The Identification of Outer Membrane Proteins and Flagella of *Campylobacter jejuni*

By D. G. NEWELL,* H. MCBRIDE AND A. D. PEARSON

Public Health Laboratory, General Hospital, Tremona Road, Southampton SO9 4XY, UK

(Received 11 October 1983)

The outer membrane proteins of five clinical isolates of *Campylobacter jejuni* were identified by ¹²⁵I-surface labelling and SDS-PAGE of outer membrane preparations. All isolates expressed a major outer membrane protein of variable molecular weight (43 000–46 000: 43K–46K). Several constant surface proteins were also identified including a 27K protein which was surface-exposed and acid-extractable but was not present in the outer membrane preparations. Isolated flagella comprised a major 62K protein and a minor 87K protein. Both proteins were absent in an aflagellate variant. The 62K protein was immunoblotted and immunoprecipitated by rabbit anti-flagella antisera.

INTRODUCTION

The thermophilic, microaerophilic enteropathogen *Campylobacter jejuni* is the most frequently reported cause of human acute bacterial gastroenteritis in the UK (Skirrow, 1982). *Campylobacter* enteritis usually presents as acute diarrhoea, generally accompanied by abdominal pain and preceded by fever and malaise (Butzler & Skirrow, 1979). The mechanism by which *C. jejuni* induces diarrhoea is unknown. Although there is no laboratory evidence for the production of an enterotoxin (Guerrant *et al.*, 1978; Manninen *et al.*, 1982) *C. jejuni* strains demonstrate invasive potential *in vitro* and *in vivo* (Manninen *et al.*, 1982; Davidson & Solomon, 1980).

Despite the prevalence of *C. jejuni* infections little is known about the surface structure of the organism. Such information is highly relevant to the study of pathogenic mechanisms as the successful establishment of an enteric infection is dependent on a number of surface components (virulence factors) which, for example, mediate attachment to the intestinal epithelial cells, preventing removal by gut motility or mucosal flow. This close association with the epithelial mucosa is a prerequisite to invasion or the effective action of enterotoxin (Giannella, 1981). Additionally, surface components may be immunogenic during infection thereby determining the host immune response to the infective agent.

This report describes the identification of the outer membrane proteins of five clinical isolates of *C. jejuni* by ¹²⁵I-surface labelling and SDS-PAGE of outer membrane preparations. Additionally, flagella have been isolated and the flagellin protein identified using flagellate and aflagellate organisms with immunoblotting and radioimmunoprecipitation techniques.

METHODS

Bacterial strains. The *C. jejuni* isolates used in this study are described in Table 1. All strains, except 81116, were from sporadic cases of campylobacter enteritis. Strain 81116 was isolated from a patient involved in an outbreak of campylobacter enteritis at a school (Palmer *et al.*, 1983). Strains were biotyped as described by Skirrow & Benjamin (1980) and kindly serotyped by Dr J. Penner using the passive haemagglutination technique (Penner & Hennessy, 1980).

Faecal isolates were purified on antibiotic selective medium at 42 °C (Skirrow, 1977) under microaerophilic conditions. Purified isolates were subcultured on blood agar containing 5% (v/v) defibrinated horse blood and 2% (w/v) agar and stored in 10% (v/v) glycerol in 1% (w/v) proteose peptone, in liquid nitrogen. For outer membrane

Table 1. *Strains of C. jejuni/coli investigated*

Strain	Source	Biotype	Serotype*
81116	Human	<i>C. jejuni</i> II	6
63923	Human	<i>C. jejuni</i> I	2
33817	Human	<i>C. jejuni</i> II	UT
68869	Human	<i>C. jejuni</i> I	UT
51180	Human	<i>C. jejuni</i> I	30

* UT, untypable.

preparations isolates were cultured on blood-free agar (2%, w/v, agar containing 0.4%, v/v, campylobacter growth supplement; Oxoid). For flagella and acid extract preparations isolates were cultured in nutrient broth: 2.5% (w/v) nutrient broth base no. 2 (Oxoid); 0.5% (w/v) yeast extract; 0.01% (w/v) cysteine; 0.08% (w/v) potassium nitrate and 0.4% (v/v) campylobacter growth supplement.

An aflagellate variant (81116 Fla⁻) was isolated from the flagellate wild-type (81116 Fla⁺) by enrichment of non-motile organisms in semi-solid nutrient gelatin medium: 1% (w/v) peptone; 0.33% (w/v) yeast extract; 0.5% (w/v) sodium chloride; 0.8% (w/v) gelatin; 0.75% (w/v) agar. Non-motile colonies were identified on nutrient gelatin medium containing 1% (w/v) agar. The variant was cloned six times to confirm its stability.

Antisera. Rabbits were immunized intramuscularly with formalin-treated (0.3% formalin) bacterial suspension (0.5 ml, A_{540} = 10.0), outer membrane preparation (20 µg protein) or purified flagella (10 µg protein) in Freund's complete adjuvant. The rabbits were boosted at 14 d intervals with a series of subcutaneous injections of the same antigen in Freund's incomplete adjuvant.

¹²⁵I-surface labelling of whole bacteria. *Campylobacter jejuni* strains, grown on blood-free agar, were washed twice by centrifugation (5000 g for 15 min at 4 °C) in phosphate-buffered saline [PBS: 0.8% (w/v) sodium chloride; 0.02% (w/v) potassium chloride; 0.115% (w/v) disodium hydrogen phosphate; 0.02% (w/v) potassium dihydrogen phosphate] with 0.01% (w/v) calcium chloride and 0.01% (w/v) magnesium chloride. Bacteria were ¹²⁵I-surface labelled using the lactoperoxidase method (Marchalonis *et al.*, 1971). Briefly, 1×10^9 c.f.u. in 500 µl PBS, with calcium and magnesium, were mixed with 5 µl of a 1 mg ml⁻¹ solution of lactoperoxidase (Sigma) and 50 µCi (1850 kBq) Na¹²⁵I (Amersham). Portions (50 µl) of 0.02 M-hydrogen peroxide were added at 2.5 min intervals and the reaction was stopped after 10 min by the addition of 10 ml 5 mM-cysteine. The specific activity of the ¹²⁵I-labelled bacteria was approximately 5 µCi mg⁻¹.

For radioimmunoprecipitation experiments bacteria were surface-labelled using 1,3,4,6-tetrachloro-3α,6α-diphenyl glycouril (Iodogen; Pierce & Warriner, Chester, UK) by the technique of Swanson (1981). Approximately 3×10^8 c.f.u. in 40 µl PBS were incubated with 5 µl of 10⁻⁵ M-potassium iodide and 500 µCi Na¹²⁵I in a small glass tube coated with 10 µg Iodogen for 10 min at 20 °C. ¹²⁵I-labelled bacteria (specific activity approximately 1 µCi µg⁻¹) were washed three times in PBS and solubilized for radioimmunoprecipitation experiments in 0.1% (w/v) SDS, 3.0% (w/v) Empigen BB (Albright & Wilson, Whitehaven, UK) in PBS at 40 °C for 1 h.

Preparation of spheroplast-derived outer membranes. Nutrient broth cultures were harvested by centrifugation (5000 g, 20 min) and resuspended in 0.75 M-sucrose, 10 mM-Tris/HCl, pH 7.8, at 4 °C to give an A_{540} of 10.0. Lysozyme was added to the suspension to give a final concentration of 0.2 mg ml⁻¹ followed by the slow addition of two volumes of 4.5 mM-EDTA, pH 7.5, at 4 °C. After 2 h of gentle stirring at 20 °C, 80–90% of the bacteria had formed spheroplasts. Spheroplast lysis and membrane separation were initiated by the addition of the following agents to the stated final concentrations: 0.05% (w/v) Brij 58 (Sigma); 14 units DNAase I ml⁻¹ (Sigma); 15 units RNAase A ml⁻¹ (Sigma) and 3 mM-magnesium chloride. After incubation for 20 min at 37 °C unlysed cells were removed by centrifugation (5000 g, 30 min) and the membrane fraction was recovered from the supernatant solution (100000 g, 2 h). The membrane pellet was washed in 3 mM-EDTA, 10 mM-Tris/acetate buffer, pH 7.5, then layered on a 20–55% (w/w) sucrose gradient containing 5 mM-EDTA and centrifuged at 90000 g for 18 h. Fractions were collected, analysed for protein and pooled as appropriate. Succinate dehydrogenase activity was determined by the reduction of 2,6-dichlorophenol indophenol coupled to phenazine methosulphate (Arrigoni & Singer, 1962; Churchward & Holland, 1976) and lipopolysaccharide estimated by the thiobarbituric acid assay for ketodeoxyoctonate (Waravdekar & Saslaw, 1969).

Preparation of Sarkosyl-insoluble outer membranes. *Campylobacter jejuni* was harvested as previously described, washed in 0.05 M-Tris/HCl buffer, pH 7.5, at 4 °C and resuspended in the same buffer to give an A_{540} of 1.0. The suspension was sonicated (4 × 30 s periods with 30 s cooling intervals) at 10 µA (Soniprep 150; MSE). Cellular debris was removed by centrifugation at 5000 g for 20 min and the crude membranes were recovered from the supernatant (100000 g, 60 min). The membrane pellet was suspended in 1% (w/v) N-lauroylsarcosine sodium salt (Sarkosyl; Sigma) in 7 mM-EDTA, pH 7.6, at 37 °C for 20 min to give a protein to detergent ratio of approximately 1:4 (w/w) (Filip *et al.*, 1973). The suspension was centrifuged at 100000 g for 2 h and the pellet re-extracted with detergent as above. The extracted membranes were washed three times in 0.05 M-Tris/HCl buffer, pH 7.5, and stored at -20 °C.

Preparation of acid extract. An acid extract was prepared from *C. jejuni* 81116 according to the method of McCoy *et al.* (1975). Bacteria (4.0 g wet weight) were washed twice in distilled water and resuspended in 100 ml 0.2 M-glycine/HCl buffer, pH 2.2. The suspension was stirred for 15 min at 20 °C then centrifuged to remove the cells (10000 g, 20 min). The supernatant was neutralized and dialysed against distilled water.

Preparation of flagella. Bacteria (5.5 g) were resuspended in 250 ml PBS with calcium and magnesium at 4 °C and homogenized in a laboratory blender fitted with disintegrating and axial flow heads (Silverson Machines, Chesham, UK) for three periods of 1 min with 30 s cooling intervals. Isolation of the flagella was achieved by centrifugation at 5000 g for 30 min to remove the cellular debris, followed by centrifugation of the supernatant solution at 100000 g for 2 h. These centrifugation steps were repeated twice. The purity of the flagella was monitored by electron microscopy of negative-stained preparations.

¹²⁵I-labelling of flagella. This was done according to the method of Der-Balain (1980). Briefly, 120 µg of flagella in 0.3 M-Tris/HCl buffer, pH 8.5, were added to 500 µCi (18 500 kBq) methyl 3,5-di[¹²⁵I]iodohydroxybenzimidate (Amersham). The mixture was gently stirred for 48 h at 37 °C. The flagella were dialysed against PBS and resuspended in 3% (v/v) Empigen BB, 0.1% (w/v) SDS in PBS. The specific activity was approximately 2 µCi mg⁻¹.

SDS-PAGE. A 10–25% (w/v) linear gradient SDS-PAGE system was used (Lambden *et al.*, 1979). Protein bands were stained with Kenacid blue R (BDH). The protein molecular weight markers were trypsin, ovalbumin, bovine serum albumin (BSA) and lysozyme.

Radioimmunoprecipitation. ¹²⁵I-labelled material was centrifuged at 10000 g for 1.5 h to remove any insoluble material and 80 µl samples (approximately 40000 c.p.s. of ¹²⁵I-solubilized bacteria or 2000 c.p.s. of ¹²⁵I-flagella) were mixed with 40 µl of a 1:2 dilution of rabbit sera for 20 min at 4 °C. Then 40 µl swollen protein A covalently coupled to Sepharose CL-4B (Sigma) swollen in 100 µl PBS containing 1 mg BSA was added to each sample. The samples were incubated at 20 °C for 1 h with mixing and the Sepharose beads were then washed five times in PBS.

Electroimmunoblotting. SDS-PAGE gels of strains 81116 Fla⁺ and 81116 Fla⁻ and of purified flagella were electroblotted on to nitrocellulose in 3.0 g Tris l⁻¹, 14.4 g glycine l⁻¹ and 20% (v/v) methanol at 60 V for 5 h. Non-specific protein binding was eliminated by incubation in 3% (w/v) BSA in 10 mM-Tris, 0.9% (w/v) sodium chloride, pH 7.4, for 2 h at 37 °C. The blots were then incubated with 1:100 rabbit antisera diluted in dilution buffer consisting of 50 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-sodium chloride, 5 mM-EDTA, 0.25% (w/v) gelatin and 0.05% (v/v) Nonidet P-40 (Sigma), for 2 h at 37 °C. After a brief wash in PBS the blots were incubated (2 h, 37 °C) with 1:1000 (in dilution buffer) peroxidase-conjugated goat anti-rabbit IgG (Miles Research Laboratories, Slough, UK) and the unbound protein was removed by extensive washing [500 mM-Tris/HCl buffer, pH 7.4, containing: 1 M-sodium chloride; 50 mM-EDTA; 0.25% (w/v) gelatin; 0.4% (w/v) sodium lauroylsarcosine]. Peroxidase was detected by incubation with 2.5 mg o-dianisidine in 100 ml 10 mM-Tris/HCl buffer, pH 7.4, containing 100 µl hydrogen peroxide (100 vol.) at 37 °C for 60 min.

RESULTS

Identification of the outer membrane proteins of C. jejuni

The surface-exposed outer membrane proteins of five *C. jejuni* isolates were ¹²⁵I-labelled using the lactoperoxidase technique (Fig. 1). A major polypeptide of variable molecular weight (43000–46000: 43K–46K) was seen in all isolates. Four minor constant proteins with molecular weights of 72K, 64K, 35K and 27K and a minor variable protein (70K–75K) were also ¹²⁵I-labelled. Iodination of surface proteins using the Iodogen technique instead of lactoperoxidase produced a similar pattern of ¹²⁵I-labelled proteins but an additional protein of 27.5K was observed. The isolation of outer membranes was considered necessary for further characterization of these surface proteins. Initially a spheroplasting technique was used to prepare outer membranes. Sucrose gradient separation of the membranous material from the lysed spheroplasts gave three protein peaks ($\rho = 1.17, 1.20$ and 1.28 g ml^{-1}) the fractions of which were pooled (P1, P2 and P3 respectively). P1 contained 58% of the succinate dehydrogenase activity and 20.9% of the LPS, whilst P3 contained 20% of the succinate dehydrogenase activity and 49% of the LPS. The SDS-PAGE profiles of these pooled fractions are shown in Fig. 2(a). P3 contained a restricted number of proteins with enhanced concentrations of the major variable protein (43K–46K) and a 62K protein. However, P3 also contained several minor protein bands which suggested some contamination with cytoplasmic membrane material. Outer membranes were therefore prepared by Sarkosyl extraction of crude membrane preparations. The membranous material retained after this extraction procedure had fewer minor proteins than fraction P3 (Fig. 2a) but otherwise had a similar protein profile. This Sarkosyl technique was used to prepare outer membranes from the four other *C. jejuni* isolates (Fig. 2b). The variable

Fig. 1. Autoradiograph of ^{125}I -surface-labelled *C. jejuni* isolates: (1), strain 51180; (2), strain 68869; (3), strain 63923; (4), strain 33817; (5), strain 81116.

43K–46K protein was the main component of all the outer membranes and will henceforth be termed the major outer membrane protein. Several constant outer membrane proteins were observed including a greatly enhanced protein with a molecular weight of 62K and minor constant proteins of 35K and 87K. Derivatization of the outer membrane material at 37 °C for 2 h, instead of 100 °C for 5 min, caused an apparent reduction in molecular weight of the major outer membrane protein, the 35K protein and several other minor proteins and an increase in molecular weight of the 87K protein.

Isolation and identification of C. jejuni flagella

Preparations of purified flagella contained a major diffuse protein band with a molecular weight of 62K (Fig. 2*a*). This protein corresponded with the 62K protein in the outer membrane preparations. Additionally a minor contaminating 87K protein was observed. Both of these proteins were absent in outer membrane preparations from an aflagellate variant. The 62K but not the 87K protein was a significant component of the acid-extractable material from whole bacteria which contained three other major proteins with molecular weights of 38K, 27K and 25K (Fig. 2*a*).

Rabbit antisera directed against the flagellate wild-type, an aflagellate variant and purified flagella were used to confirm the identity of the flagella protein, using immunoblotting techniques. A 62K protein was detected by antisera directed against the flagellate organism or purified flagella but not by antisera directed against the aflagellate variant (Fig. 3). However, some labelling of the 62K protein in the aflagellate variant was observed with anti-flagella antisera.

Fig. 2. (a) SDS-PAGE of *C. jejuni* strain 81116: (1), whole cell protein profile; (2), crude membrane preparation; (3), sucrose gradient fraction P₁ (cytoplasmic membrane); (4), sucrose gradient fraction P₂; (5), sucrose gradient fraction P₃ (outer membrane); (6), Sarkosyl-insoluble outer membrane preparation; (7), isolated flagella; (8), acid-extractable material. (b) SDS-PAGE of Sarkosyl-insoluble outer membrane preparations from *C. jejuni* isolates: (1), strain 33817; (2), strain 63923; (3), strain 68869; (4), strain 51180.

Attempts to ¹²⁵I-label undissociated flagella using Iodogen or lactoperoxidase were unsuccessful. However, when purified flagella were reacted with methyl 3,5-di[¹²⁵I]iodo-hydroxybenzimidate a single ¹²⁵I-labelled protein band of 62K was obtained. This protein was immunoprecipitated with rabbit antisera directed against purified flagella or the flagellate wild-type but not by control rabbit serum. A small amount of this 62K was also precipitated by antisera directed against the aflagellate variant. In some immunoprecipitation experiments a small amount of the 43K protein was also precipitated.

DISCUSSION

The outer membrane proteins of five clinical isolates of *C. jejuni* have been identified by ¹²⁵I-surface labelling and by the SDS-PAGE of outer membrane preparations isolated by sucrose gradient centrifugation of spheroplasts and Sarkosyl extraction of crude membranes. A major protein, of variable molecular weight (43K–46K), was consistently observed in the outer membrane preparations of all isolates. Logan & Trust (1982) found a similar protein (band e) to be characteristic of type strains and wild-type isolates of *C. jejuni* and *C. coli* and suggested that this is a porin or matrix protein. Previous antibody protection studies on the major outer membrane proteins of *C. fetus* (McCoy *et al.*, 1976) suggested that these proteins were unexposed. In contrast, ¹²⁵I-surface labelling studies indicate that the major outer membrane protein of *C. jejuni* is surface-exposed.

Fig. 3. Electroimmunoblots of (1) strain 81116 Fla⁺, (2) isolated flagella and (3) strain 81116 Fla⁻ incubated with rabbit antisera directed against (a) strain 81116 Fla⁺, (b) strain 81116 Fla⁻ and (c) flagella.

A surface-exposed and heat-modifiable minor outer membrane protein had a constant molecular weight of 35K in the five *C. jejuni* strains investigated. This protein was absent in outer membrane preparations from three *C. coli* strains which expressed a 41K protein of similar properties (unpublished data). The relationship of these two proteins to species differences between *C. jejuni* and *C. coli* (Owen & Leaper, 1981; Belland & Trust, 1982; Skirrow & Benjamin, 1980) is under investigation.

A 27K protein was ¹²⁵I-surface-labelled in all the isolates investigated. This protein was not, however, detected by SDS-PAGE in the outer membranes prepared by either technique, but was present in an acid-extractable material. Using the same extraction conditions McCoy *et al.* (1975) isolated an antiphagocytic microcapsular component from the surface of *C. fetus*. However, neither conventional transmission electron microscopy nor staining techniques using colloidal thorium, ruthenium red or India ink indicate the presence of a capsular structure on *C. jejuni* (unpublished data).

Purified flagella contained a major 62K and a minor 87K protein. Both proteins were absent in outer membrane preparations from an aflagellate variant. Although 62K is a high molecular weight for flagellin it is not inconsistent with the larger flagellins of some *Escherichia coli* and *Salmonella* strains (Lawn, 1977; Kondah & Hotani, 1974). The identity of *C. jejuni* flagellin as the 62K protein was confirmed using immunoblotting and radioimmunoprecipitation techniques with rabbit antisera directed against purified flagella, the flagellate wild-type strain or an aflagellate variant. Small amounts of flagellin were detected in the aflagellate variant by immunoblotting and immunoprecipitation suggesting that the variant can synthesize flagellin but not assemble flagella. Although amino acid analysis of purified flagella indicates the presence of tyrosine and histidine (unpublished data), intact flagella could not be ¹²⁵I-labelled

using the lactoperoxidase or Iodogen techniques, suggesting an inaccessibility of these residues. However, flagella could be ^{125}I -iodinated by incubation with methyl 3,5-di[^{125}I]iodohydroxybenzimidate. This iodination technique produced radio-labelled flagella suitable for radio-immunoprecipitation experiments.

At present little is known about the antigenicity of *C. jejuni* surface components. Recent studies have shown that LPS (Walder & Foresgen, 1982) and the acid-extractable surface components (Rautelin & Kosunen, 1983) are major surface antigens which may be relevant in serotyping and serodiagnosis. Preliminary investigations (Newell, 1983) indicate that the major outer membrane protein, a 27K surface protein and flagella are also immunogenic during naturally acquired human campylobacter enteritis. Furthermore, the antibodies produced against these proteins are cross-reactive with other serotypes of *C. jejuni*. Further work is in progress to investigate the importance of these proteins in the pathogenesis of, and the immune response to, *C. jejuni* infections.

We thank Dr John Heckels and Professor Peter Watt for many helpful discussions.

REFERENCES

- ARRIGONI, O. & SINGER, T. P. (1962). Limitations of the phenazine methosulphate assay for succinic and related dehydrogenases. *Nature, London* **193**, 1256–1258.
- BELLAND, R. J. & TRUST, T. J. (1982). Deoxyribonucleic acid sequence relatedness between thermophilic members of the genus *Campylobacter*. *Journal of General Microbiology* **128**, 2515–2522.
- BUTZLER, J. P. & SKIRROW, M. B. (1979). Campylobacter enteritis. *Clinics in Gastroenterology* **8**, 737–765.
- CHURCHWARD, G. G. & HOLLAND, I. B. (1976). Envelope synthesis during the cell cycle in *Escherichia coli* B/r. *Journal of Molecular Biology* **105**, 245–261.
- DAVIDSON, J. A. & SOLOMON, J. B. (1980). Onset of resistance to pathogenic strains of *Campylobacter fetus* subspecies *jejuni* in the chick embryo. In *Aspects of Developmental and Comparative Immunology - I*, pp. 289–294. Edited by J. B. Solomon. Oxford: Pergamon Press.
- DER-BALIAN, G. P. (1980). Imidoester iodination of immunoglobulins to high specific activity. *Analytical Biochemistry* **106**, 411–416.
- FILIP, C., FLETCHER, G., WULFF, J. F. & EARHART, C. F. (1973). Solubilization of the cytoplasmic membrane of *Escherichia coli* by the ionic detergent sodium lauryl sarcosinate. *Journal of Bacteriology* **115**, 717–722.
- GIANNELLA, R. A. (1981). Pathogenesis of acute bacterial diarrhoeal disorders. *Annual Review of Medicine* **32**, 341–357.
- GUERRANT, R. L., LAHITA, R. G., WINN, W. C. & ROBERTS, R. B. (1978). Campylobacteriosis in man: pathogenic mechanisms and review of 91 bloodstream infections. *American Journal of Medicine* **65**, 584–592.
- KONDAH, H. & HOTANI, H. (1974). Flagellin from *Escherichia coli* K/2. Polymerization and molecular weight in comparison with *Salmonella* flagellins. *Biochimica et biophysica acta* **336**, 117–139.
- LAMBLEN, P. R., HECKELS, J. E., JAMES, L. T. & WATTS, P. J. (1979). Variations in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. *Journal of General Microbiology* **114**, 305–312.
- LAWN, A. M. (1977). Comparison of the flagellins from different flagellar morphotypes of *Escherichia coli*. *Journal of General Microbiology* **101**, 112–130.
- LOGAN, S. M. & TRUST, T. J. (1982). Outer membrane characteristics of *Campylobacter jejuni*. *Infection and Immunity* **38**, 898–906.
- MANNINEN, K. I., PRESCOTT, J. F. & DOHOO, I. R. (1982). Pathogenicity of *Campylobacter jejuni* isolates from animals and humans. *Infection and Immunity* **38**, 46–52.
- MARCHALONIS, J. J., CONE, R. E. & SANTER, V. (1971). A probe for accessible surface proteins of normal and neoplastic lymphocytes. *Biochemical Journal* **124**, 921–927.
- MCCOY, E. C., DOYLE, D., BURDA, K., CORBEIL, L. B. & WINTER, A. J. (1975). Superficial antigens of *Campylobacter (Vibrio) fetus*: characterisation of an antiphagocytic component. *Infection and Immunity* **11**, 517–525.
- MCCOY, E. C., WILTBERGER, H. A. & WINTER, A. J. (1976). Major outer membrane protein of *Campylobacter fetus*: physical and immunological characterisation. *Infection and Immunity* **13**, 1258–1265.
- NEWELL, D. G. (1983). The surface protein antigens, including flagella of *Campylobacter jejuni*. In *Campylobacter II*, pp. 66. Edited by A. D. Pearson, M. B. Skirrow, B. Rowe, J. R. Davis & D. M. Jones. London: P.H.L.S.
- OWEN, R. J. & LEAPER, S. (1981). Base composition, size and nucleotide sequence similarities of genome deoxyribonucleic acids from species of the genus *Campylobacter*. *FEMS Microbiology Letters* **12**, 395–400.
- PALMER, S. R., GULLY, P. R., WHITE, J. M., PEARSON, A. D., SUCKLING, W. G., JONES, D. M., RAWES, J. C. L. & PENNER, J. L. (1983). Water-borne outbreak of campylobacter gastroenteritis. *Lancet* **i**, 287–290.
- PENNER, J. L. & HENNESSY, J. N. (1980). Passive haemagglutination technique for serotyping *Campylobacter fetus* subspecies *jejuni* on the basis of

- soluble heat-stable antigens. *Journal of Clinical Microbiology* **12**, 732-737.
- RAUTELIN, H. & KOSUNEN, T. U. (1983). An acid extract as a common antigen in *Campylobacter coli* and *Campylobacter jejuni* strains. *Journal of Clinical Microbiology*, **17**, 700-701.
- SKIRROW, M. B. (1977). Campylobacter enteritis: a 'new' disease. *British Medical Journal* **2**, 9-11.
- SKIRROW, M. B. (1982). Campylobacter enteritis - the first five years. *Journal of Hygiene* **89**, 175-184.
- SKIRROW, M. B. & BENJAMIN, J. (1980). Differentiation of enteropathogenic campylobacter. *Journal of Clinical Pathology* **33**, 1122.
- SKIRROW, M. B. & BENJAMIN, J. (1982). The classification of 'thermophilic' campylobacters and their distribution in man and domestic animals. In *Campylobacter: Epidemiology, Pathogenesis and Biochemistry*, pp. 40-44. Edited by D. G. Newell. Lancaster: MTP Press.
- SWANSON, J. (1981). Surface exposed protein antigens of the gonococcal outer membrane. *Infection and Immunity* **34**, 804-816.
- WALDER, M. & FORSGREN, A. (1982). Enzyme-linked immunosorbent assay (ELISA) for antibodies against *Campylobacter jejuni* and its clinical application. *Acta pathologica, microbiologica et immunologica scandinavica* **90**, 423-433.
- WARAVDEKAR, V. S. & SASLAW, L. D. (1959). A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malonaldehyde thiobarbituric acid reaction. *Journal of Biological Chemistry* **234**, 1945-1950.