains designated as group F were quite distinct because two isomers of 16:1 were present. The methyl esters of 16:1 ω7-cis and 16:1 ω5-cis do not separate on all fused-silica capillary columns, and the presence of this second isomer may not always be evident (10). In addition, the methyl esters of unsaturated straight-chain acids and branched-chain hydroxy acids may coelute (10), and hydrogenation (10, 18) and trifluoroacetylation (10, 18) experiments are needed to confirm which type of acid is present. Additional confirmation of the identity can be made by mass spectrometry (18).

These GLC results demonstrate the usefulness of cellular fatty acid analysis for the differentiation of suspected *Campylobacter* strains. In at least three instances, GLC analyses of CLO indicated that new or previously unrecognized species ("*C. cinaedi*," *C. cryaerophila*, and *C. hyointestinalis*) were being isolated from human infections. Since *Campylobacter* species are being detected with increasing frequency from clinical sources, more rapid methods for screening and characterizing these isolates are needed. GLC of cellular fatty acids provides a relatively fast and simple method, as cells from one culture plate are usually more than adequate for the analysis. In addition, automated GLC instrumentation allows analysis of large numbers of samples with minimal operator interaction. The fatty acid profiles can be used alone or in combination with conventional test results to provide a strong presumptive identification even with strains which have atypical phenotypic characteristics. Unidentified CLO with similar profiles can be grouped for further testing.

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