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Analysis of *Campylobacter jejuni* Antigens with Monoclonal Antibodies

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To develop monoclonal reagents for antigenic analysis and serotyping of *Campylobacter* spp., hybridoma cell lines were produced by fusion of mouse myeloma cells and spleen cells from mice immunized with Formalin-treated *Campylobacter jejuni* organisms. An enzyme immunoassay was used for preliminary screening of the cell culture supernatants and ascites. Twenty-nine clones which reacted with the immunogen were obtained. Seven of these clones were positive in passive hemagglutination tests with sheep erythrocytes coated with boiled saline extract of whole bacteria; four of these reacted with the purified polysaccharide preparation and with the autoclaved saline extract, but not with lipopolysaccharide prepared from the immunogen strain. Two of the antipolysaccharide clones agglutinated live bacteria in slide tests. Four additional clones gave positive slide agglutination tests with live bacteria, but in tube testing no clones agglutinated Formalin-treated bacteria. No cross-reactions with unrelated bacteria were seen, but several clones reacted in the enzyme immunoassay with many of the 24 *Campylobacter* strains studied. The clone which gave the highest mean enzyme immunoassay values with *Campylobacter coli* and *C. jejuni* strains also reacted with *Campylobacter fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* strains. This clone also gave the highest enzyme immunoassay value with an acid glycine extract of the immunogen, which indicates the presence of common antigens in the extract. The results suggest that monoclonal antibodies may be used to devise serotyping schemes for *Campylobacter* spp.

The recent surge of interest in *Campylobacter* infections has prompted studies of methods to identify and detect *Campylobacter* strains for epidemiological purposes. Although biochemical tests and differences in antibiotic susceptibility have been effective for identifying species and subspecies, they have limited ability to differentiate strains within subspecies (6, 8). Further characterization and typing of *Campylobacter coli* and *Campylobacter jejuni* strains have been based on slide and latex agglutination tests with live bacteria (7, 12) and the passive hemagglutination (PHA) technique with heated saline extracts (11, 17).

Traditionally, the antisera used for typing live bacteria are absorbed with boiled organisms before assay (7, 12). Therefore, serotyping with these reagents is based on heat-labile antigens. The precise nature of these flagellar and capsular antigens as well as that of heat-stable antigens has not been defined. In addition, various cross-reactions have been observed among type strains and clinical isolates, indicating the complexity of both heat-labile and heat-stable antigens (7, 11, 12, 17). The advantages offered by monoclonal antibodies in the study of antigenic components of bacteria and in the development of diagnostic reagents (3, 23) prompted us to explore their application for *Campylobacter* spp. We have established 29 hybridoma cell lines producing monoclonal antibodies that reacted with *C. jejuni* 143483, and we have characterized their behavior in several different immunoassays.

MATERIALS AND METHODS

***Campylobacter* strain used as immunogen.** *C. jejuni* 143483 was originally isolated from a human stool specimen in Edinburgh, United Kingdom, by Watson et al. (26). The bacteria were oxidase-, catalase-, and hippurate-positive, H_2S-negative, nalidixic acid-susceptible, curved gram-negative rods (19, 20).

Antigen preparation. The bacteria for the antigenic preparations were grown on Mueller-Hinton agar medium supplemented with IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.) at 43°C in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2.

Immunizations. After a 48-h incubation, the bacteria were harvested in sterile saline containing 0.5% Formalin, incubated at 37°C for 4 h, and then stored at 4°C. For injections, the suspension was adjusted with saline to a concentration of 10^{10} CFU/ml according to the McFarland scale. Two BALB/c mice were injected with 0.2 ml of the suspension intraperitoneally on day zero and intravenously on day 21.

Production of hybridomas. The immune spleen cells were fused with myeloma cells 4 days after the second immunization by the method of Galfre et al. (5), with minor modifications (2). When the cells had reached 50% confluency in the majority of the wells showing cell growth, the supernatants were collected and assayed by enzyme immunoassay (EIA) with whole bacteria as described below. The cells from the wells in which antibody production against the immunogen strain was observed were grown in vitro to stabilize the lines. For ascites production, 0.4×10^6 to 2.0×10^6 cells of these hybridoma lines were inoculated intraperitoneally into BAB 14 mice. As soon as ascites formation was observed (about 3 weeks), the ascitic fluids were withdrawn. The ascites cells were stored in liquid nitrogen, and the fluids were stored in small samples at −20°C.

Testing of the hybridoma product. Determination of the isotype. The ascitic fluids were diluted 1:30 to 1:40 in phosphate-buffered saline (PBS, no. D-026, Orion Diagnostica, Helsinki, Finland) and examined by immunodiffusion on 1% agar slides with antisera prepared in rabbits against purified mouse immunoglobulin G1, G2a, G2b, and G3 (no. 8403-04-06 and 8405-08, Litton Bionetics, Kensington, Md.).

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Electrophoresis. The ascitic fluids were also analyzed by electrophoresis on cellulose acetate plates (Helena Laboratories, Beaumont, Tex.) at pH 8.6 (180 V, 15 min). The plates were stained with Ponceau S stain (Gelman Instrument Co., Ann Arbor, Mich.).

EIA. For the detection of antibodies in cell culture supernatants and ascites, the method of Voller et al. (25) was used with minor modifications. Briefly, the procedure was as follows. Live whole bacteria were harvested in saline, and the density of the suspension was adjusted with a spectrophotometer to give an optical reading of 0.05 at 650 nm. This suspension was used to coat cuvettes, which were incubated overnight at 37°C. After three washings with PBS-Tween, the culture supernatants (diluted 1:5) or ascites samples (diluted from 1:50 to 1:500) were added to the cuvettes, which were incubated for 2 h at room temperature. After three washings, alkaline phosphatase-labeled anti-mouse immunoglobulin (Orion Diagnostica) was added, and the cuvettes were again incubated for 2 h at room temperature. After three further washings, freshly prepared *p*-nitrophenylphosphate substrate solution (1 mg/ml) was added, and the cuvettes were then incubated at 37°C for 30 to 100 min (until the positive mouse serum control included in the series gave a spectrophotometer reading of about 1). The reaction was stopped with 3 M NaOH, and readings were made with a spectrophotometer at 400 nm. All individual EIA values were calculated as the means of two parallel determinations. The cell culture supernatants were tested once, and the ascitic fluids were tested two to four times. Determination of cross-reactions with other bacteria was also made by EIA. Twenty *C. coli* and *C. jejuni*, two *Campylobacter fetus* subsp. *venerealis*, and two *C. fetus* subsp. *fetus* strains (7) were tested once as above. In addition, single strains of *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Yersinia enterocolitica*, and *Pseudomonas aeruginosa*, all isolated from clinical samples, were run once in the EIA as described above for the immunogen strain.

The EIA used for the detection of antibodies directed against an acid extract of whole cells were carried out by using an antigenic preparation obtained by treating live bacteria with 0.2 M glycine-hydrochloride buffer, pH 2.2, as described by McCoy et al. (15). The neutralized, dialyzed, and then lyophilized material was dissolved in PBS (3 μg/ml) to coat the cuvettes, which were incubated with the antigen at 37°C overnight. The assay was composed as described above.

EIA for antibodies against LPS. Lipopolysaccharide (LPS) was extracted with hot phenol water, and the nucleic acids were removed as described by Staub (21). The lyophilized powder was characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10). No protein bands could be visualized with Coomassie blue when 40 μg of the preparation was applied per lane. The few fast-migrating bands seen in a silver-stained preparation (24) were similar to those of an LPS from a rough *Salmonella typhimurium*, with no evidence of the wash board-like bands (16) observed when an LPS preparation from a smooth *S. typhimurium* was applied for comparison (*S. typhimurium* LT2, strain SH2183, rough, chemotype Ra, complete core (16), and *S. typhimurium* LT2, strain SH9013, smooth; the LPS preparations were obtained by courtesy of M. Sarvas, Institute of Public Health, Helsinki, Finland). The contents of reducing sugars (4) in our LPS preparation were lower than those of the LPS from the rough and smooth *Salmonella* strains (7, 45, and 72%, respectively, when determined with D-glucose as standard). For the EIA, the dried LPS preparation was dissolved in PBS (1 μg/ml) to coat the cuvettes. Further assay was done as described above.

Slide agglutination. One drop of ascites diluted 1:5 in saline was placed on a glass slide. Material from a smooth colony was taken with a toothpick and gradually mixed with the ascites. The slide was tilted for 2 min, and the reaction was read with a magnifying glass. Negative reactions were recorded as −, and positive ones as + (weak), ++ (moderate), and +++ (strong and very strong).

Tube agglutination. Twofold dilutions of the ascites samples were made in saline; 0.2 ml of each dilution was mixed with an equal amount of the antigen preparation (formalinized antigen, 0.5% formalinized saline at 35°C overnight, or antigen heated at 100°C for 1 h in saline and stored in 0.5% formalinized saline). The tubes with formalinized antigen were incubated for 4 h at 37°C, and those with heated antigen were incubated for 20 h at 56°C (1). Readings were made with a magnifying glass.

PHA tests with sheep erythrocytes coated with saline extracts of bacteria. The technique described by Penner and Hennessy (17) was used for autoclaved, boiled, and unheated extracts. For each preparation, growth from four plates was harvested in 4 ml of saline. For the unheated antigen, the suspension was extracted at room temperature in a small flask stirred with a magnetic rotator for 1 h. The boiled extract was heated in a water bath at 100°C for 1 h, and the autoclaved extract was heated for 30 min at 120°C. Each preparation was centrifuged, and the supernatant was used (diluted 1:10 in PBS) to sensitize sheep erythrocytes. The extracts were mixed with an equal volume of a 1% suspension of sheep erythrocytes in PBS and incubated for 1 h at 37°C. The sensitized erythrocytes were washed three times in PBS and used as an 0.5% suspension. Twofold dilutions of the ascites samples were made in PBS, and 0.025 ml of each dilution was mixed with an equal volume of the sensitized erythrocytes in microtitration plates with U-shaped wells. The plates were shaken, incubated at 35°C for 1 h, stored overnight at 4°C, and then read for agglutination of erythrocytes.

PHA test for antibodies against polysaccharides. A polysaccharide preparation was made by the method of Liu et al. (13) from bacteria grown as above. The presence of reducing sugars was determined spectrophotometrically at 490 nm by the method of Dubois et al. (4), with D-glucose as the standard preparation. Our material contained 90% reducing sugars. When 100 μg of the polysaccharide preparation was added to a lane in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis run (10), no protein bands could be visualized with Coomassie blue. For the PHA test, the dried powder was dissolved in saline to a concentration of 5 μg/ml. This solution was used to sensitize an equal volume of a 1% suspension of sheep erythrocytes in PBS. The tests were carried out as described above for the saline extracts.

RESULTS

After fusion, the cells were seeded into 420 wells (2×10^5 cells per well). Cell growth was observed in 273 wells, and antibody production was observed in 51 wells. After additional culturing in vitro, the 29 surviving clones were injected into BAB 14 mice (0.2×10^6 to 2.0×10^6 cells per mouse). Each hybridoma cell line, except cell line 14, produced monoclonal immunoglobulins as determined by electrophoresis on cellulose acetate plates (cell line 14 had and additional band). The isotypes for the monoclonal antibodies are listed in Table 1.

TABLE 1. Isotypes of ascitic fluids and EIA values of representative runs with the immunogen

Ascites no.	Isotype	Optical density of:	
		Live bacteria antigen (1:50)[a]	Acid extract antigen (1:100)
1	γ1	1.15	0.10
2	γ2b	1.75	0.06
3	γ3	2.59	0.19
4	γ2b	1.93	0.12
5	γ3	1.13	0.08
6	γ2a	2.57	0.67
7	γ1	0.30	0.09
8	γ2b	1.74	0.19
9	γ1	2.51	0.17
10	γ2b	0.99	0.06
11	γ2a	1.12	0.05
12	γ2b	0.64	0.03
13	γ1	1.99	0.16
15	γ1	2.15	0.11
16	γ1	0.53	0.07
17	γ1	1.43	0.15
18	γ1	0.57	0.15
19	γ1	2.39	0.23
20	γ2b	1.45	0.06
21	γ2a	0.76	0.05
22	γ2a	2.39	0.20
23	γ1	1.68	0.12
24	ND[b]	1.50	ND
25	ND	0.60	0.05
26	γ3	0.86	0.06
27	γ2a	2.14	0.32
28	γ1	0.95	0.07
29	ND	1.10	0.09
30	γ1	0.47	0.01
Control		0.35	0.01

[a] Numbers in parentheses show dilution of ascitic fluid.
[b] ND, Not determined.

Eight ascitic fluids reacted very strongly with whole live bacteria in the EIA (Table 1). Two of these clones, 3 and 9, produced antibody in high titers which reacted strongly in the PHA tests with saline extracts of unheated, boiled, and autoclaved whole bacteria as well as with purified polysaccharide preparation. In addition, these two antibodies reacted in slide agglutination tests with live bacteria (Table 2). Four additional clones also reacted with live bacteria in slide agglutination tests. None of the 29 clones reacted in tube agglutination tests with formalinized whole bacteria, but three clones were positive in tube agglutination tests with a boiled suspension of whole bacteria; two of these also reacted strongly in PHA tests with boiled saline extract, but no other reactions were observed for the third clone. In all, PHA tests with boiled saline extracts gave positive reactions with seven ascitic fluids, some of which were poorly reactive in the EIA with whole bacilli as the antigen. When the saline extracts were autoclaved and used in PHA tests, the same four clones that reacted in this test also reacted with purified polysaccharide.

None of the monoclonal antibodies reacted with the LPS antigen (data not shown). A strong reaction was seen with ascites 14, but this product was not monoclonal. Ascites 14 did not react in PHA tests.

Clone 6 produced antibodies which reacted strongly in the EIA with the acid glycine extract preparation (Table 1), but this reaction did not correlate with any other tests carried out with the immunogen strain.

Reactivity of the ascites samples against other *Campylobacter* spp. and other unrelated bacteria was studied by the EIA (Table 3). None of the antibodies reacted with unrelated bacteria, but several clones bound to several or many of the *Campylobacter* strains, including *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus*. Clone 6 reacted with all *Campylobacter* strains tested and gave the highest average EIA value of all clones with each subspecies.

DISCUSSION

Antibodies in primary hybridoma culture fluids could be screened and detected successfully by using a suspension of live bacteria as the antigen in the EIA. Most of the 30 clones grown in mouse ascites produced antibodies detectable by the same assay, and 12 produced antibodies detectable by other tests as well. These tests included both slide agglutination of live bacteria and PHA with heated saline extracts as antigen, which are the basic methods for serotyping with conventional antisera (7, 11, 12, 17).

The unique specificity of the monoclonal antibodies makes them an excellent tool for exploring the details of complex antigens like those of bacteria. We obtained four clones that reacted with the purified polysaccharide, thus implying the presence of carbohydrate antigens in *Campylobacter* spp. Two of these clones, 3 and 9, gave a positive slide agglutination reaction as well, indicating that carbohydrates may be an important group of antigens which contribute to slide agglutination by unabsorbed polyvalent sera. At least a part of the carbohydrate antigens are loosely bound to the cell wall. This was shown by their presence in the saline extracts prepared at room temperature, as demonstrated by the PHA test with these two clones. By extracting the bacteria with boiling saline, additional antigens which reacted with several clones in the PHA test were obtained. Some of these antigens resisted autoclaving. The fact that the four antipolysaccharide clones reacted with the boiled and autoclaved saline extracts shows that both the extract and the polysaccharide have similar antigenic determinants. Since Penner et al. (17) and Lauwers et al. (11) extracted the serotyping antigens for PHA with boiling saline, it is apparent that polysaccharide antigens are involved in their typing reactions. The finding of three further clones which reacted with boiled saline extracts but not with the polysaccharide in PHA indicates the complexity of the PHA antigen used in serotyping. These additional heat-stable antigens may be chemically something other than carbohydrates, or they may be carbohydrates which do not have the same antigenic determinants as those with which our antipolysaccharide clones reacted. Since two of these three clones agglutinated boiled bacteria in the tube tests, there must also be similar antigenic determinants which remain in the cell wall, but their chemistry remains to be determined.

The failure of the antipolysaccharide clones to react with the LPS preparation is in accord with the low carbohydrate content of the LPS. This was shown by the sodium dodecyl sulfate-polyacrylamide gel analysis, in which our LPS resembled that from a rough *Salmonella* strain known to lack carbohydrate chains. A similar LPS was recently extracted from some *Campylobacter* strains by Logan and Trust (14). The only ascites (no. 14, not monoclonal) which reacted strongly with the LPS did not give a positive PHA reaction.

TABLE 2. Agglutination reactions of ascitic fluids with different antigenic preparations of the immunogen

Ascites no.	Test results[a]					
	Slide agglutination[c]	Tube agglutination[d]	PHA[b]			
			Saline extract	Boiled extract	Autoclaved extract	Polysaccharide
1				1:1,280		
2						
3	+++		1:5,120	1:1,280	1:5,120	1:2,560
4		1:800		1:1,280		
5				1:640	1:2,560	1:640
6						
7						
8						
9	+++		1:5,120	1:640	1:5,120	1:640
10						
11						
12						
13						
15						
16		1:3,200				
17						
18	++					
19						
20				1:80	1:160	1:320
21						
22						
23						
24		1:1,600		1:1,280		
25						
26	+					
27	+					
28	++					
29						
30						

[a] Negative reaction was obtained unless otherwise indicated.
[b] First dilution studied, 1:40.
[c] Live bacteria antigen; ascitic fluid diluted 1:5.
[d] Boiled bacteria antigen; first dilution studied, 1:100.

This is inconsistent with the idea that the antigen responsible for the PHA serotyping could be the LPS as such. In addition to the two antipolysaccharide clones, there were four clones which gave a slide agglutination reaction with live bacteria. None of the clones, however, agglutinated formalinized bacteria in the tube test, which has been presumed to detect antiflagellar antibodies (1). These findings indicate that, besides the flagellum-dependent reaction, there must be other antigen-antibody pairs which contribute to the slide agglutination of live bacteria. It is apparent that very little is known of the heat-labile antigens on which some preliminary typing schemes are based (7, 12) and that great care is needed when the polyvalent typing sera are absorbed with heat-stable antigens.

Monoclonal antibodies can also be used to investigate the common antigens by studies of their reactivity with other bacterial strains. None of our clones reacted with the few unrelated bacteria tested, but many clones reacted with several *Campylobacter* ssp. One of the clones gave exceptionally high EIA values with all 24 *Campylobacter* strains tested, including two *C. fetus* subsp. *venerealis* and two *C. fetus* subsp. *fetus* strains. The same clone gave a high EIA value with the acid extract prepared from the immunogen as described by McCoy et al. for *C. fetus* subsp. *fetus* (15). This extract was reported by McCoy et al. to be superficial, antiphagocytic, and responsible for the non-agglutinability of the strains in O-type antisera. They also suggested that it might be a virulence factor. Our interest in this antigenic preparation was further increased when we found that not only all 21 rabbits immunized with different *Campylobacter* strains, but also the majority of human patients with *Campylobacter* enteritis, had antibodies which cross-reacted with this extract from our immunogen strain (9, 18). A similar antigen extracted from two *Campylobacter* strains was successfully used in serological studies on human serum samples by Svedhem et al. (22).

The facts that monoclonal reagents have high specificity and that they react in tests used in the preliminary serotyping systems reported are encouraging for further exploration of their use in developing serotyping systems based on well-defined antigens. The clones reacting with the polysaccharides are the most promising in this context, but it will be interesting to study also the other clones which react, for example, in PHA tests with strains that cross-react with the immunogen when typed with polyvalent sera.

The monoclonal reagents also seems to offer an excellent

TABLE 3. Cross-reactions of some ascitic fluids with 24 *Campylobacter* strains and 5 other bacterial strains

Ascites (1:100)	Optical density in EIA					
	C. jejuni and *C. coli*[a]	*C. fetus* subsp. *venerealis*		*C. fetus* subsp. *fetus*		Other bacteria[b]
		13823	14840	13014	14865	
1	0.48 ± 0.25	0.31	0.54	0.26	0.50	0.09 ± 0.03
3	0.76 ± 0.43	0.34	0.75	0.43	0.71	0.16 ± 0.02
6	1.54 ± 0.63	1.13	2.03	1.33	2.00	0.01 ± 0.02
8	0.65 ± 0.40	0.41	0.81	0.42	0.61	0.14 ± 0.03
9	0.43 ± 0.26	0.13	0.31	0.15	0.30	0.09 ± 0.03
13	0.62 ± 0.31	0.27	0.54	0.30	0.47	0.16 ± 0.04
15	0.43 ± 0.26	0.18	0.46	0.25	0.36	0.07 ± 0.04
19	0.82 ± 0.48	0.46	1.26	0.56	1.53	0.10 ± 0.02
22	0.92 ± 0.45	0.44	0.87	0.44	0.74	0.14 ± 0.03
23	0.49 ± 0.31	0.15	0.43	0.12	0.36	0.06 ± 0.01
27	0.69 ± 0.42	0.39	0.93	0.46	0.81	0.07 ± 0.01

[a] Mean ± standard deviation for 20 strains.
[b] Mean ± standard deviation for five strains.

way for characterizing and purifying those bacterial antigens for which earlier methods have proved inadequate. The acid glycine extract found to cross-react with most other *Campylobacter* spp. demonstrates that, despite the high antigenic variability revealed by serotyping, there seems to be common antigens. Further studies are needed to determine whether acid extracts from one or two strains, or at most a small number, would be sufficiently cross-reactive to react with all *Campylobacter* strains and thus to be usable in serological studies of clinical material.

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LITERATURE CITED

1. **Abbott, J. D., B. Dale, J. Eldridge, D. M. Jones, E. M. Sutcliffe.** 1980. Serotyping of *Campylobacter jejuni/coli*. J. Clin. Pathol. **33**:762–766.
2. **Bång, B. E., M. Hurme, K. Juntunen, and O. Mäkelä.** 1981. Studies of monoclonal and polyclonal anti-digoxin antibodies for serum digoxin radioimmunoassay. Scand. J. Clin. Lab. Invest. **41**:75–78.
3. **Coates, A. R. M., J. Hewitt, B. W. Allen, J. Ivanyi, and D. A. Mitchison.** 1981. Antigenic diversity of Mycobacterium tuberculosis and Mycobacterium bovis detected by means of monoclonal antibodies. Lancet **ii**:167–169.
4. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28**:350–356.
5. **Galfre, G., S. C. Howe, C. Milstein, G. W. Butcher, and J. C. Howard.** 1977. Antibodies to major histocompatibility antigens produced by hybrid cells lines. Nature (London) **266**:550–552.
6. **Hébert, G. A., D. G. Hollis, R. E. Weaver, M. A. Lambert, M. J. Blaser, and C. W. Moss.** 1982. 30 years of campylobacters: biochemical characteristics and a biotyping proposal for *Campylobacter jejuni*. J. Clin. Microbiol. **15**:1065–1073.
7. **Kosunen, T. U., D. Danielsson, and J. Kjellander.** 1982. Serology of *Campylobacter fetus* ss. *jejuni*. 2. Serotyping of live bacteria by slide, latex and co-agglutination tests. Acta Pathol. Microbiol. Scand. Sect. B **90**:191–196.
8. **Kosunen, T. U., S. Pöllänen, M. L. Hänninen, and D. Danielsson.** 1982. Are the *Campylobacter jejuni* strains from different species similar? p. 45–49. *In* D. G. Newell (ed.) Campylobacter. MTP Press, Lancaster, United Kingdom.
9. **Kosunen, T. U., H. Rautelin, T. Pitkänen, A. Pönkä, and T. Pettersson.** 1983. Antibodies against an acid extract from a single campylobacter strain in hospitalized campylobacter patients. Infection **11**:189–191.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Lauwers, S., L. Vlaes, and J. P. Butzler.** 1981. Campylobacter serotyping and epidemiology. Lancet **i**:158–159.
12. **Lior, H., D. L. Woodward, J. A. Edgar, L. J. Laroche, and P. Gill.** 1982. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J. Clin. Microbiol. **15**:761–768.
13. **Liu, T.-Y., E. C. Gotschlich, E. K. Jonssen, and J. R. Wysocki.** 1971. Studies on the meningococcal polysaccharides. I. Composition and chemical properties of the group A polysaccharide. J. Biol. Chem. **246**:2849–2858.
14. **Logan, S. M., and T. J. Trust.** 1982. Outer membrane characteristics of *Campylobacter jejuni*. Infect. Immun. **38**:898–906.
15. **McCoy, E. C., D. Doyle, K. Burda, L. B. Corbeil, and A. J. Winter.** 1975. Superficial antigens of *Campylobacter (Vibrio) fetus*: characterization of an antiphagocytic component. Infect. Immun. **11**:517–525.
16. **Palva, E. T., and P. H. Mäkelä.** 1980. Lipopolysaccharide heterogeneity in *Salmonella typhimurium* analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Eur. J. Biochem **107**:137–143.
17. **Penner, J. L., and J. N. Hennessey.** 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J. Clin. Microbiol. **12**:732–737.
18. **Rautelin, H., and T. U. Kosunen.** 1983. An acid extract as a common antigen in *Campylobacter coli* and *Campylobacter jejuni* strains. J. Clin. Microbiol. **17**:700–701.
19. **Skirrow, M. B., and J. Benjamin.** 1980. Differentiation of enteropathogenic campylobacter. J. Clin. Pathol. **33**:1122.
20. **Smibert, R. M.** 1974. Genus II. *Campylobacter* Sebald and Véron, 1963, 907, p. 207–212. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Company, Baltimore.
21. **Staub, E. M.** 1967. Preparation of cell wall antigens from Gram-negative bacteria. Methods Immunol. Immunochem. **1**:28–34.
22. **Svedhem, Å., H. Gunnarsson, and B. Kaijser.** 1982. Serological diagnosis of *Campylobacter jejuni* infections using the enzyme-linked immunosorbent assay principle, p. 118–121. *In* D. G. Newell (ed.), Campylobacter. MTP Press, Lancaster, United Kingdom.
23. **Tam, M. R., T. M. Buchanan, E. G. Sandström, K. K. Holmes, J. S. Knapp, A. W. Siadak, and R. C. Nowinski.** 1982. Serological classification of *Neisseria gonorrhoeae* with monoclonal antibodies. Infect. Immun. **36**:1042–1053.
24. **Tsai, C.-M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
25. **Voller, A., D. Bidwell, and A. Bartlett.** 1976. Microplate enzyme immunoassays for the immunodiagnosis of virus infections, p. 506–512. *In* N. R. Rose and H. Friedman (ed.), Manual of clinical immunology, 1st ed. American Society for Microbiology, Washington, D.C.
26. **Watson, K. C., E. J. C. Kerr, and S. M. McFadzean.** 1979. Serology of human campylobacter infections. J. Infect. **1**:151–158.