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Lipopolysaccharide Characteristics of Pathogenic Campylobacters

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Most *Campylobacter jejuni* strains are sensitive and most *Campylobacter fetus* strains are resistant to the bactericidal activity in normal human serum. We purified lipopolysaccharides from *Campylobacter* strains to determine whether their composition and structure relate to serum susceptibility. The lipopolysaccharide of two serum-sensitive strains was best isolated by the Galanos procedure, but for two serum-resistant strains a cold-ethanol extraction was optimal. For each lipopolysaccharide preparation, the ratio of 2-keto-3-deoxyoctonate to protein was increased by 100 to 1,000-fold over that of whole cells. For serum-resistant strains, total carbohydrates were a high proportion of lipopolysaccharide weight; for serum-sensitive strains, 2-keto-3-deoxyoctanate was a high proportion of total carbohydrates. By polyacrylamide gel electrophoresis, the lipopolysaccharide of serum-sensitive strains appeared rough, but for serum-resistant strains a smooth-type ladder was seen, with a minimal core region and several high-molecular-weight complexes. Proteinase K-treated whole-cell lysates showed polyacrylamide gel electrophoresis profiles similar to that of pure lipopolysaccharide. Proteinase K-treated whole-cell lysates from seven serum-sensitive *C. jejuni* strains all had rough profiles, and five serum-resistant *C. fetus* strains all had smooth profiles. These studies indicate that lipopolysaccharide composition may be an important determinant of serum susceptibility among *Campylobacter* species and that serum resistance is usually associated with a smooth-type lipopolysaccharide.

Campylobacter jejuni is now recognized as one of the most common bacterial causes of acute gastroenteritis (3). *Campylobacter fetus* subsp. *fetus*, an uncommon pathogen, usually causes systemic human illnesses such as endocarditis, meningitis, arthritis, septic abortions, and abscesses (27). In contrast to *C. jejuni*, *C. fetus* usually causes disease in debilitated and immunosuppressed patients (15). Despite intensive recent inquiry, major questions concerning the pathogenic mechanisms involved in *Campylobacter* infections remain unanswered (3, 4). There are suggestions that endotoxin (7), enterotoxins (30), and invasive properties (22) may represent causative factors in the variable severity of the illnesses produced by these infections. We have recently shown that *C. fetus* isolates are resistant to the bactericidal activity of normal human serum, whereas *C. jejuni* isolates are sensitive (M. J. Blaser, P. F. Smith, and P. A. Kohler, J. Infect. Dis., in press); however, the molecular basis for this susceptibility is not known.

An important outer membrane constituent of gram-negative organisms is lipopolysaccharide (LPS), the endotoxic moiety (26, 33, 35). The chemical composition, immunochemistry, and role in serum resistance of the LPS of *Enterobacteriaceae* have been well characterized (14, 35). In general, organisms with smooth-type LPS are serum resistant, whereas those with rough-type LPS are serum sensitive (35). Biological (7) and chemical (21) studies have indicated that LPSs are present in strains of *C. jejuni*, but little is known of their characteristics. Such information might be used to determine their roles in virulence of *Campylobacter* infections and may form a sound basis for a serological typing system or for development of vaccines. For these reasons we studied the LPS of *Campylobacter* strains. We sought to determine whether different chemotypes of LPS are present in *C. fetus* and *C. jejuni* strains and whether these may have an impact on susceptibility of these strains to normal human serum.

MATERIALS AND METHODS

Bacterial strains. The *Campylobacter* strains used in this study were either obtained from the culture collection of the Denver Veterans Administration Medical Center *Campylobacter* laboratory or were the prototype strains used in the serotyping system of Penner and Hennessy on the basis of heat-stable hemagglutinating antigens (25). All *Campylobacter* strains used in this study (see Table 1) had been identified to species level by standard criteria (11, 15). Most strains had been passaged at least 10 times on sheep blood agar plates (PASCO, Wheat Ridge, Colo.). All strains were maintained frozen at −70°C in brucella broth containing 15% glycerol. Working stocks of strains were obtained by culturing the freezer stock on sheep blood agar plates, followed by transfer to the same media. Colonies were selected after examination in reflected light. Cultures were grown statically at 37°C for *C. fetus* or 42°C for *C. jejuni* and incubated in an atmosphere of 5% oxygen–10% carbon dioxide–85% nitrogen (15) for 48 h. Cells from plates were harvested in sterile distilled water and centrifuged twice at 3,000 × *g* for 20 min at 25°C, and the pellet was frozen at −20°C until the extractions were made. For comparison, we used two strains of *Salmonella minnesota* (*S. minnesota* WT218 and *S. minnesota* Re595) which were grown on the same media at 37°C for 24 h under aerobic conditions.

Serum susceptibility. The susceptibility of the test *Campylobacter* strains to the bactericidal activity present in normal human serum was assessed in a standardized assay as previously described (Blaser et al., in press). Pooled serum from healthy adults, frozen at −70°C and thawed immediately before use, was used in all assays. In brief, 24-h cultures of test strains were harvested and added to 10% serum in Hanks balanced salts solution with medium 199 (Flow Laboratories, Inc., McLean, Va.) for final concentra-

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TABLE 1. *Campylobacter* strains used in LPS studies

Species and strain designation	Source (site)	Log_{10} killing in standardized assay
C. jejuni		
VA 81-93 (PEN1)	Human (feces)	2.61
VA 81-94 (PEN2)	Human (feces)	1.62
VA 81-95 (PEN3)	Human (feces)	0.69
VA 83-85	Human (feces)	3.19
VA 83-86	Human (feces)	3.78
VA 83-100	Human (feces)	ND[a]
VA 84-19	Human (CSF[b])	<0.05
VA Moore	Human (feces)	0.54
VA Mosley	Human (feces)	0.47
C. fetus subsp. *venerealis*		
VA 80-81	Bull (prepuce)	<0.05
VA 81-169 (ATCC 19438)	Sheep fetus (brain)	3.69
C. fetus subsp. *fetus*		
VA 80-109	Human (blood)	<0.05
VA 81-170 (ATCC 27374)	Heifer (vaginal mucus)	2.5
VA 81-173	Human (blood)	<0.05
VA 81-200	Human (feces)	<0.05
VA 82-40	Human (blood)	<0.05
VA 83-97	Human (feces)	<0.05

[a] ND, Not determined.
[b] CSF, Cerebrospinal fluid.

tions of 10^3 to 10^5 cells per ml on a microtiter plate. After incubation at 37°C for 60 min, 50-μl portions were plated in triplicate, and colonies were counted after 48 h of incubation. Differences in pre- and postincubation counts were transformed into log_{10} kill determinations (see Table 1). Serum sensitivity was defined as greater than a 1.0-log_{10} kill, serum resistance was defined as less than a 0.1-log_{10} kill, and relative sensitivity was intermediate.

LPS preparation. The LPS from *S. minnesota* WT218 was prepared by the hot phenol-water method of Westphal and Jann (38) (Westphal method), and subsequent purification steps were performed essentially as described by Hanson and Phillips (9). The LPS from *S. minnesota* Re595 was prepared by the method of Galanos et al. (8) (Galanos method). For each of four representative *Campylobacter* strains, we attempted to extract the LPS by both the Westphal and Galanos methods. Because of difficulties encountered with the Galanos method for *Campylobacter* LPS, we used the following modifications. In brief, the pellet of cells was washed with 95% ethanol–acetone and twice with anhydrous ether, and the cells were dried in vacuo. Five milliliters of the 90% phenol–chloroform–petroleum ether extraction mixture was added to the dried bacteria, the mixture was vortexed for 2 min and centrifuged at 3,000 × *g* for 15 min, and the supernatant was decanted into a glass tube. After two reextractions, the chloroform and petroleum ether were removed by bubbling nitrogen, the phenol crystals were removed, and the LPS was precipitated and washed as described previously (8).

Because of low yields with both the Westphal and Galanos methods, the LPS from some *C. fetus* strains were prepared by the cold-ethanol extraction procedure of Darveau and Hancock (5), with the following modifications. In brief, the bacterial suspension was sonicated (Branson Instruments, Danbury, Conn.) for five 30-s bursts at a probe intensity of 7.5, instead of the cells being ruptured with a French press; DNase and RNase were added only once, since no residual DNA or RNA was found; and the final LPS pellet was subjected to a hot phenol-water extraction (38) to remove contaminating protein. The water phase was dialyzed for 24 h against distilled water, and the dialysate was lyophilized.

Analytical methods. Protein concentrations were measured by the modification of Markwell et al. (17) of the method of Lowry et al. For determination of the 2-keto-3-deoxyoctonate (KDO) concentrations of fractions, the thiobarbituric acid method (37) with the modifications described by Keleti and Lederer (16) was used with 3-deoxyoctulosonic acid-ammonium salt (Sigma Chemical Co., St. Louis, Mo.) as the standard. This assay measured KDO concentrations over a range of 0.125 to 10 μg/100 μl. Nucleic acid concentrations in the preparations were ascertained by UV absorbance at 200 to 300 nm with *Escherichia coli* DNA (type VIII; Sigma), and yeast RNA (type XI; Sigma) as standards; by this method, the lower limits of detection for DNA and RNA were 1.0 and 2.5 μg/ml, respectively. Carbohydrate concentrations were determined by the phenol-sulfuric acid procedure with glucose as the standard (9). The *Limulus* amoebocyte lysate assay was used for detection of the endotoxic activity (20) of *Campylobacter* LPS in comparison with LPS from *E. coli* O111 (List Biological Laboratories, Campbell, Calif.) and U.S. standard endotoxin (Associates of Cape Cod, Inc., Woods Hale, Mass.).

PAGE of LPS. LPS preparations and *S. minnesota* LPS standards (List Biological Laboratories) were analyzed by polyacrylamide gel electrophoresis (PAGE) in a modified Laemmli gel system (1) as previously described (2). Discontinuous PAGE was done in 1.5-mm-thick slab gels with a 4.5% stacking gel and separating gels ranging from 10 to 20% acrylamide. The LPS preparations were suspended in sample buffer containing sodium dodecyl sulfate (5%), bromophenol blue (0.003%), glycerol (20%), dithiothreitol (0.5%), and Tris base (1.57%) at pH 6.8 and then boiled at 100°C for 3 min. Samples with fractions calculated to have 30 to 60 ng of KDO were applied to each gel lane; in some gels, each lane was loaded with 1 to 3 μg of LPS (dry weight). Electrophoresis was carried out with a constant current of 35 mA for about 2 h, and the temperature was maintained at 8°C by circulating cold water. After electrophoresis, gels were fixed and LPS was resolved by the silver stain of Tsai et al. (36) with the modifications of Hitchcock (13) and Hitchcock and Brown (14).

PAGE of whole cells. We also used the method of Hitchcock and Brown (14) with minor modifications to detect LPS in whole cells. In brief, the organisms were suspended in cold Dulbecco phosphate-buffered saline (pH 7.2) to 32% of transmittance at 450 nm (Coleman Junior; Coleman Inc., Maywood, Ill.), and 1.5 ml of this suspension was centrifuged at 12,000 × *g* for 275 s. The pellets were solubilized in 200 μl of lysing buffer containing 2% sodium dodecyl sulfate, 4% 2-mercaptoethanol, 10% glycerol, 1 M Tris (pH 6.8), and 0.0027% bromophenol blue, and lysates were then heated at 100°C for 10 min. Protein digestion was done with 60 μg of proteinase K (Boehringer Mannheim Inc., Indianapolis, Ind.) solubilized in 25 μl of lysing buffer and incubated at 60°C for 60 min. Before the preparations were loaded into the well for electrophoresis, all samples were boiled for 5 min. The conditions for electrophoresis and staining were the same as those described above. Because profiles were identical regardless of whether 2% sodium dodecyl sulfate was incorporated into the stacking and separating gels, we routinely ran gels without sodium dodecyl sulfate (14).

TABLE 2. Comparative analysis of LPSs extracted from *Campylobacter* and *Salmonella* cells[a]

Bacterial cells	Extraction procedure	% of cells (dry wt)		Ratio A (μg of KDO/μg of protein)[b]	Yield of LPS	% of LPS by wt			Ratio B (μg of KDO/μg of protein)[b]	Ratio B/ ratio A	KDO/ CHO
		KDO	Protein			KDO	Protein	CHO[c]			
C. jejuni PEN1	Galanos	ND[d]	ND	0.0062	1.3	5.7	1.5	25	3.80	612.9	0.23
	Westphal	0.16	21.6	0.0074	8.3	3.1	2.7	10.66	1.15	155.4	0.30
C. fetus											
81-170	Galanos	ND	ND	0.0036	0.9	0.37	0.5	2.33	0.74	205.6	0.16
	Westphal	ND	ND	0.0032	5.1	0.27	0.8	1.66	0.34	106.3	0.16
82-40	Cold ethanol[e]	0.098	25.8	0.0038	4.2	3.2	2.0	32.0	1.6	421.1	0.10
80-109	Cold ethanol[e]	0.098	27.4	0.0036	3.0	4.3	1.4	56.4	3.07	852.8	0.08
S. minnesota											
Re595	Galanos	ND	ND	0.0065	2.4	20.2	5.0	20.5	4.04	621.5	0.99
WT218	Westphal	0.100	15.84	0.0063	4.0	2.6	2.0	13	1.3	206.3	0.20

[a] All values shown are the means of three separate determinations.
[b] Ratios A and B were calculated as the proportions of KDO to protein in whole cells (dry weight) (ratio A) and in purified LPS (ratio B).
[c] CHO, Total carbohydrate.
[d] ND, Not determined.
[e] Modification of the procedure of Darveau and Hancock (5); see text for methods.

RESULTS

Yields of LPS. We attempted to purify LPS from four *Campylobacter* strains and from the two *Salmonella* strains for purposes of comparison. Two of the *Campylobacter* strains were serum sensitive (*C. jejuni* PEN1 and *C. fetus* 81-170) and two were serum resistant (*C. fetus* 82-40 and *C. fetus* 80-109) (Table 1). Initially we used both the Galanos and Westphal extraction methods for all strains but found that, for the two serum-resistant *C. fetus* strains, the Galanos method yielded no detectable LPS. The yield from the Westphal method was minimal for these two strains regardless of whether cells were pretreated with pronase. Therefore, we used a modification of the cold-ethanol extraction procedure of Darveau and Hancock (5), which was more successful. However, when we used only the procedure suggested by Darveau and Hancock, protein represented more than 20% of the final product, even though we used the heating step described (5). For that reason we added a hot-phenol extraction to the procedure to completely remove contaminating proteins. With the final extraction methods employed (see Table 2), LPS yields ranged from 0.9 to 8.3%. For the two serum-sensitive strains, the yields obtained by the Westphal method were greater than those obtained by the Galanos method; however, use of the Galanos method resulted in greater purity (see ratio B, Table 2). Yields of LPS from the two serum-resistant *C. fetus* strains by the cold-extraction method were intermediate, and yields for the *Salmonella* strains were similar to those previously reported (5, 8).

Chemical composition of LPS preparations. The chemical composition of the LPS preparations obtained is shown in Table 2. For preparations from each strain with purified LPS concentrations ranging from 1.0 to 3.0 mg/ml, DNA and RNA were undetectable. Protein contamination of each preparation ranged from 0.5 to 2.7% (wt/wt). For each of the extraction methods finally used (Table 2), we found a ratio of KDO to protein that was increased by 106- to 852-fold over that of whole cells. The proportion of LPS represented by the carbohydrate fraction varied from 1.6 to 56.4%, but the greatest carbohydrate proportions were in the serum-resistant strains, as compared with the serum-sensitive strains. Conversely, the proportion of the total carbohydrate represented by KDO was higher in the serum-sensitive strains (16.0 to 29.5%) than in the serum-resistant strains (7.7 to 10.0%). For *C. fetus* strain 81-170, the cold-ethanol extraction gave a yield of LPS that was 10-fold higher, with 35% carbohydrate and 2.05% KDO, suggesting that neither the classical Westphal (38) nor the Galanos (8) method was optimal for this strain.

***Limulus* gelation.** When U.S. standard endotoxin was used, the lowest dilution that gelled the lysate was 50 pg

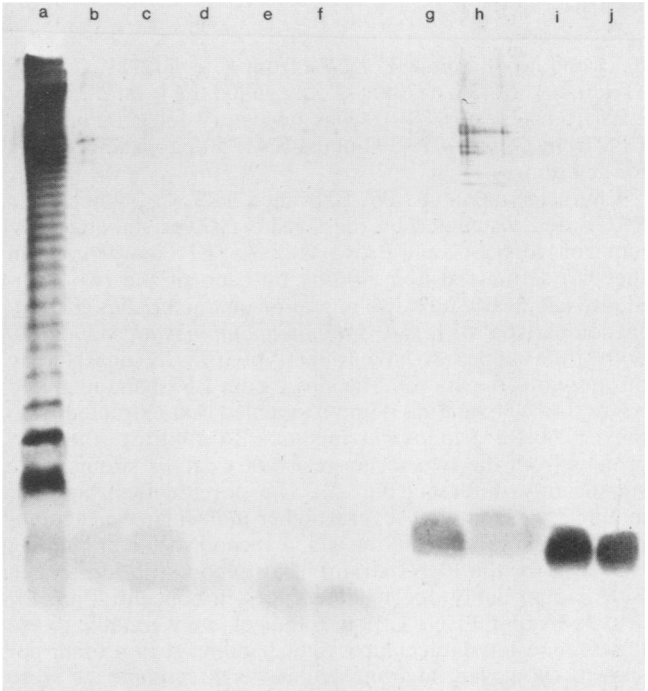

FIG. 1. Silver stain of 15% polyacrylamide gel with purified LPS. Lanes a through f, LPS from *S. minnesota* WT218, Ra, Rb, Rc, Rd, and Re, respectively; lanes g and h, LPS from *C. fetus* 81-170 from the Westphal and Galanos extractions, respectively; lanes i and j, LPS from *C. jejuni* PEN1 from the Westphal and Galanos extractions, respectively.

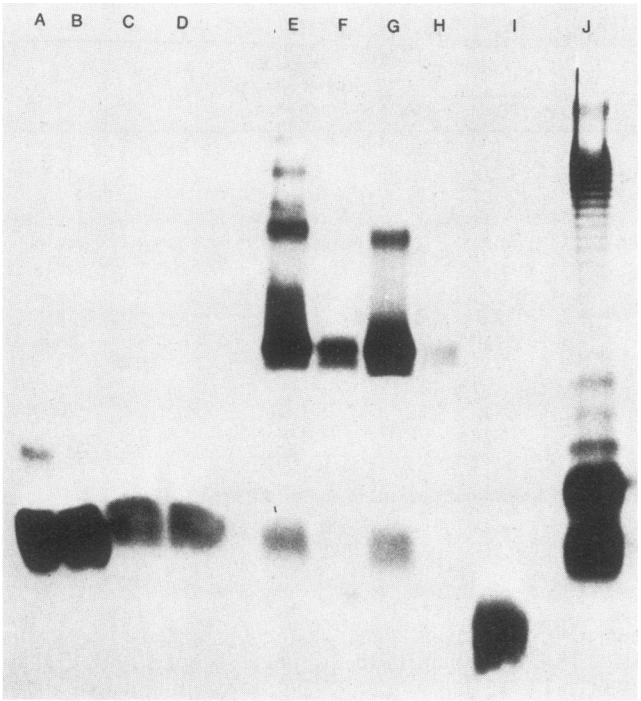

FIG. 2. Silver stain of 15% polyacrylamide gel with purified LPS. Lanes A and B, *C. jejuni* PEN1 from the Galanos and Westphal extractions, respectively; lane C and D, *C. fetus* 81-170 from the Galanos and Westphal extractions, respectively; lanes E and F, *C. fetus* 82-40 from the cold-ethanol and Westphal extractions, respectively; lanes G and H, *C. fetus* 80-109 from the cold-ethanol and Westphal extractions, respectively; lane I, *S. minnesota* Re595 from the Galanos extraction; lane J, *S. minnesota* WT218 from the Westphal extraction.

(0.25 endotoxin units). For LPS from *E. coli* O111, *C. fetus* 81-170, and *C. fetus* 82-40, 12.5 pg gelled the lysate; 25 pg of LPS from *C. fetus* 80-109 was necessary for gelation. For LPS from *C. jejuni* 79-193 and PEN1, 50 pg was necessary for gelation.

Characterization of LPS. Extracted LPS was subjected to PAGE and visualized by the sensitive silver staining technique of Hitchcock and Brown (14). The LPS extracted from the two serum-sensitive strains by each of the two techniques had nearly identical migration characteristics (Fig. 1). In comparison with the *Salmonella minnesota* standards, both strains appear to have rough-type LPS, as suggested by our previous results (2). The single-core LPS band migrated below 14,000 (see Fig. 3) and resembled that extracted from the Ra or Rb *Salmonella* mutants. In contrast, the LPS profiles from the two serum-resistant *C. fetus* strains were significantly different (Fig. 2). The core region was only minimally present, but several higher-molecular-weight complexes could be seen. In this gel, a second region of banding was seen for the PEN1 strain. That this was not present in Fig. 1 is probably due to differences in concentration. By heavily loading PEN1 LPS into the gel, we were able to see this second low-molecular-weight banding region (data not shown); however, high-molecular-weight complexes were not resolved for any of the serum-sensitive strains on this or any other gel.

We determined the effects of sodium hydroxide and treatment with 4 M urea on the electrophoretic profiles. NaOH treatment reduced the size of LPS aggregates without altering antigenic (O) determinants, presumably by removing ester-linked fatty acids from lipid A (23). Treatment produced a slight increase in the mobility of the core LPS of *C. jejuni* 79-193 and a reduction in the number of *C. fetus* 80-109 side chains. Urea, a chaotropic agent (10, 19), markedly increased the mobility of the *C. jejuni* LPS visualized in proteinase K whole-cell lysates but had little effect on the high-molecular-weight complexes of serum-resistant *C. fetus* (data not shown).

PAGE of proteinase K-treated whole-cell lysates. We next compared the PAGE profiles of the purified LPS from the four strains with the profiles of whole-cell lysates treated with proteinase K by the method of Hitchcock and Brown (14). The profiles of LPS obtained from whole cells were similar to those obtained from purified LPS (Fig. 3). Notable exceptions were the Westphal extracts from the serum-resistant strains (Fig. 3, lanes I and L). That many bands seen in the other preparations were not seen here correlates with the low efficiency of the Westphal method for extracting LPS from these strains. Some minor differences may have been due to differences in LPS concentrations loaded onto gels; however, the greatest differences were seen for strain PEN1. Varying the acrylamide concentration from 20 to 10% did not change the distribution of the bands seen (data not shown). Because 15% acrylamide gels produced the best resolution of the core region, we used this concentration in all subsequent gels. We also determined the effect of adding 40 mM EDTA to the PAGE running buffer (5) and of adding 2 mM EDTA to the stacking gel on the electrophoretic profile of proteinase K-treated whole-cell lysates. EDTA binds divalent cations, which have been shown to stabilize the LPS core structure (32). The core regions were more clearly resolved with EDTA than without EDTA, but the higher-molecular-weight complexes seen for the *C. fetus* strains were not altered, and no new complexes were seen for the *C. jejuni* strains (data not shown).

Varying the concentration of proteinase K-treated whole-cell lysates in PAGE produced a greater effect on the electrophoretic profile than varying the concentration of the purified LPS preparations (data not shown). Increasing the developing time for the silver stain affected the LPS profile of some proteinase K-treated whole-cell lysates (Fig. 4). After 20 min of development, the presence of low-molecular-weight (<20,000) bands (see Fig. 2, 3, and 5) was more pronounced. This prolonged developing time permitted resolution of several high-molecular-weight bands for *C. fetus* 80-81 but for none of the 9 *C. jejuni* strains in this study or for 26 other strains recently examined. However, the increase in developing time produced significant background that made the observation of the complete profile difficult.

For most of the 16 *Campylobacter* strains studied, the profiles of the proteinase K-treated whole-cell lysates correlated with serum status; a rough-type LPS was seen for the serum-sensitive and relatively sensitive strains, and a smooth-type LPS was seen for the serum-resistant strains (Fig. 5). Four other serum-sensitive or intermediately sensitive *C. jejuni* strains (Moore, Mosley, 83-86, and 83-85) had a rough-type LPS, and one other serum-resistant *C. fetus* strain (83-97) had a smooth-type LPS (data not shown). There were two exceptions, *C. fetus* 80-81 and *C. jejuni* 84-19, both of which were serum resistant but which showed a rough-type LPS.

DISCUSSION

As expected for gram-negative organisms and confirming earlier reports (2, 6, 21), lipopolysaccharides are present in

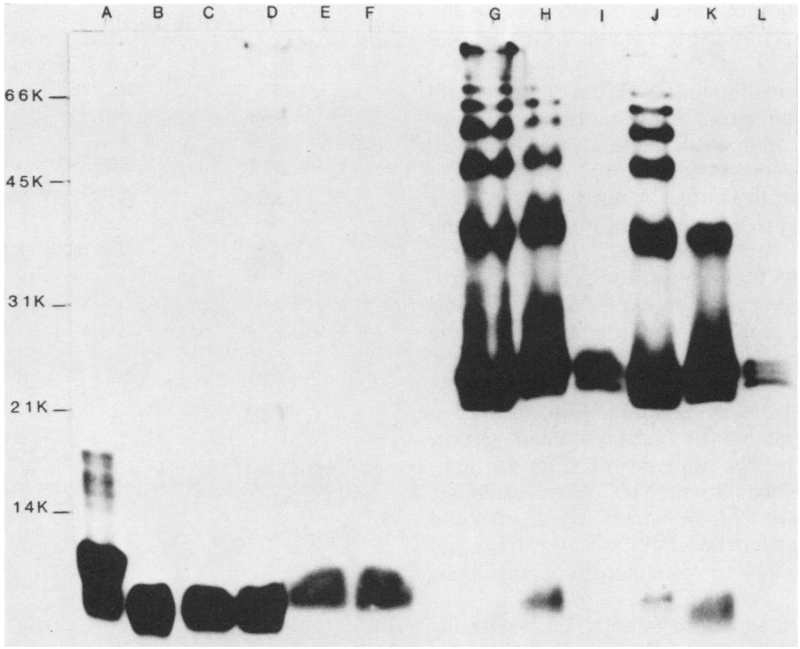

FIG. 3. Silver stain of 15% polyacrylamide gel with purified LPS and proteinase K-treated whole-cell lysates from *C. jejuni* and *C. fetus*. Lane A, *C. jejuni* PEN1 LPS proteinase K; lanes B and C, *C. jejuni* PEN1 LPS from the Galanos and Westphal extractions, respectively; lane D, *C. fetus* 81-170 proteinase K; lanes E and F, *C. fetus* LPS from the Galanos and Westphal extractions, respectively; lane G, *C. fetus* 80-109 LPS proteinase K; lanes H and I, *C. fetus* 80-109 LPS from the cold-ethanol and Westphal extractions, respectively; lane J, *C. fetus* 82-40 LPS proteinase K; lanes K and L, *C. fetus* 82-40 LPS from the cold-ethanol and Westphal extractions, respectively.

pathogenic campylobacters, and by a variety of extraction procedures LPS yields have been shown to be similar to those obtained from other gram-negative organisms (5, 8). The *Limulus* amoebocyte lysate assay provides a comparative in vitro estimate of the endotoxicity of LPS extracts. We confirmed earlier observations (7, 39) that endotoxin activity is present in *Campylobacter* LPS. Fumarola and colleagues have found that the endotoxic activity in *C. jejuni* cells is less than that for *E. coli* in the *Limulus* amoebocyte lysate assay (7). Using purified LPS, we confirmed this result and further showed that the endotoxic activity of LPS from serum-resistant and serum-sensitive *C. fetus* was similar to that of *E. coli*. Whether differences in endotoxicity between *C. fetus* and *C. jejuni* play a role in the pathogenesis of infection is unknown.

On the basis of the following criteria, we believe that the purified LPS obtained from serum-sensitive strains most resembles the rough chemotypes of enterobacterial LPS: (i) the Galanos method was better than the Westphal method for the extraction of LPS from the two serum-sensitive strains; this method was originally described for obtaining LPS from rough *Enterobacteriaceae* strains with hydrophobic LPS, for which the Westphal extraction was not effective (8); (ii) the proportion that total carbohydrate represented in these LPS preparations was lower than that in serum-resistant strains; (iii) the proportion of KDO to total carbohydrate was high in the serum-sensitive strains; and (iv) by PAGE, the LPS of serum-sensitive strains had similar migration characteristics to the LPS of rough chemotypes of *S. minnesota*. However, the results of prolonging the developing time suggested that short O side chains may be present in low concentrations. Furthermore, the Penner serotyping system is based on heat-stable hemagglutinating antigens (25). Finally, the cold-ethanol method, not the Galanos method, was best for LPS extraction from *C. fetus* 81-170 and showed higher carbohydrate concentrations. Therefore, although predominantly possessing characteristics similar to those of rough chemotypes of enterobacterial LPS, the LPS of serum-sensitive *C. jejuni* isolates cannot be adequately categorized by the rough-smooth dichotomy of the *Enterobacteriaceae*.

The purified LPS from serum-resistant *C. fetus* strains most closely resemble those of smooth-type *Enterobacteriaceae* by the following criteria: (i) the Galanos method was completely ineffective for isolating LPS from these strains; (ii) the percentage of total carbohydrate in these strains was higher than that in serum-sensitive strains; (iii) the proportion of KDO to total LPS carbohydrate was low; and (iv) the profiles for the LPS in the serum-resistant strains were significantly different from those of the serum-sensitive strains, with a minimal core region and several higher-molecular-weight complexes. These resembled the washboard patterns typical of smooth *Enterobacteriaceae* strains, but fewer complexes were present, and no complexes migrating at a molecular weight of less than about 25,000 were noted. Although it has predominantly smooth characteristics, the *C. fetus* LPS differs from those from smooth enterobacterial strains.

The silver-stained profiles of purified LPS and the profiles of proteinase K-treated whole-cell lysates were similar in all *Campylobacter* strains we studied, as has been described for *Salmonella* (14), *Pseudomonas cepacia* (18), *Neisseria meningitidis*, and *Neisseria gonorrhoeae* (24) strains. The finding of a second region of banding in some of the proteinase K-treated *C. jejuni* whole-cell lysates, but not in purified LPS, may be due to differences in concentration or to contaminating proteins because we used the silver LPS-protein staining method described by Hitchcock and Brown

(14). Alternatively, resolution of the other bands may be due to less catalysis of polysaccharide side chains in the whole-cell lysates.

When we studied 16 strains, including serum-resistant and serum-sensitive *C. fetus* and serum-resistant and serum-sensitive *C. jejuni*, by the proteinase K method, we found an excellent correlation between proteinase K-treated whole-cell lysate profiles and serum status. When PAGE profiles are compared, there appear to be degrees of roughness in the serum-sensitive and relatively sensitive *C. jejuni* strains. These data were similar to those observed for the *Enterobacteriaceae*, of which several chemotypes of *Salmonella* and *Escherichia coli* are known, and might explain the different degrees of serum susceptibility in these strains. However, there were two exceptions. A serum-resistant *C. fetus* subsp. *venerealis* strain of animal origin showed a rough profile but was resistant to normal human serum. However, by increasing the developing time (Fig. 4), high-molecular-weight banding was seen. The other exception was the only serum-resistant *C. jejuni* strain, which also had a rough profile, indicating that non-LPS structural characteristics may confer the ability to resist serum bacteriolysis (35).

The profiles of the seven serum-resistant *C. fetus* strains were different from the profile of the LPS from the wild-type *S. minnesota* strain. We found less washboard, which may mean that variability in O side chain length was less frequent than in the wild-type *S. minnesota* LPS. The inability of EDTA to significantly affect the campylobacter LPS banding patterns suggested that, similar to that reported for gonococcal LPS (19), ionic interactions did not play a major role in the aggregation of the LPS. That urea had little effect suggests that the high-molecular-weight complexes are not LPS aggregates; however, the effects of sodium hydroxide treatment suggest that some aggregation was occurring. The lack of a classical washboard pattern also may explain why the Westphal method (38) for extraction of smooth-type hydrophilic LPS was inefficient. Another reason for the low yield with this method might be the presence of an outermost surface layer; a glycoprotein-containing microcapsule has been described for *C. fetus* (40). However, we did not find any increase in LPS yield when we treated the cells with pronase, as suggested by Naess and Hofstad (21).

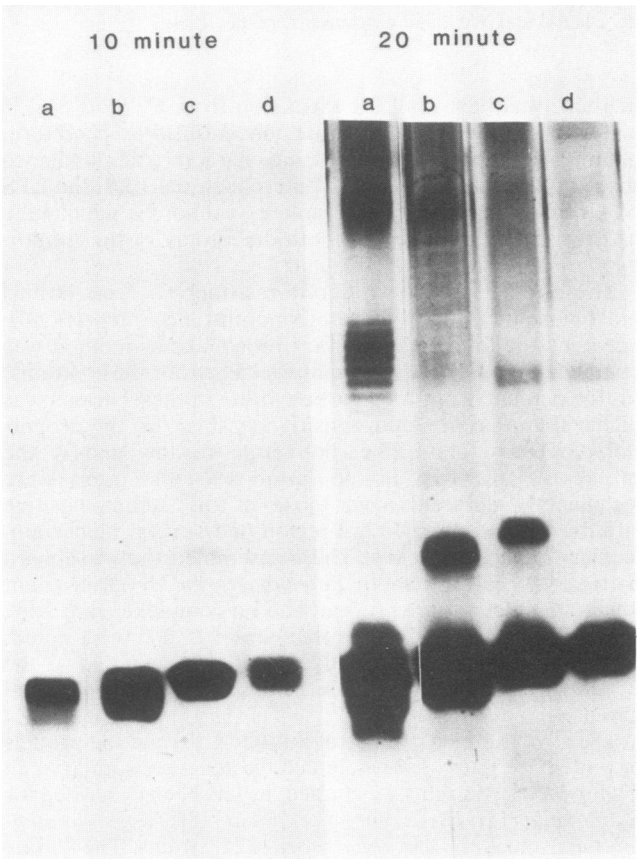

FIG. 4. Effect of variation in developing time on LPS profiles. Proteinase K-treated whole-cell lysates of *C. fetus* 80-81 (lane a), *C. jejuni* 84-19 (lane b), *C. jejuni* 79-193 (lane c), and *C. jejuni* 83-100 (lane d) run in a 15% polyacrylamide gel and stained with silver were examined after 10 and 20 min of color development.

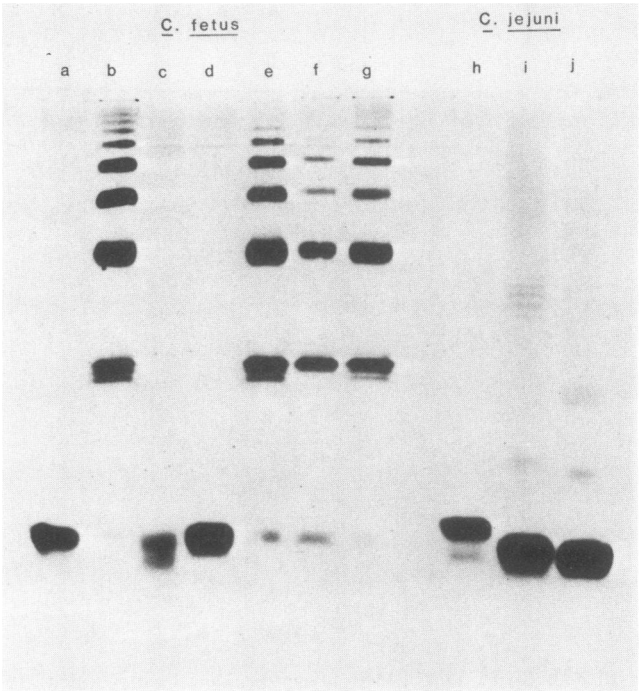

FIG. 5. Silver stain of *C. fetus* and *C. jejuni* LPS. Samples consisting of 5 μl of proteinase K-treated whole-cell lysates of *C. fetus* and *C. jejuni* were electrophoresed on a 15% polyacrylamide gel. *C. fetus* strains: lane a, 80-81; lane b, 80-109; lane c, 81-169; lane d, 81-170; lane e, 81-173; lane f, 81-200; lane g, 82-40. *C. jejuni* strains: lane h, PEN1; lane i, PEN2; lane j, PEN3.

Serum resistance may be mediated by several determinants in the outer membrane (28, 29, 35). An outer membrane protein is the principal determinant for *N. gonorrhoeae* strains that cause disseminated disease (12), whereas LPS has the principal role in the resistance of most *Enterobacteriaceae* (31, 34). In the case of campylobacters, our results suggest that LPS structure also may be a major determinant of serum resistance; however, the finding of variant strains suggests that other factors also may have a role. Work is in progress in our laboratory to clarify this point.

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