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Basis for Serological Heterogeneity of Thermostable Antigens of *Campylobacter jejuni*

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Lipopolysaccharides (LPS) were extracted from eight strains of *Campylobacter jejuni* and purified by enzyme treatment to remove traces of RNA, DNA, and protein. This material was used to sensitize sheep erythrocytes for the passive hemagglutination assay that is presently used to serotype *C. jejuni*. The results confirmed that the thermostable antigen typing scheme is based on LPS (O) antigens. The LPS after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining was found to consist of a series of slow migrating bands which could not be eliminated by treatment with NaOH, urea, or EDTA. However, the use of LPS double labeled with ^{14}C and ^{32}P yielded evidence that the bands of high molecular weight were indeed aggregations of low-molecular-weight LPS molecules.

Campylobacter jejuni and *Campylobacter coli* have become recognized as major causes of human bacterial enteritis (40, 41) and interest in the epidemiology of infections due to these organisms has led to the development of serotyping systems. Schemes that differentiate strains through differences in the specificities of surface antigens (13), thermolabile antigens (21), and thermostable antigens (19, 35) have been described. Currently, attention is being directed towards characterizing the biological and biochemical properties of the antigens to seek insight into the basis for the extensive serological heterogeneity noted to occur among strains of these species. At the outset, the thermostable antigens were thought to be lipopolysaccharide (LPS), the somatic O antigens common to gram-negative species (34), and investigations at the molecular level that support this view have recently been forthcoming (22, 27–29). Although the LPS of *C. jejuni* and *C. coli* appear similar to LPS of the *Enterobacteriaceae* in that both have considerable serological heterogeneity, it appears that, structurally, LPS in *C. jejuni* and *C. coli* may be quite unique. In the present report, enzyme-purified LPS was used to study the serological specificity of the O antigen by passive (indirect) hemagglutination (PHA) and to characterize the structure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining. Evidence is provided to show that the thermostable antigen typing scheme is based on O antigens composed of LPS which have structural features different from the LPS of other gram-negative bacterial species.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. The strains of *C. jejuni* used in this study are listed in Table 1. Also, the smooth strain of *Escherichia coli* O:111B4 and the Ra (SL3749) and Rc (SL3748) mutants of *Salmonella typhimurium* were used.

Stock cultures were maintained at −70°C in 15% glycerol–1% proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.) and, when required, strains were thawed, plated on brucella agar (Difco), and incubated for 48 h under reduced oxygen conditions in a CO_2 incubator. The *E. coli* and *S. typhimurium* strains were cultured on tryptic soy agar plates at 37°C.

Radiolabeling of bacteria. To label cellular DNA, RNA, and protein, cells were incubated for 24 h on brucella agar, washed, and diluted to 10^9 CFU/ml, and 500 μl was spread onto fresh brucella agar plates containing 10 μCi of L-[^{35}S]-methionine, [5,6-^{3}H]uridine, or [*methyl*-^{3}H]thymidine (Amersham Radiochemicals, Oakville, Ont.). Cells were harvested after 24 h of incubation.

To double label cells, bacteria were cultured for 24 h on brucella agar, harvested, diluted to 10^9 CFU/ml, and inoculated into 50 ml of brucella broth with FPB supplement (Oxoid Canada Ltd., Napean, Ont.), 125 μCi of [U-^{14}C]protein hydrolysate (56 mCi/matom of carbon; Amersham), 150 μCi of [U-^{14}C]glycine (Amersham), and 1 mCi of ^{32}P (orthophosphoric acid, carrier-free; New England Nuclear Corp., Boston, Mass.). Flasks were gassed for 5 s with carbon dioxide, incubated at 37°C with shaking, and harvested 12 h later.

LPS extraction and purification. Bacteria incubated for 48 h on brucella agar plates under microaerophilic conditions (8% CO_2) in a carbon dioxide incubator were harvested with sterile normal saline (0.85% NaCl) and washed twice (8,000 × *g*, 10 min, 4°C) in saline. Crude LPS from strains of *C. jejuni* and *E. coli* was extracted by the hot phenol-water technique (45). After dialysis against distilled water, the crude material was concentrated by lyophilization and then purified by enzyme treatment. A 50-mg portion of LPS was dissolved in 5 ml of 0.1 M Tris hydrochloride buffer (pH 7.5) containing RNase A (1 mg/ml; Sigma Chemical Co., St. Louis, Mo.) and DNase II (1 mg/ml; Sigma) and incubated at 37°C for 18 h. Proteinase K (0.1 mg/ml; Boehringer Mannheim Canada Ltd., Dorval, Que.) was added and incubation was allowed to proceed for an additional 4 h. Incubation of this mixture at 65°C for 4 h terminated the reaction. After overnight dialysis against distilled water, the LPS was pelleted at 100,000 × *g* for 18 h, redissolved in distilled water, and lyophilized. LPS from the two strains of *S. typhimurium* were prepared according to a previously described method (5).

PHA titrations. The PHA technique was used to titrate *C. jejuni* antisera prepared in a previous study (34). Two types

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TABLE 1. Strains of *C. jejuni* used in this study

Strain	Origin	PHA serotype (Penner system)
HSC7360	Reference strain	1
C142	Reference strain	8
TGH4936	Reference strain	16
MK15	Reference strain	17
D67	Reference strain	44
IVS	Patient stool isolate	1/44
IVB	Patient blood isolate	1/44
17J	Patient stool isolate	17/8

of antigenic material were used to sensitize erythrocytes that had been washed three times in phosphate-buffered saline (PBS; 10 mM Na_2HPO_4, pH 7.0, in 0.85% NaCl). Purified LPS was prepared as described and diluted to 0.25 mg/ml in PBS prior to use. The second type of antigenic material was a saline extract prepared by heating washed cell suspensions for 1 h at 100°C and removing cell debris by centrifugation (8,000 × *g*, 10 min), followed by dilution of the supernatant in PBS (1:10). For sensitization of erythrocytes, a 1% suspension of washed erythrocytes was added to the antigenic preparation (1:1, vol/vol) and incubated for 1 h at 37°C. The sensitized erythrocytes were centrifuged, washed three times in PBS, and suspended to a final volume equivalent to a 0.5% erythrocyte suspension. Twofold dilutions were performed with a Medimixer (Flow Laboratories, Rockville, Md.) in microtitration plates (microtiter; Dynatech Laboratories, Inc., Alexandria Va.) with U-shaped wells containing 0.25 ml of PBS. A volume of 25 μl of sensitized erythrocytes was added to each well of diluted antisera, and the plates were shaken, incubated at 37°C for 1 h, and stored overnight at 4°C. The plates were read by examining wells for agglutination of erythrocytes and the highest dilution of antiserum showing agglutination was taken as the titer. The initial dilution of the antisera was 1:40 and the absence of agglutination at this dilution was considered a negative reaction.

SDS-PAGE and silver staining of LPS. Preparations were electrophoresed on an SDS-PAGE system (18), using a stacking gel of 7% acrylamide and a separating gel of 14% acrylamide. In some experiments, gels were prepared as a 7 to 20% linear gradient of acrylamide (33) and, where specified, 4 M urea (Fisher Scientific Co., Fairlawn, N.J.), 2 mM EDTA (disodium EDTA; BDH Chemicals Canada Ltd., Toronto, Ont.), or higher concentrations of SDS (1%; Bio-Rad Laboratories, Richmond, Calif.) were incorporated. Gels were stained with Coomassie brilliant blue R (Sigma) or stained with silver (44). Autoradiography was performed on ^{32}P-labeled dried gels at −70°C for 8 to 48 h, using Cronex Xtra Life intensifying screens (E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.) and Kodak AR X-ray film.

Characteristics of electrophoresed LPS. Various concentrations of extracted LPS from *C. jejuni* Pen 1 and *E. coli* O:111B4 were electrophoresed. One lane of each preparation was silver stained to determine the location of bands, and corresponding areas of an unstained lane were excised, loaded into the slot of another gel, re-electrophoresed, and silver stained. Bacteria double labeled with ^{32}P and ^{14}C were used to prepare pure LPS by the phenol-water method (45) which was electrophoresed in triplicate by SDS-PAGE. One sample was silver stained, another was dried and used for autoradiography, and the third was sliced into 1-mm-thick pieces and counted in scintillation fluid to determine the ratio of $^{14}C/^{32}P$ counts.

In another set of experiments, pure LPS samples (2 mg/ml) were treated with 0.1 N NaOH, incubated at 37°C for 18 h, and dialyzed against distilled water, and portions were added to solubilizing buffer prior to SDS-PAGE.

Biochemical assays. Protein concentrations were determined by the method of Lowry et al. (23), using lysozyme (Sigma) as a standard. 2-Keto-3-deoxyoctonate (Sigma) was analyzed by the method of Karkhanis et al. (17).

High-pressure liquid chromatography analysis. LPS (500 μg) was exposed to 2.5 μg of DNase II (240 U/mg of protein; Calbiochem, San Diego, Calif.), 2.5 μg of RNase A (type 1A; 70 kU/mg of protein; Sigma), 2.0 μg of alkaline phosphatase (type VII, 960 U/mg of protein; Sigma), and 2.5 μg of snake venom phosphodiesterase 1 (type VII, 0.4 U/mg of protein; Sigma) for 21 h at 37°C. Samples were deproteinated with 1 N perchloric acid and neutralized with 2 N NaOH, and the insoluble precipitates were removed by centrifugation. Treated LPS (100 μg) was injected into a μBondapak C_{18} reverse-phase column (Waters Associates, Inc., Milford, Mass.) and eluted isocratically with 5 mM $NH_4H_2PO_4$–6% methanol (pH 4.8) at a flow rate of 1 ml/min. The Waters model U6K injector, model M-45 solvent delivery system, and model 440 absorbance detector were used.

RESULTS

Properties of purified LPS from *C. jejuni*. To evaluate the efficiency of previously reported procedures for extracting LPS from *C. jejuni*, LPS was prepared from cells cultured in the presence of [^{35}S]methionine, [^{3}H]uridine, or [*methyl*-^{3}H]thymidine and the levels of residual radioactivity were measured after extraction and purification of LPS (Table 2). The material obtained by the hot phenol-water method (45) was found to contain protein. This was indicated by the presence of 5.3% of the radioactivity incorporated due to growth in [^{35}S]methionine-containing medium, the visualization of bands after Coomassie brilliant blue staining of SDS-polyacrylamide gels, and the detection of 9 μg of protein per mg of LPS (23). Protein was readily removed by proteinase K treatment.

LPS obtained from cells cultured in the presence of [^{3}H]uridine contained 22% of the radioactivity initially incorporated (Table 2). A diffuse band of high molecular weight, identifiable by silver staining and autoradiography (data not shown), was removed by treatment with RNase, which suggested that it was composed of RNA.

Experiments to determine the presence of DNA in the LPS (Table 2) indicated that radioactivity was retained at low levels not only after extraction (8.1%) but also after treatment with DNase (5.6%). This prompted further examination of the LPS, using high-pressure liquid chromatogra-

TABLE 2. Purification of LPS from strain Pen 1, using cells labeled with [^{3}H]uridine, [^{3}H]thymidine, and [^{35}S]methionine

Label	% Radioactivity		
	Whole cells	Impure LPS	Pure LPS
L-[^{35}S]methionine (protein)	100	5.3	ND[a]
[5,6-^{3}H]uridine (RNA)	100	22	ND
[*methyl*-^{3}H]thymidine (DNA)	100	8.1	5.6

[a] ND, None detectable.

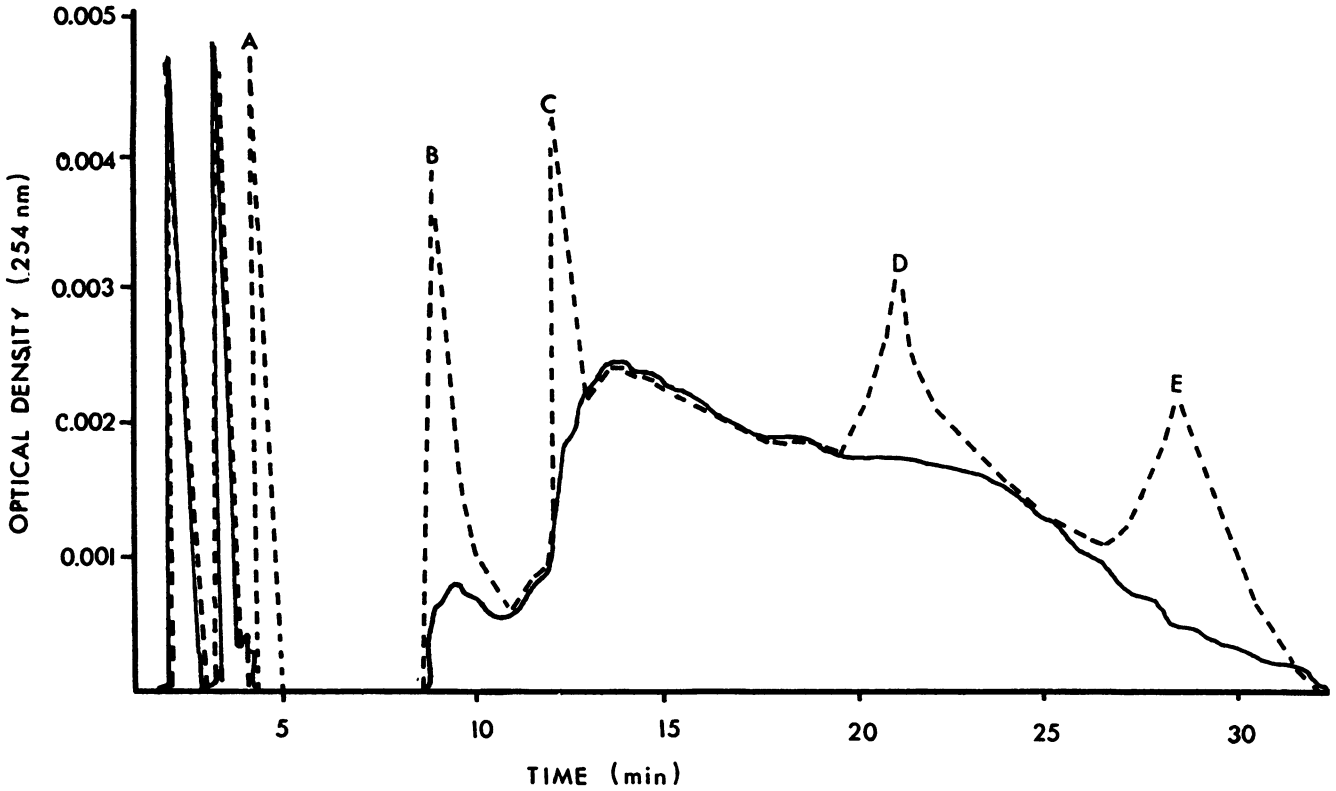

FIG. 1. High-pressure liquid chromatogram of purified LPS from *C. jejuni* strain Pen 1. Symbols: —, enzyme-treated LPS; ------, enzyme-treated LPS containing standards—A, uridine; B, guanosine; C, thymidine; D, adenosine; E, deoxyadenosine.

phy to quantitate the nucleosides present (Fig. 1). DNase-treated LPS with and without the standards uridine, guanosine, thymidine, adenosine, and deoxyadenosine were passed through the high-pressure liquid chromatography column. The area of each peak in the LPS sample which had a retention time the same as a nucleoside standard was calculated, and by using a standard curve of concentration versus peak area, it was found that 1.0 μg of LPS contained 188.4 pg of DNA and <50 pg of RNA, amounts which would be expected to be below limits detectable by the silver staining procedure used in SDS-PAGE analysis of LPS.

Labeling of cellular protein, RNA, and DNA and monitoring of the radioactivity present in LPS enabled us to determine that there was substantial contaminating protein and RNA as well as small amounts of DNA in extracted LPS. These contaminants were removed with proteinase K, RNase, and DNase to yield a purified preparation. The presence of LPS in this material was confirmed by 2-keto-3-deoxyoctonate analysis (1.4 μg/100 μg of LPS).

Antigenic specificity of purified LPS. To examine whether the serological specificity of LPS had suffered alteration through extraction and purification, LPS was extracted from the eight strains of *C. jejuni* listed in Table 1 and purified to remove contaminating protein, RNA, and DNA. The LPS was used to sensitize sheep erythrocytes for PHA titrations against antisera specific for five *C. jejuni* strains used in the serotyping system. The results of this experiment are illustrated in Table 3. The titers obtained in routine serotyping in which erythrocytes are sensitized with supernatants obtained by heating bacterial suspensions for 1 h at 100°C are included for comparison. Except for one case (reactions of antiserum 16), titers obtained with LPS were higher than titers obtained with the heat-extracted antigenic material. Generally, the specificity of the reactions were the same regardless of the material used to sensitize the cells, but a few more low-titered cross-reactions were noted with the purified LPS. It was evident from these results that extraction and purification procedures had not adversely affected the antigenic specificity of the LPS but had instead resulted

TABLE 3. Serotyping of *C. jejuni* strains by PHA with thermostable cell extract and purified LPS

Antigen extracted from *C. jejuni* strains[a]	Titer with given antiserum no.[b]				
	1	44	8	17	16
Sheep erythrocytes sensitized with heated extracts					
1	2,560	—	160	—	—
44	160	1,280	—	—	—
IVS	640	160	—	—	—
IVB	1,280	320	—	—	—
8	—	—	320	40	—
17	—	—	—	640	—
17J	—	—	160	320	—
16	—	—	—	—	640
Sheep erythrocytes sensitized with purified LPS					
1	40,960	640	40	—	—
44	40	10,240	40	—	—
IVS	2,560	1,280	80	—	—
IVB	2,560	1,280	—	—	—
8	40	640	2,560	—	—
17	—	80	320	1,280	—
17J	—	—	320	640	—
16	—	—	—	—	640

[a] See Table 1.
[b] Titers are expressed as reciprocals. —, Titers of <1:40.

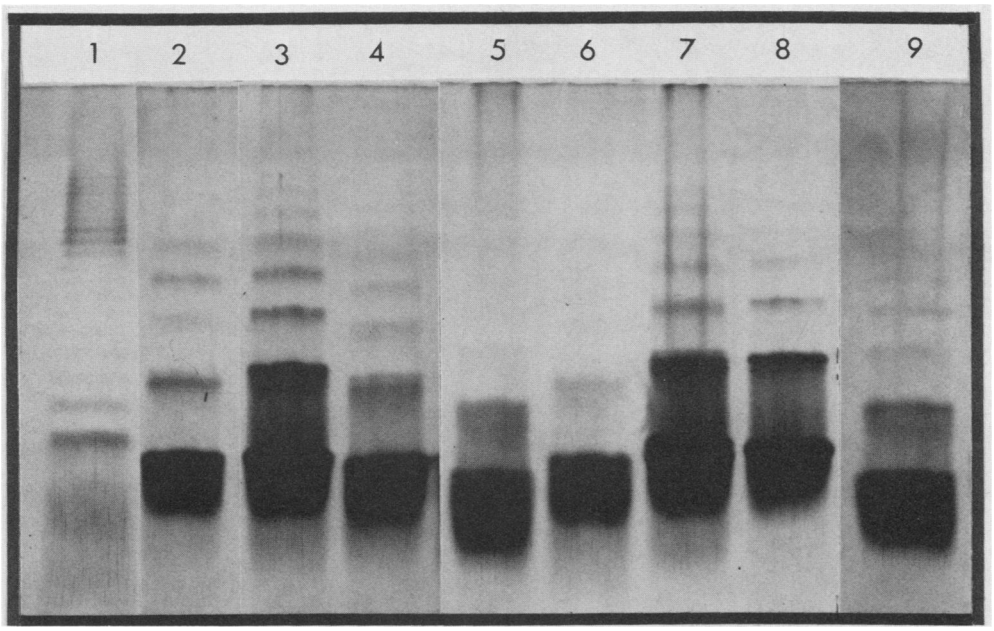

FIG. 2. Silver-stained polyacrylamide gel of purified LPS from strains of *C. jejuni*. A 20-μg portion of LPS was loaded onto a 14% separating–7% stacking gel. Lane 1, *E. coli* O:111B4; lane 2, *C. jejuni* Pen 1; lane 3, *C. jejuni* IVB; lane 4, *C. jejuni* IVS; lane 5, *C. jejuni* Pen 8; lane 6, *C. jejuni* Pen 16; lane 7, *C. jejuni* Pen 17; lane 8, *C. jejuni* 17J; lane 9, *C. jejuni* Pen 44.

in preparations with increased sensitivity to the antibodies in the typing antisera.

Examination of *C. jejuni* LPS by SDS-PAGE and silver staining. Purified LPS obtained from five *C. jejuni* serotype reference strains, three patient isolates, and an *E. coli* O:111B4 strain were subjected to SDS-PAGE and stained with silver (44). *C. jejuni* LPS reflected a migration pattern not unlike that of *E. coli* O:111B4. The profile consisted of a fast migrating band that corresponded to lipid A with core polysaccharide and a series of slower migrating bands that on first inspection resembled bands characteristic of LPS with O side chains (Fig. 2).

When five different concentrations of LPS were subjected to SDS-PAGE and silver staining (Fig. 3A), it was clear that increasing the concentration led to a greater number of the slow migrating bands. Since this effect could be accounted for on the basis of low numbers of LPS molecules with O side chains or, alternatively, on the basis of aggregations,

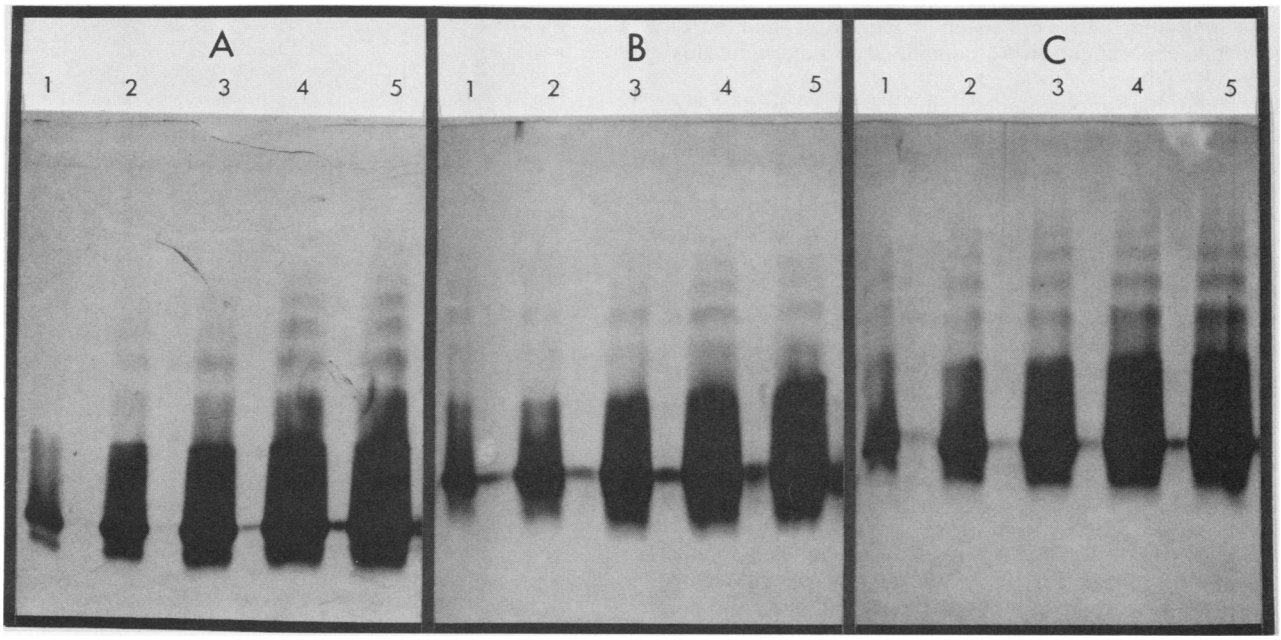

FIG. 3. Silver-stained polyacrylamide gels of different concentrations of purified LPS from *C. jejuni* strain Pen 1. (A) 14% separating–7% stacking standard gel system; (B) 14–7% gel plus 1% SDS; (C) 14–7% gel plus 4 M urea. Lanes 1 to 5: 5, 10, 25, 50, and 75 μg of LPS.

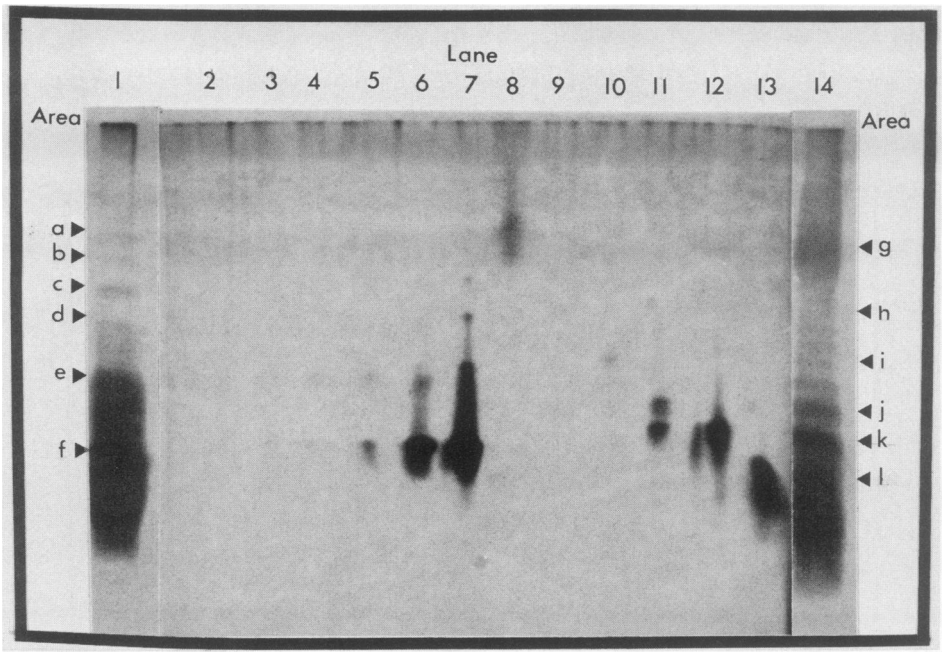

FIG. 4. Silver-stained polyacrylamide gels of *C. jejuni* Pen 1 and *E. coli* O:111B4. Lane 1, 20 μg of *C. jejuni* Pen 1 LPS; lanes 2 to 7, re-electrophoresis of *C. jejuni* Pen 1 LPS excised from areas of the gel corresponding to a to f in lane 1; lanes 8 to 13, re-electrophoresis of *E. coli* O:111B4 LPS excised from areas of the gel corresponding to g to l in lane 14; lane 14, 20 μg of *E. coli* O:111B4 LPS.

electrophoresis was conducted under a variety of conditions to assess the effects of adding 1% SDS (Fig. 3B), 4 M urea (Fig. 3C), or 2 mM EDTA (data not shown) to the polyacrylamide gels. These conditions were expected to reduce or eliminate aggregates (12, 20, 32) but the results failed to indicate significant differences from those obtained with the standard gel system (Fig. 3A). Pretreatment of LPS with 0.1 N NaOH before electrophoresis did not change the number of bands observed in the gel but did cause a slight decrease in the molecular weight of the bands (data not shown). On the basis of these results, it could not be concluded whether the slow migrating bands consisted of aggregates, and therefore further experiments were conducted to determine this possibility.

Individual high-molecular-weight bands separated by SDS-PAGE were excised and re-electrophoresed in separate lanes of another gel (Fig. 4). Bands cut from the gel of LPS from *E. coli* O:111B4 (Fig. 4, lane 14) were re-electrophoresed in their corresponding original position (Fig. 4, lanes 8 to 13). This was unlike the case of *C. jejuni* (lane 1) in which individual bands cut from the top of the gel migrated to the low-molecular-weight area of the gel (lanes 2 to 7), similar in position to that of the lipid A with core polysaccharide. This suggested, as did the results of a similar experiment performed by Logan and Trust (22), that the slow migrating bands were aggregates, but did not rule out the possibility that LPS of *C. jejuni* was degraded as a result of the experimental manipulations or second exposure to SDS. For this reason, an experiment involving double labeling of cells with ^{14}C and ^{32}P was conducted. Cells were radiolabeled, and purified LPS was prepared and electrophoresed by SDS-PAGE. Increases in the size of O side chains should correspond to increases in the $^{14}C/^{32}P$ ratios (10). The value of the lowest-molecular-weight band was set at unity and the values of the other two bands are presented as a fraction of that value (Fig. 5). Since results showed no significant differences in the ratios obtained from the various bands, it was taken as convincing evidence that upper bands observed in polyacrylamide gels of *C. jejuni* LPS were due to aggregation of low-molecular-weight LPS.

It appeared, therefore, that aggregates resulted from electrophoresis of high concentrations of LPS. To prevent this from occurring and to enable characterization of LPS from various *C. jejuni* strains, no more than 5 μg of LPS was

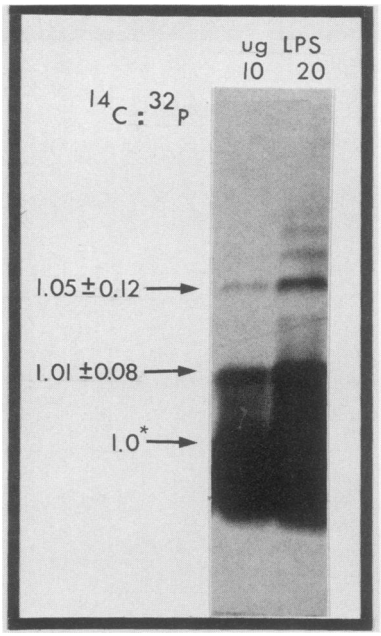

FIG. 5. Silver-stained polyacrylamide gel of ^{14}C- and ^{32}P-double-labeled LPS extracted and purified from *C. jejuni* strain Pen 1. $^{14}C/^{32}P$ ratios are presented for the three bands indicated.

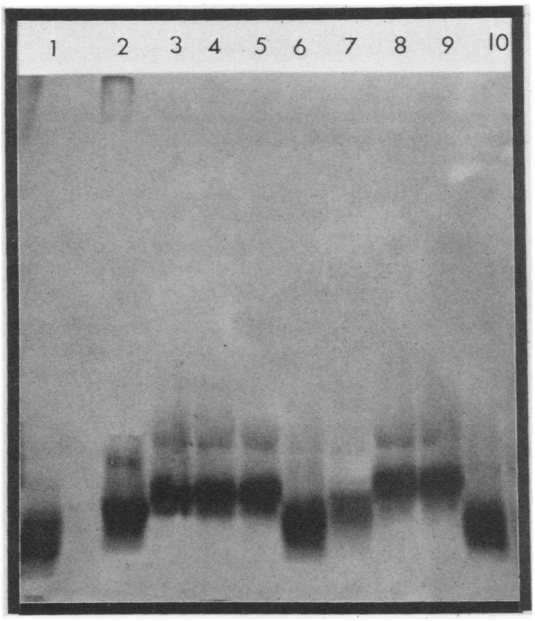

FIG. 6. Silver-stained polyacrylamide gradient gel (7 to 20% acrylamide) of purified LPS from different strains of *C. jejuni* and *S. typhimurium*. Lane 1, *S. typhimurium* Rc mutant; lane 2, *S. typhimurium* Ra mutant; lane 3, *C. jejuni* IVB; lane 4, *C. jejuni* IVS; lane 5, *C. jejuni* Pen 8; lane 6, *C. jejuni* Pen 16; lane 7, *C. jejuni* Pen 17; lane 8, *C. jejuni* Pen 17; lane 9, *C. jejuni* 17J; lane 10, *C. jejuni* Pen 44.

loaded per lane of a gradient gel. Under these conditions, the electrophoresis profiles of all strains showed a fast migrating band (Fig. 6). Of considerable interest was the observation that strains of the same serotype had bands of the same molecular weight. This was consistent with our previous studies which showed that both isolates of serotype 17 were identical strains (25, 36). Although two of the isolates of serotype 1 were from the same patient, the serotype reference strain 1 was isolated in a different hospital at least 5 years earlier. Moreover, except for LPS from strains 8 and 44, all of the LPS molecules from the serotype reference strains migrated at different rates (Fig. 6). With respect to molecular weights, it should be noted that LPS from all *C. jejuni* strains had molecular weights higher than that of the *S. typhimurium* Rc mutant. LPS from serotype reference strains 8 and 44 were approximately equal to that of the *S. typhimurium* Ra mutant, whereas LPS from strains 1, 16, and 17 had somewhat higher molecular weights. From these results it was evident that in at least some cases the migration rate of LPS was characteristic of the serotype.

DISCUSSION

Thermostable antigens extracted from *C. jejuni* and *C. coli* have been used in PHA to differentiate strains involved in epidemiological investigations (2, 24, 37). These antigens were believed to be LPS or O antigens because of their biological properties (34). These properties include thermostability, the ability to be readily extracted from bacterial cells by methods used to extract O antigens from other gram-negative bacteria (20), the ability to sensitize mammalian erythrocytes, making them specifically agglutinable in antisera (31), and their occurrence in a wide spectrum of antigenic specificities. However, the molecular basis for the serological heterogeneity has not been systematically investigated.

It should be noted that PHA is particularly well suited for identifying the specificities of the LPS molecule. Mammalian erythrocytes have lipoglycoprotein receptors that bind to the fatty acids of the lipid A moiety (11, 42). Moreover, LPS that sensitizes erythrocytes may be extracted in several ways. For routine serotyping of *C. jejuni* in our laboratory, antigenic material is extracted by heating saline suspensions of bacteria for 1 h at 100°C. Although Buck et al. (4) provided evidence that there were proteins present in the heated cell extracts, they are probably antigenically altered due to the heat treatment. PHA titrations yielded essentially the same results when the antigens were prepared by the method of Lieve et al. (20), where EDTA is used to release LPS along with proteins (34). To investigate further a possible role for proteins in the PHA serotyping system, LPS was extracted by the phenol-water method and enzyme purified to remove RNA, DNA, and protein. When this protein-free LPS was used to sensitize the erythrocytes, the titration results were essentially the same as the results obtained when erythrocytes were sensitized with antigenic material prepared by heating saline suspensions of whole cells (Table 2). This indicated that LPS, but not proteins, carrying the significant antigenic determinants in PHA. It should be noted that PHA has been used in determining O specificities for other species (6, 7, 31) and was cited to be more specific than procedures which rely on agglutination of bacterial cell suspensions (3, 8, 30). For *C. jejuni*, PHA is particularly appropriate because O specificities cannot be reliably identified with procedures involving agglutinations of heated bacterial suspensions because of their tendency to autoagglutinate and produce nonspecific reactions (1, 9, 15, 34). Moreover, the use of purified LPS as the antigenic material for sensitizing erythrocytes resulted in higher titers, thereby enhancing the sensitivity of the test (Table 2). This suggests that O specificities may be more accurately defined with purified LPS and this approach will be pursued in future studies to resolve complexities in the serotyping system (35).

That the thermostable antigens described in the serotyping schemes are actually LPS was also indicated by other workers. Naess and Hofstad (27), Logan and Trust (22), and Perez and Blaser (38) showed that *Campylobacter* sp. had phenol-water-extractable LPS, but these workers did not use the PHA system to show directly that the thermostable antigens in the serotyping scheme were LPS. However, Jones et al. (16) isolated a low-molecular-weight component from polyacrylamide gels of phenol-water-extracted LPS and found it to be active in PHA. It now seems appropriate that the traditional designation of thermostable antigens as described in the serotyping systems (8, 35) be considered synononymous with LPS or O antigens.

Although *C. jejuni* and *C. coli* are known to possess a multiplicity of LPS specificities, current knowledge on the composition and structure of LPS has not permitted a clear understanding of the basis for this serological diversity. Further characterization of the LPS is essential to shed light on this question and to enable the serologist to appreciate differences between strains that produce incomplete LPS (rough strains) and those with complete LPS molecules (smooth strains) needed for producing typing antisera.

In the present study, phenol-water-extracted and enzyme-purified LPS was examined by SDS-PAGE and silver staining. When low concentrations of LPS were loaded onto gels, a fast migrating band of low molecular weight was seen for each of the *C. jejuni* strains. There was no indication of a common core type among the five serotypes to which the eight strains belonged, as the fast migrating band for strains

of different serotypes migrated at slightly different rates. However, the same rates of migration were noted for strains of the same serotype. Therefore, differences in serotype corresponded to differences in molecular weights of the fast migrating LPS which suggested that serological heterogeneity was to some extent governed by the structure and composition of this low-molecular-weight LPS.

The series of slow migrating bands seen in gels with higher concentrations of LPS were found to be present even when gels were electrophoresed under a variety of conditions. These results are not in accord with those reported for LPS from *Neisseria* sp. (26), where the presence of urea in the gel or pretreatment of LPS with NaOH abolished high-molecular-weight bands. The decrease in molecular weight of the material in the slower migrating bands after extraction from the gels and re-electrophoresis as well as the absence of significant differences in the $^{14}C/^{32}P$ ratios of high- and low-molecular-weight bands is indicative of LPS aggregation. Results of experiments with LPS from proteinase K-digested whole cells as performed by Logan and Trust (22) and confirmed in the present study (data not shown) led to the same conclusion. Naess and Hofstad (29) found that acetic acid-degraded LPS from various strains of *C. jejuni* and *C. coli* fell into two classes after elution on Bio-Gel and gas liquid chromatographic analysis. Some strains appeared to have LPS consisting of only lipid A and core sugars while others contained lipid A and core sugars with 10 to 25 hexose residues per three residues of heptose, indicating the presence of short O side chains. In the present study, it was shown that the molecular weight of at least three serotype reference strains was greater than the *S. typhimurium* Rc mutant which is consistent with the concept of a core with a short side chain (28, 29). Variations among strains observed in this study in the color of the silver-stained gels (data not shown) could reflect differences in the sugars comprising the LPS of the various serotype reference strains.

Two structures of LPS may now be postulated to account for the serological heterogeneity. One possibility is that the LPS has a structure termed lipooligosaccharide (39), like that of *Neisseria meningitidis* (43), *Neisseria gonorrhoeae* (26), and *Haemophilus influenzae* (14), consisting of lipid A to which is attached a short oligosaccharide. Considerable variability in the composition of the oligosaccharide component could dictate a wide range of specificities. Alternatively, the results of Naess and Hofstad (28, 29) permit the concept of LPS as consisting of lipid A with a core resembling those of the *Enterobacteriaceae* but to which is attached a single repeat unit rather than a long O side chain. This would reflect the semirough mutant, and serological diversity could be attributable to differences in the composition of a small oligosaccharide analogous to the single repeat unit of the semirough mutant. These *Campylobacter* species would be conceived as lacking activity of the enzyme(s) required to polymerize the second and subsequent repeat units to produce long O side chains. For a final resolution of the basis for the serological heterogeneity of *Campylobacter* LPS, it is essential that components constituting the short oligosaccharide be determined for strains of different serotypes.

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