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Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. nov., Respectively

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Different types of studies have shown that *Campylobacter pylori* does not belong in the genus *Campylobacter*. Ribonucleic acid sequencing has indicated that *C. pylori* might belong in the genus *Wolinella*, but we describe five major groups of taxonomic features of the genus *Wolinella* that differ markedly from those of *C. pylori*, including ultrastructure and morphology, cellular fatty acids, menaquinones, growth characteristics, and enzyme capabilities, indicating that *C. pylori* should not be included in the genus *Wolinella*. Thus, we propose the establishment of a new genus, *Helicobacter*; *C. pylori* should be transferred to this genus as *Helicobacter pylori* comb. nov., and *H. pylori* NCTC 11637 (= ATCC 43504) is the type strain. The gastric spiral organism from ferrets has been elevated recently from *Campylobacter pylori* subsp. *mustelae* to *Campylobacter mustelae*. We describe the similarities and differences between *C. mustelae* and *C. pylori* compared with other campylobacters, and we propose that *C. mustelae* should be included in the new genus *Helicobacter* as *Helicobacter mustelae* comb. nov. (type strain, ATCC 43772).

In April 1982 in the Microbiology Department of Royal Perth Hospital, Perth, Western Australia, culture of endoscopic biopsy specimens of human gastric mucosa yielded a spiral organism with some features of the genus *Campylobacter*, which was named *Campylobacter pyloridis* (34). The specific epithet was grammatically incorrect, and the name was later corrected to *Campylobacter pylori* (33). However, in 1985 we reported that the cellular fatty acids and major ultrastructural features of *C. pylori* were very different from those of all other campylobacters, and this suggested that *C. pylori* did not belong in this genus (24). We also reported that methylated menaquinone 6 (MK-6), which is characteristic of all other campylobacters, is absent in *C. pylori* (22); in addition, the antibiotic susceptibilities of *C. pylori* are different from those of other campylobacters (20). *C. pylori* could not be included in the genus *Spirillum*, a genus which consists of rigid spiral organisms (24). Four studies of the 5S and 16S ribosomal ribonucleic acid sequences of *C. pylori* compared with those of other campylobacters indicated clearly that *C. pylori* should not be included in the genus *Campylobacter* (28, 38, 41, 44), but may be more closely related to *Wolinella succinogenes*. Thompson et al. (44) suggested that *C. pylori* should be placed in the genus *Wolinella*, but Romaniuk et al. (41) and Lau et al. (28) noted sufficient differences between *W. succinogenes* and *C. pylori* to justify separate genera. The Committee on Reconciliation of Approaches to Bacterial Systematics stated that "the depth in a ribonucleic acid dendrogram at which a given hierarchical line is to be drawn may vary along different major branches of the dendrogram and will be strongly influenced by phenotypic consistency at each level" (46). In this paper we describe marked phenotypic inconsistencies between *C. pylori* and *W. succinogenes* and delineate five major groups of taxonomic features of these bacteria (ultrastructural features, cellular fatty acid profiles, respiratory quinones, growth characteristics, and enzyme capabilities) which were also regarded as important by the Subcommittee for Taxonomy of Methanogenic Bacteria (3). Our data indicate that *C. pylori* should not be included in the genus *Wolinella*, but should be a member of a new genus.

Ribonucleic acid sequencing of "*Flexispira rappini*" has indicated a close relationship between this organisms and *C. pylori* (B. J. Paster, personal communication). "*F. rappini*" is not a spiral organism, but is urease positive. In this paper we describe the chemotaxonomic differences between *C. pylori* and "*F. rappini*," which confirm the original suggestion that "*F. rappini*" should not be in the same genus as *C. pylori* (J. H. Bryner, Campylobacter IV. Proc. 4th Int. Workshop Campylobacter Infect., p. 440–442, 1988).

From the stomachs of ferrets a spiral organism similar to *C. pylori* was isolated in Boston, Mass. (16). This organism was named *Campylobacter pylori* subsp. *mustelae*, because deoxyribonucleic acid (DNA)-DNA hybridization showed a relationship with *C. pylori* at the subspecies level (17). However, in Perth, our DNA-DNA hybridization studies yielded a much lower degree of homology, indicating a more distant relationship; this finding was subsequently confirmed by the original workers. Other important taxonomic features distinguish *C. pylori* and the ferret organism, and the latter organism has now been elevated to species status as *Campylobacter mustelae* (15).

C. pylori is an important human pathogen, causing type B gastritis of stomachs (13), and is probably a major predisposing cause of duodenal ulcers (19, 25). *C. pylori* gastritis is widespread in many countries in the world (31) and may be one of the most common chronic human infections. Thus, if *C. pylori* should not be in the genus *Campylobacter*, from the viewpoint of medicine there is great urgency in assigning a new genus name to this organism, so that the definitive name can become commonly used. This would also avoid the current confusion when reference is made to "campylobacter infections," which usually refer to diarrhoea due to

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Campylobacter jejuni and allied campylobacters and not to stomach symptoms due to *C. pylori*. Furthermore, similar spiral organisms have been found in the stomachs of the monkeys *Macaca mulatta* (36) and *Macaca nemestrina* (5), and in Perth we have recently isolated similar organisms from *Macaca fascicularis* and from *Papio* species (unpublished data). These bacteria are candidates for inclusion in the new genus, for which we propose the name *Helicobacter*. The name *Helicobacter* refers to the morphology of the organisms, which are helical in vivo but often rodlike in vitro; also, the name *Helicobacter* is euphonious and has the advantage that it is similar to the name *Campylobacter*.

In this paper we report the guanine-plus-cytosine (G+C) contents, DNA-DNA hybridization data, ultrastructural features, cellular fatty acid profiles, respiratory menaquinones, growth characteristics, and enzyme capabilities of members of the new genus *Helicobacter* and of the two species which we propose for it, *Helicobacter pylori* and *Helicobacter mustelae*.

MATERIALS AND METHODS

Bacterial strains and growth of stock cultures. *C. pylori* (designated *H. pylori* as a result of this study) NCTC 11637^T (T = type strain) was isolated in Perth, Australia, in 1982, where 69 other isolates were also obtained from human endoscopic specimens. Two isolates of *H. pylori* from the United Kingdom were supplied by F. J. Bolton, Public Health Laboratory, Preston, United Kingdom, and six isolates from the United States were provided by Donna Morgan, Norwich Eaton Pharmaceuticals, Norwich, N.Y. *C. mustelae* (designated *H. mustelae* as a result of this study) ATCC 43772^T was isolated from the stomach of a ferret and was supplied by James Fox, Boston, Mass. *H. mustelae* NCTC 12032 was isolated in the United Kingdom. Two strains of *H. mustelae* (strains FM1 and FM2) were supplied by Dennis Jones, Manchester, United Kingdom, and strain FPD1 was supplied by Diane Newell, Porton, United Kingdom. In Western Australia, we isolated *H. mustelae* FP1 from a ferret. Thus, six isolates of *H. mustelae* were studied. *C. jejuni* NCTC 11351 and *W. succinogenes* NCTC 11488^T were obtained from the National Collection of Type Cultures, London, United Kingdom. "*F. rappini*" 1893 was supplied by J. H. Bryner, Ames, Iowa.

The organisms were maintained on brain heart infusion agar (BHIA) (Oxoid Ltd., London, England) supplemented with 7% horse blood lysed by saponin and were incubated in vented jars containing a microaerophilic atmosphere (5% O_2, 7% CO_2, 8% H_2, 80% N_2) for 2 to 3 days (21), by which time all of the strains had grown. The strains were stored at −70°C in 1% peptone water containing 25% glycerol.

DNA base composition. DNAs were extracted by the Marmur method (32) with the addition of protease treatments after cell lysis by sodium dodecyl sulfate and again after ribonuclease treatment. The G+C contents were determined by the T_i method of Sly and co-workers (42), using a model 2600 microprocessor-controlled spectrophotometer fitted with a thermal programmer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

DNA hybridization. DNA hybridization was performed by two methods. The nylon filter blot method (method P) was used in Perth, Australia (15). Briefly, DNA was extracted and purified as described by Majewski and Goodwin (31); samples were denatured by boiling and blotted in triplicate onto a positively charged nylon membrane (Biotrace; Gelman Sciences, Inc., Ann Arbor, Mich.), and the membranes were baked at 80°C for 1 to 2 h to immobilize the DNA. Portions (50 ng) of DNAs from *H. pylori* NCTC 11637^T and *H. mustelae* ATCC 43772^T and NCTC 12032 were labeled with α-[^{32}P]cytidine triphosphate by random priming. Nylon membranes were sealed in plastic bags, and prehybridization and hybridization were performed at 65°C in a shaking water bath. The membranes were air dried and autoradiographed. Radioactivity was assayed by dividing the membranes into 1-cm^2 pieces, each containing one DNA blot. These pieces were placed into glass vials and dissolved by adding 1-ml portions of concentrated formic acid; 9 ml of scintillation fluid (Instagel; Packard Instrument Co., Inc., Downer's Grove, Ill.) was added to each vial, which was then counted with a liquid scintillation counter (model 1219; LKB Instruments, Inc., Broma, Sweden). A homologous control reaction (labeled and unlabeled DNAs from the same strain) was used to represent 100% relatedness.

In Queensland, Australia, the spectrophotometric renaturation rate method for DNA hybridization (method Q) was performed as described by De Ley and co-workers (11) and modified by Huss and co-workers (26). The temperatures of renaturation were calculated by using the method of Gillis and co-workers (18) to be 68°C for *H. mustelae* NCTC 12032 and 66°C for *H. pylori* NCTC 11637^T. The results of the spectrophotometric method have been reported to correlate well with the results of the membrane filter method at levels of relatedness above 30%, but are unreliable at levels of relatedness below 30% (26). Two hybridization methods were used to provide confirmation of our original disagreement with world experts on the species position of *H. mustelae*.

Ultrastructural studies. Cell morphology and ultrastructural features of *H. pylori*, *H. mustelae*, *W. succinogenes*, and *C. jejuni* were determined by examining shaken broth cultures. A 3-ml portion of brain heart infusion broth (Oxoid) supplemented with 10% horse serum and 0.25% yeast extract was dispensed into a 20-ml container, which was placed in a jar with a microaerophilic atmosphere. The jar was shaken overnight, which yielded a heavy growth of bacteria in the liquid medium after 18 h. The organisms were subcultured into fresh shaken fluid medium on two occasions, and the third culture was used to provide preparations for thin-section electron microscopy as described previously (1). The presence of an external glycocalyx in *H. pylori*, *H. mustelae*, *C. jejuni*, and *W. succinogenes* was investigated by using the tannic acid method in conjunction with glutaraldehyde-osmium fixation, which provides stabilization and contrast enhancement of polysaccharide-rich bacterial surface material (30). The gastric antral biopsy specimens or bacterial cultures were fixed in 2.5% cacodylate-buffered glutaraldehyde at room temperature for 2 to 24 h. The material was then washed in 0.16 M cacodylate buffer (pH 7.2) and incubated in a freshly made solution of tannic acid (laboratory no. T-0215; Sigma Chemical Co., St. Louis, Mo.) in the same buffer at pH 7.2 for 24 h at 4°C. Specimens were again washed in cacodylate buffer, and the bacterial suspensions were pelleted by centrifugation in 10% bovine serum albumin. Subsequent processing (in a Sakura model REM-208B EM Processor) included postfixation with 1% osmium tetroxide, dehydration, and infiltration with epoxy resin. Thin sections were mounted on naked copper grids and viewed, after staining with uranyl acetate and lead citrate, by using a Philips model EM 410(HM) electron microscope.

Cellular fatty acid analysis. The strains of bacteria were cultured for 2 to 4 days on BIHA supplemented with 7% saponin-lysed blood; however, only if a heavy, healthy

growth was obtained were the cells harvested and processed. Bacteria from old or poorly growing cultures tended to give variable results and were discarded. Fatty acid methyl esters were prepared and analyzed by using the method of Goodwin et al. (23). This included saponification by sodium hydroxide, methylation with sulfuric acid in methanol, extraction of the fatty acid methyl esters with hexane, and concentration under a stream of nitrogen. The samples were stored at −20°C until they were analyzed by gas chromatography with a model 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a flame ionization detector and a Hewlett-Packard model 3393A integrator. Separation was achieved on a Superox-20M capillary column (0.53 mm by 10 m) by using a column flow rate of 2.6 ml/min, with the injector temperature at 250°C (to ensure efficient vaporization), the detector temperature at 250°C, and the oven temperature at 60°C for 1 min and then increased by 5°C/min to 200°C, which was maintained for 10 min. Major peaks were identified by comparison with the retention times of known standards and by mass spectroscopy. The area under each peak was calculated with the integrator to determine the percentage of each fatty acid in the sample. Extractions from the various isolates were replicated three times to ensure that the results were reproducible. We used a long temperature program to ensure that all relevant cellular fatty acids were detected.

Analysis of respiratory quinones. The method used to analyze respiratory quinones was that described by Goodwin et al. (22).

Growth conditions and enzyme capabilities. Growth at various temperatures was tested on BHIA containing 7% horse blood, 1% IsoVitaleX (BBL Microbiology Systems Cockeysville, Md.), and 0.25% yeast extract, and the ability to grow in the presence of air enriched with 10% CO_2 was tested in a CO_2 incubator with 98% humidity. Growth was also tested on the same medium supplemented with a final concentration of 3.5% NaCl, 0.5 or 1% glycine, or 1% bile. In addition, growth was tested on the peptone-starch-dextrose (PSD) medium of Dunkelberg et al. (14) and on campylobacter blood-free selective agar base (2), which contains charcoal, casein, and 0.1% deoxycholate. Growth anaerobically at 37°C was tested in a jar that was evacuated and filled three times with a gas mixture containing 10% CO_2, 10% H_2, and 80% N_2 and also in an anaerobic chamber. Failure to grow after 4 days of incubation was considered a negative result.

Urease was tested in Christensen urea broth (29); hippurate hydrolysis was tested by the method of Lennette et al. (29); nitrate production was tested by the method of Cowan and Steel (10); and H_2S production was tested in triple sugar iron agar containing 0.25% yeast extract.

Enzyme capabilities were tested by the method of Mégraud et al. (35) in a microaerophilic atmosphere. A suspension equivalent to a McFarland standard of 7 to 8 was prepared in a saline solution, and 0.1 ml was used to inoculate each of the API ZYM microtubes (API System, La Balme les Grottes, France). Motility was determined by phase-contrast microscopy (40). To determine motility in broth, the organisms were shaken overnight in brain heart infusion broth in a microaerophilic atmosphere (1).

Antibiotic susceptibilities were determined by disk diffusion on BHIA containing 7% lysed horse blood, with incubation for 1 day for *C. jejuni*, *W. succinogenes*, and "*F. rappini*" and for 2 days for *H. pylori* and *H. mustelae*.

TABLE 1. Levels of DNA relatedness of *H. pylori* and *H. mustelae* strains

Test strain	% Relatedness to reference DNA from[a]:				
	H. mustelae ATCC 43772^T (method P)[b]	*H. mustelae* NCTC 12032		*H. pylori* NCTC 11637^T	
		Method P	Method Q	Method P	Method Q
H. mustelae ATCC 43772^T	100			7	30
H. mustelae NCTC 12032	75		100	1	44
H. mustelae FP1	96			8	
H. pylori NCTC 11637^T	2	<5	49	100	100

[a] Hybridization analyses were conducted at optimal renaturation temperatures, which were calculated by using the method of Gillis et al. (18) and were 68°C for strain NCTC 12032 and 66°C for strain NCTC 11637^T.
[b] See text for explanation of methods.

RESULTS

DNA base composition. The G+C content of *H. mustelae* ATCC 43772^T was 36.5 ± 0.8 mol%, the G+C content of strain NCTC 12032 was 40.6 ± 0.57 mol%, and the G+C content of isolate FP1 from Western Australia was 35.9 ± 0.46 mol%.

DNA hybridization. Table 1 shows the results determined by the filter blot and spectrophotometric hybridization methods, which indicate the levels of relatedness of the *H. mustelae* strains to one another and to *H. pylori*. In 65°C reactions in which the filter blot method was used, *H. mustelae* strains were 75 to 100% interrelated and exhibited 2 to 8% relatedness to *H. pylori*. When the spectrophotometric method at 66 or 68°C was used, *H. pylori* was 30 to 49% related to *H. mustelae*.

Ultrastructural features. The major ultrastructural features of *H. pylori* have been described previously (24). They are summarized in Table 2 together with the features of *H. mustelae* NCTC 12032, *W. succinogenes* NCTC 11488, and *C. jejuni* NCTC 11351. The flagella of *H. pylori* and *H. mustelae* are multiple and sheathed and have distinctive, terminal bulbs; the cell wall is smooth, and the ends are rounded. These features are different in *W. succinogenes* and *C. jejuni*. In pure cultures, as well as in parallel gastric biopsy specimens, *H. pylori* and *H. mustelae* regularly exhibit a prominent electron-dense glycocalyx or capsulelike

TABLE 2. Ultrastructural features of *H. pylori*, *H. mustelae*, *C. jejuni*, and *W. succinogenes*

Strain	Cell wall membrane	Flagella	Flagellar sheath	Flagellar bulb	Glycocalyx (in vitro in shaken broth)
C. jejuni NCTC 11351	Rugose	Single, bipolar	Absent	Absent	Scanty
H. pylori NCTC 11637^T	Smooth	Multiple, unipolar	Present	Present	Present
H. mustelae NCTC 12032	Smooth	Multiple, bipolar and lateral	Present	Present	Present
W. succinogenes NCTC 11488	Variable	Single, unipolar	Absent	Absent	Scanty

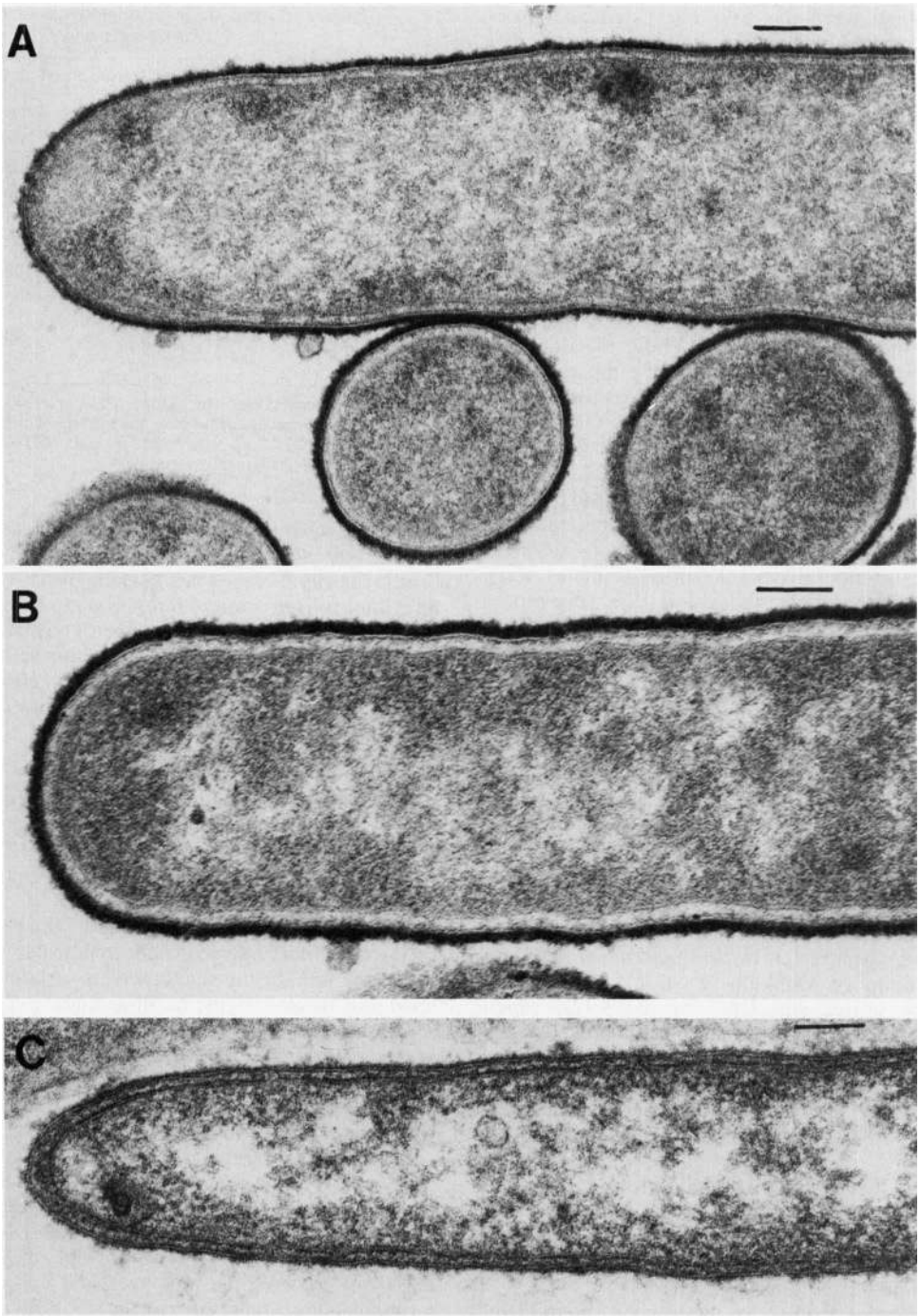

FIG. 1. Ultrastructural appearance of cultured cells of *H. pylori* NCTC 11637^T (A), *H. mustelae* FM2 (B), and *W. succinogenes* NCTC 11488 (C) after processing with tannic acid for stabilization of the external polysaccharidic glycocalyces. The electron-opaque surface layer is comparatively sparse in *W. succinogenes* NCTC 11488. Thin-section electron microscopy. Bars = 100 nm.

coat external to the cell wall unit membrane (Fig. 1A and B); this structure was quite inapparent in specimens processed conventionally for thin sectioning. The glycocalyx is up to 40 nm thick, has a radial periodicity of about 14 nm, and is generally thicker in vivo than in cultured organisms. By comparison, *W. succinogenes* (Fig. 1C) and *C. jejuni* have only a very scanty glycocalyx.

Cellular fatty acid analysis. The cellular fatty acid profiles of four isolates of *H. pylori* and six isolates of *H. mustelae* are shown in Table 3. The profile of *H. pylori* is characterized by a very high percentage of 14:0 acid (>35%), a low percentage of 16:0 acid (<8%), and the presence of 3-OH-18:0 acid, while *H. mustelae* has a moderate concentration of 14:0 acid (<18%), a very small amount of 15:0 acid (2 to 4%), and a large amount of 16:0 acid (>27%) but no 3-OH-18:0 acid. The profile of *W. succinogenes* indicates a very small amount of 14:0 acid (<5%), a moderate amount of 16:0 acid (17%), a very high percentage of 16:1 acid (33%),

TABLE 3. Cellular fatty acids of *H. pylori*, *H. mustelae*, and *W. succinogenes*

Strain[a]	% of[b]: 12:0 acid[c]	14:0 acid	3-OH 14:0 acid	15:0 acid	16:0 acid	16:1 acid	3-OH 16:0 acid	18:0 acid	18:1 acid	18:2 acid	3-OH 18:0 acid	19:0 cyc acid
H. pylori strains												
NCTC 11637^T	T	41	—	—	5	T	5	5	16	4	2	17
NCTC 11638	T	41	—	—	7	—	5	7	9	2	3	16
Q188	T	37	—	T	5	T	5	8	17	3	2	17
72R	T	36	—	—	6	T	7	11	17	2	3	17
H. mustelae strains												
NCTC 12032	T	15	—	2	32	—	2	T	23	7	—	15
ATCC 47332^T	—	13	—	2	30	—	2	T	17	5	—	16
Ferret MI	T	17	—	3	28	—	4	T	17	9	—	13
Ferret PD1	—	15	—	3	36	—	2	5	23	6	—	10
Ferret M2	T	14	—	4	29	—	2	3	14	7	—	8
Ferret P1	T	14	—	2	34	—	2	T	23	5	—	15
W. succinogenes NCTC 11488	5	3	T	T	17	33	T	T	28	9	—	—

[a] ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.
[b] Values are percentages of total fatty acids and are arithmetic means. —, Not detected or less than 0.5%; T, 0.5 to 1.9%.
[c] Number of carbon atoms: number of double bonds. cyc, Cyclopropane fatty acid; 3-OH, hydroxy group at carbon 3.

and no 3-OH-18:0 or 19:0 cyc acid. This profile is clearly different from the profiles of *H. pylori* and *H. mustelae*.

Respiratory quinones. *H. pylori* possesses only MK-6 and lacks the methylated MK-6 (22) which is found in all campylobacters (7) and also in *W. succinogenes* at a level of 20 to 50% (8). We found that *H. mustelae* NCTC 12032 possesses a small amount (5%) of methylated MK-6 and that "*F. rappini*" 1893 does not possess methylated MK-6.

Growth conditions and enzyme capabilities. In Table 4 the major biochemical characteristics of *H. pylori* and *H. mustelae* are compared with those of *C. jejuni*, *W. succinogenes*, and "*F. rappini*". *W. succinogenes* was catalase and urease negative, which differentiates this species from *H. pylori* and *H. mustelae*. *W. succinogenes* was a weak producer of H_2S in triple sugar iron agar, but *H. pylori* and *H. mustelae* were not. *W. succinogenes* lacked gamma-glutamyltranspeptidase

TABLE 4. Biochemical and cultural characteristics of *C. jejuni*, *H. pylori*, *H. mustelae*, *W. succinogenes*, and "*F. rappini*"

Characteristic	*C. jejuni* NCTC 11351	*H. pylori* NCTC 11637^T	*H. mustelae* NCTC 12032	*W. succinogenes* NCTC 11488	"*F. rappini*" 1893
Oxidase	+[a]	+	+	+	+
Catalase	+	+	+	−	+
Urease	−	+	+	−	+
Hippurate hydrolysis	+	−	−	−	−
Nitrate reduction (microaerophilic)	+	−	+	+	−
H_2S production in triple sugar iron agar	−	−	−	W	−
Gamma-glutamyltranspeptidase	−	+	+	−	+
Alkaline phosphatase	W	+	+	−	−
Motility in brain heart infusion broth	+	+	+	+	+
Motility from agar plate	+	−	−	−	−
Growth microaerophilically at:					
25°C	−(2)[b]	−(4)	−(4)	−(2)	−(2)
30°C	+(1)	+(2)	+(2)	−(2)	−(2)
37°C	+(1)	+(2)	+(2)	+(1)	+(1)
42°C	+(1)	−(4)	+(2)	W(2)	+(1)
Growth in CO_2 inhibitor	+(1)	+(2)	−(4)	−(2)	−(2)
Growth anaerobically at 37°C	+	−	W	+	+
Growth on blood agar containing 3.5% NaCl	−	−	−	−	−
Growth on 0.5% glycine	+	+	+	−	+
Growth on 1% glycine	+	−	+	−	W
Growth on 1% bile	+	−	−	+	+
Growth on PSD	+	+	−	+	+
Growth on charcoal–Casein–0.1% deoxycholate	+	−	−	+	−
Susceptibility to nalidixic acid (30-μg disk)	S	R	S	R	R
Susceptibility to cephalothin (30-μg disk)	R	S	R	R	R
Susceptibility to metronidazole (5-μg disk)	R	S	S	S	R

[a] +, Positive; −, negative; W, weak; S, susceptible; R, resistant.
[b] The numbers in parentheses are numbers of days.

and alkaline phosphatase, which were possessed by *H. pylori* and *H. mustelae*. *W. succinogenes* failed to grow at 30°C and on 0.5% glycine, whereas *H. pylori* and *H. mustelae* grew at 30°C and on 0.5% glycine. *W. succinogenes* grew on BHIA supplemented with 1% bile and on the campylobacter-selective medium (2) containing charcoal, casein, and 0.1% deoxycholate; *H. pylori* NCTC 11637^T and *H. mustelae* NCTC 12032 failed to grow on these media. We found that *W. succinogenes* was oxidase positive, although in the original description this species was defined as oxidase negative (43).

The number of strains tested to determine the biochemical features of *H. pylori* (Table 4) varied between 25 and 78 strains, and in each test all of the isolates gave the same reaction. We have reported previously (31) that when four strains were repeatedly subcultured in semisolid agar over many months and years, they lost their urease activity and that two isolates lost their catalase activity. We found that these urease-negative strains (including strain Q188 [Table 3]) were able to grow on 1% bile. The six strains of *H. mustelae* were tested for each of the biochemical differences. All of the isolates gave the same reaction with the following exceptions: only one strain showed growth in air enriched with CO_2, and five strains grew on 1% bile; strain NCTC 12032 failed to grow on bile (Table 4). None of the strains grew on PSD agar, in contrast to *H. pylori*. One strain of *H. mustelae* lost its urease activity (strain FM1), and three other strains showed much weaker urease activity after relatively few subcultures. *H. mustelae* grew only weakly in an anaerobic jar, but grew well in the anaerobic chamber, which could not be guaranteed to be oxygen free.

DISCUSSION

Recently, Fox and co-workers (17) described the ferret gastric organism as a new subspecies, *C. pylori* subsp. *mustelae*. However, in Western Australia we found that the level of DNA-DNA hybridization between *H. pylori* and the ferret organism was less than 15% at 65°C. We exchanged strains with D. J. Brenner at the Centers for Disease Control, Atlanta, Ga., who subsequently agreed with our findings, and the ferret organism has been elevated to species status as *C. mustelae* (15). Other major taxonomic features of the ferret organism are consistent with its designation as a species separate from *H. pylori*. Ultrastructurally, *H. mustelae* is shorter than *H. pylori* and has both bipolar and lateral flagella (Table 2); *H. pylori* has only unipolar flagella. The cellular fatty acid profile of *H. mustelae* contains a lower percentage of 14:0 acid and a greater percentage of 16:0 acid than the profile of *H. pylori* (Table 3). The latter species contains 3-OH-18:0 acid, as originally reported by Lambert and co-workers (27). Differences in growth characteristics and enzyme capabilities between *H. pylori* and *H. mustelae* (Table 4) include nitrate reduction by *H. mustelae* and not by *H. pylori*. *H. pylori* grows on PSD agar and in air enriched with CO_2, but not anaerobically and variably at 42°C. *H. mustelae* does not grow on PSD agar or in air enriched with CO_2, but grows well at 42°C and weakly in an anaerobic atmosphere. *H. pylori* is susceptible to cephalothin, but most strains are resistant to nalidixic acid, while *H. mustelae* is susceptible to nalidixic acid and resistant to cephalothin. Tompkins and co-workers (45) reported that *H. mustelae* fails to grow anaerobically, but all six of our strains grew anaerobically.

The phylogenetic position of *H. pylori*, as determined by 16S ribosomal ribonucleic acid sequencing, clearly indicates that this species should be excluded from the genus *Campylobacter* (28, 38, 41, 44). Ribosomal ribonucleic acid sequencing has also indicated that *H. pylori* is closely related to *W. succinogenes*; Thompson et al. have suggested that *H. pylori* should be transferred to the genus *Wolinella* (44). However, Romaniuk et al. (41) and Lau et al. (28) noted significant differences between *W. succinogenes* and *H. pylori* and concluded that these organisms should be in separate genera. Computerized ribonucleic acid data can be handled in at least two different ways; one yields a simple dendrogram, such as the dendrogram of Thompson et al. (44), but a more sophisticated "treeing" calculation might indicate that *H. pylori* is not closely related to *W. succinogenes*. Owen (37) identified 14 phenotypic differences between *H. pylori* and *W. succinogenes* and argued cogently that these species should be in separate genera. In this study we delineated five major groups of taxonomic features which clearly differentiate *H. pylori* and *H. mustelae* from *W. succinogenes* and *C. jejuni*. These features were also considered important by the Subcommittee for Taxonomy of Methanogenic Bacteria (3); they are ultrastructural features, cellular fatty acid profiles, respiratory quinones, growth characteristics, and enzyme capabilities. We suggest that a premature decision to assign *H. pylori* to the genus *Wolinella* would result in that genus being the home of a very diverse group of bacteria. The removing of diversity from one genus by introducing it into another would not be the solution to the taxonomic problems of *H. pylori* and *H. mustelae*; thus, the concept of a new genus seems to be the most reasonable strategy at this time (J. L. Penner, personal communication). Reclassification now seems fully justified and necessary.

Brenner (4) has stated that "phenotype should take precedence over DNA relatedness at the genus level. Even if one can arrive at a genetic definition for genus (I doubt that one can, since genus is a synthetic ranking that was designed for convenience), the primary consideration for a genus is that it contain biochemically similar species, that are convenient or important to consider as a group, and that must be separated from one another at the bench." There are many differences in biochemical features and growth characteristics between *H. pylori* and *W. succinogenes* (Table 4), which indicate that these species should not be in the same genus. *W. succinogenes* is catalase negative and urease negative, whereas *H. pylori* and *H. mustelae* are catalase positive and urease positive. *W. succinogenes* does not possess gamma-glutamyltranspeptidase or alkaline phosphatase, which are possessed by *H. pylori* and *H. mustelae*. *W. succinogenes* does not grow at 30°C or on 0.5% glycine, but grows on charcoal–casein–0.1% deoxycholate; *H. pylori* and *H. mustelae* have exactly the opposite characteristics.

Paster and Dewhirst (38) have argued that differences in DNA G+C contents should not be a primary consideration for excluding species from the same genus, and we agree. These authors emphasized the importance of methylated MK-6 to campylobacters and stated that it is a valuable chemotaxonomic marker; *W. succinogenes* contains this menaquinone, while *H. pylori* does not.

We conclude that these phenotypic differences outweigh at the genus level the genomic relatedness suggested by 16S ribosomal ribonucleic acid sequencing and justify the transfer of *H. pylori* and *H. mustelae* to a new genus.

"*F. rappini*" is another organism which is urease positive and possibly genomically closely related to *H. pylori*. However, it is a straight organism, not a spiral organism, with

marked periplasmic fibers, and a G+C content of 33 mol%, and it grows at 43°C (Bryner, *Campylobacter* IV. Proc. 4th Int. Workshop Campylobacter Infect.). These characteristics are quite different than those of *H. pylori* and *H. mustelae*. Table 4 shows the biochemical differences between "*F. rappini*" and *H. pylori* and *H. mustelae*. "*F. rappini*" does not possess alkaline phosphatase or grow at 30°C, whereas *H. pylori* and *H. mustelae* possess this enzyme and grow at 30°C. "*F. rappini*" is resistant to metronidazole (5 μg), whereas the other two organisms are susceptible.

In conclusion, the low degree of relatedness between *H. pylori* and any previously described genus except the genus *Wolinella*, together with the possession of a set of chemotaxonomic properties different from those of the genus *Wolinella* and neighboring taxa, provides a strong case for the designation of this organism as the type species of a new genus, which we name *Helicobacter*, with *Helicobacter pylori* comb. nov. as the type species. Spiral organisms identified as *H. pylori* have been isolated from the stomachs of rhesus monkeys, *M. mulatta* (36), and *M. nemestrina* (5). We have determined that the DNA base composition of the organism from *M. nemestrina* is 44 mol% G+C, and therefore we include this value in the G+C range for the genus *Helicobacter*. The significance of bacterial polysaccharidic glycocalyces in microbial-host interactions, whether pathogenic or autocthonous, has been comprehensively reviewed previously (9). The presence of a glycocalyx is revealed ultrastructurally after stabilization with either ruthenium red (9) or tannic acid (30), but the latter method gives higher contrast enhancement. The thick glycocalyces of *H. pylori* and of *H. mustelae* are important structural features of the organisms, although their chemical compositions have yet to be determined precisely. Organisms grown on solid media rarely produce a glycocalyx.

Description of *Helicobacter* Goodwin et al. gen. nov. *Helicobacter.* (He. li. co. bac' ter; Gr. n. *helix*, a spiral; N. L. masc. n. *bacter*, a staff; N. L. masc. n. *Helicobacter*, a spiral rod). Helical, curved, or straight unbranched cells, 2.5 to 5 μm long and 0.5 to 1.0 μm wide, having rounded ends and spiral periodicity (23). External glycocalyx produced in vitro in shaken broth cultures. Gram negative, microaerophilic. Rapid, darting motility by means of multiple sheathed flagella that are unipolar or bipolar and lateral, with terminal bulbs. Endospores are not produced. Slow growth in brain heart infusion broth and other liquid media unless shaken; growth in 2 to 5 days on brain heart infusion blood agar and chocolate agar. Growth also occurs on BHIA supplemented with charcoal or corn starch (6).

Colonies are nonpigmented, translucent, and 1 to 2 mm in diameter (5, 6, 34, 36). Optimal growth at 37°C; growth at 30°C but not at 25°C; variable growth at 42°C. Variable growth in air enriched with 10% CO_2 and anaerobically. No growth in the presence of 3.5% NaCl. Growth in the presence of 0.5% glycine and 0.04% triphenyltetrazolium chloride. Catalase and oxidase positive. Urea is rapidly hydrolyzed. The major isoprenoid quinone is MK-6; variable possession of methylated MK-6. H_2S production is negative on triple sugar iron agar and variable on lead acetate paper. Variable reactions for nitrate reduction and hippurate hydrolysis. Exhibits alkaline phosphatase and gamma-glutamyltranspeptidase activities; variable leucine arylamidase activity (35). Susceptible to penicillin, ampicillin, amoxicillin, erythromycin, gentamicin, kanamycin, penicillin, rifampin, and tetracycline (20). Resistant to vancomycin, sulfonamides, and trimethoprim. Variable resistance to nalidixic acid, cephalothin, metronidazole, and polymyxin (12). G+C content, 35 to 44 mol% (melting temperature method).

Isolated from the gastric mucosa of primates and ferrets, and some organisms in the genus may be associated with gastritis and peptic ulceration. The type species is *Helicobacter pylori* (Marshall et al., 1984) comb. nov.

Description of *Helicobacter pylori* comb. nov. *Helicobacter pylori* (basionym, *Campylobacter pylori* Marshall and Goodwin, 1987) (py. lo' ri. Gr. masc. n. *pyloros*, gate keeper; L. masc. n. *pylorus*, lower part of the stomach; L. gen. n. *pylori*, of the pylorus). Four to six unipolar flagella. In addition to the characteristics described above, *H. pylori* has a G+C content of 36 to 38 mol% (34). Growth in the presence of air enriched with 10% CO_2 and on PSD agar. No growth anaerobically at 37°C or on 1% glycine. The cellular fatty acid profile includes 3-OH 18:0 acid, more than 35% 14:0 acid, less than 10% 16:0 acid, and more than 4% 3-OH 16:0 acid (27). Does not reduce nitrates. Susceptible to cephalothin (30 μg). Isolated from the gastric mucosa of primates. Found in human cases of gastritis and gastric and duodenal ulcers. Causative for type B gastritis; the role of this organism in duodenal ulceration is probably as a predisposing cause, not a precipitating cause (19, 25). The type strain is Royal Perth Hospital isolate 13487 (= NCTC 11637 = ATCC 43504), which has a G+C content of 37.1 mol% (34).

Description of *Helicobacter mustelae* comb. nov. *Helicobacter mustelae* (basionym, *Campylobacter mustelae* Fox et al., 1989) (mus. tel' ae. L. gen. n. *mustelae*, of a ferret). Lateral and bipolar flagella. In addition to the genus characteristics described above, the G+C content is 35 to 41 mol%. The cellular fatty acid profile includes less than 18% 14:0 acid, more than 25% 16:0 acid, and no 3-OH 18:0 acid. Grows at 42°C microaerophilically and anaerobically with CO_2 at 37°C. No growth in air enriched with 10% CO_2 or on PSD agar. Variable growth on 1% glycine (17). Reduces nitrates. Susceptible to nalidixic acid (30 μg) but resistant to cephalothin (30 μg). Does not exhibit leucine arylamidase activity (45). Isolated from the gastric mucosa of ferrets. Causes gastritis and peptic ulceration in adult animals. The type strain is strain R85-13-6 (= ATCC 43772) (15), which has a G+C content of 35.5 ± 0.8 mol%.

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