The influence of the O and K antigens of *Klebsiella aerogenes* on surface hydrophobicity and susceptibility to phagocytosis and antimicrobial agents

P. WILLIAMS, P. A. LAMBERT, C. G. HAIGH AND M. R. W. BROWN*

Microbiology Research Group, Department of Pharmaceutical Sciences, Aston University, Aston Triangle, Birmingham B4 7ET

Summary. The surface hydrophobicity of *Klebsiella aerogenes* is influenced by the presence of capsular (K) and lipopolysaccharide (O) antigens. Loss of both K and O antigens (K^-O^-), but not the K antigen alone (K^-O^+), increased surface hydrophobicity and susceptibility to phagocytosis. Unheated serum (i.e., containing complement) increased the surface hydrophobicity and phagocytosis of the K^-O^+ and K^-O^- strains, but not of the K^+O^+ encapsulated parent strain. Despite the altered susceptibility to phagocytosis caused by the presence or absence of the K and O antigens, their loss did not influence sensitivity to a range of hydrophilic, hydrophobic or cationic antimicrobial agents.

Introduction

Exopolysaccharides and lipopolysaccharides play an important role in bacterial pathogenesis (Robbins *et al.*, 1980; Wicken and Knox, 1980; Costerton and Marrie, 1983). Electronmicroscopy of bacteria grown *in vivo* demonstrates the presence of an extensive polysaccharide matrix surrounding the cells (Costerton and Marrie, 1983). This glyco-calyx is involved in adhesion, in nutrient uptake, and in resistance to host defences and antimicrobial agents (Robbins *et al.*, 1980; Slack and Nichols 1982; Costerton and Marrie, 1983). *Klebsiella* species are predominantly encapsulated organisms (Sutherland, 1977); they are often resistant to many antimicrobial drugs and pose severe problems in immunocompromised patients (Umsawasdi *et al.*, 1973; Montgomerie, 1979; Casewell, 1982). *Klebsiella* infections have characteristic epidemiological and clinical features and their importance in the hospital environment has been stressed (Montgomerie, 1979; Casewell, 1982; Young, 1982). In view of the problem of antibiotic resistance and the high mortality rates associated with *klebsiella* infections, Cryz (1983) has drawn attention to the need to develop immunoprophylactic and immunotherapeutic agents. Type-specific protection can be achieved by vaccination with the homologous capsular polysaccharide (Cryz, 1983). Jones and Roe (1984), however, showed that culture-filtrate vac-

cines prepared from 12 selected serotypes produced full protection against 71 serotypes. To explain this cross-protection, a clearer understanding of the role of the O and K antigens and outer membrane proteins in relation to host defences and as potential immunogens is required. The most virulent strains of the genus *Klebsiella* appear to possess K1 or K2 capsular antigens in combination with an O1 LPS (Mizuta *et al.*, 1983). Recently, we have shown the importance of these antigens in protecting *klebsiella* against complement-mediated serum killing and phagocytic ingestion (Williams *et al.*, 1983). Physico-chemical properties and in particular surface hydrophobicity play an important role in determining the interaction between bacteria and phagocytes (van Oss, 1978; Stendahl, 1983). The present investigation was performed to characterise the sensitivity of *K. aerogenes* to phagocytosis in physico-chemical terms and to determine whether loss of the capsule or the O antigen or both influences phagocytosis. We specifically investigated the role of complement in modifying surface hydrophobicity and thereby influencing phagocytic ingestion, and we also studied the influence of the O and K antigens on antimicrobial susceptibility.

Materials and methods

Bacterial strains

K. aerogenes strain NCTC 5055 (K2,O1) was obtained from the National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. Mutants M10 (K^-O^+) and M10B (K^-O^-)

Received 28 Mar. 1985; accepted 11 May 1985.

Correspondence should be sent to M. R. W. Brown.

derived by nitrosoguanidine mutagenesis from strain NCTC 5055 were kindly donated by Dr I. R. Poxton and Dr I. W. Sutherland, Edinburgh (Poxton and Sutherland, 1976). The absence of fimbriae in the three strains was confirmed by their failure to agglutinate guinea-pig erythrocytes (Duguid, 1959). Bacteria were grown for 18 h on nutrient agar (Lab M, Ford Lane, Salford, M6 6PB) at 37°C, and resuspended in the appropriate buffer. Growth on solid medium was used to reduce the loss of the capsular polysaccharide that follows growth in liquid media in a shaking incubator.

Antimicrobial agents

Polymyxin B sulphate was obtained from Burroughs Wellcome, London (generously donated by Dr J. Gurney); tetracycline hydrochloride from Lederle, Gosport, Hants; azlocillin from Bayer UK, Haywards Heath, W. Sussex; rifampicin from Ciba, Horsham, Surrey; cefuroxime from Glaxo, Greenford, Middlesex; chlorhexidine digluconate and cetrime from ICI Chemicals, Macclesfield, Cheshire and benzalkonium chloride from Sigma, P.O. Box 14508, St Louis, MO, USA.

Determination of antibiotic susceptibility

A range of concentrations of each antimicrobial agent was prepared in nutrient-agar plates. Bacteria were diluted in saline 0.9% w/v to a concentration of 10^4 or 10^3 cfu/ml, and 0.1-ml samples spread on at least three replicate plates. Colonies were counted after incubation at 37°C for 18 h and the concentration of agent required to reduce the viable-cell number by 90% was calculated.

Preparation and electrophoresis of outer membranes

Bacteria were harvested by centrifugation, broken by sonication and treated with sodium lauryl sarcosinate 2% w/v to prepare outer membranes (OMs) as described by Filip *et al.* (1973). OM proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on 15% acrylamide gels by the method of Lugtenberg *et al.* (1975). Gels were fixed and stained with Coomassie brilliant blue and photographed.

Pre-opsonisation of bacteria

For some hydrophobicity and phagocytosis experiments, bacteria suspended in 10 mM phosphate-buffered saline (pH 7.2) to an absorbance at 470 nm of 1.0 (E_{470} 1.0) were incubated for 15 min at 37°C with 1.0 ml of fresh or heat-inactivated pooled human or guinea-pig serum before resuspension in the appropriate buffer. In some experiments heat-inactivated guinea-pig serum was pre-absorbed with the bacteria as described previously (Williams *et al.*, 1983).

Hydrophobicity studies

Hydrophobic interaction chromatography (HIC) was

performed on phenyl- and octyl-sepharose CL4B (Pharmacia) according to the method of Smyth *et al.* (1978). Pasteur pipettes containing glass wool sinters at the constriction were filled with the sepharose stock suspension to a height above the sinter of 4 cm (1.0 ml packed gel volume) and washed with 10 ml of water followed by 10 ml of 2 M or 4 M NaCl in 10 mM sodium phosphate buffer (pH 7.2). After washing, 0.1 ml of bacterial suspension (E_{470} 1.0) was carefully added to the top of the column. The column was eluted with 2 ml of 2 M or 4 M NaCl in 10 mM sodium-phosphate buffer; the eluate was collected and its E_{470} recorded.

Bacterial adherence to hydrocarbons (BATH) was demonstrated by the following method. Bacteria were resuspended in phosphate-urea-magnesium buffer (pH 7.2) as described by Rosenberg *et al.* (1980) and Rosenberg (1984): 1.2 ml of a suspension of E_{470} 0.3 was placed in a round-bottomed glass test tube (10 mm diameter) and various volumes of octane or hexadecane were added; after incubation for 10 min at room temperature, the tubes were mixed by vigorous vortexing for 120 s (Whirlimixer, Fisons, Loughborough, Leics). After allowing 20 min for the separation of aqueous and hydrocarbon phases, the aqueous phase was carefully removed and its E_{470} recorded.

Phagocytosis

Rat polymorphonuclear leukocytes (PMNs) were used in a luminol-enhanced chemiluminescence (CL) assay (Allen, 1977; Easmon *et al.*, 1980; Robinson *et al.*, 1984). PMNs were obtained by peritoneal lavage from male Wistar rats (250 g) 4 h after intraperitoneal injection of mussel glycogen (Sigma) 2% w/v. The lavage fluid was 25 ml of ice-cold Hanks's balanced salt solution (HBSS) containing heparin 10 U/ml. PMNs were washed three times in ice-cold HBSS and resuspended to 2.5×10^6 cells/ml in Eagle's Minimum Essential Medium modified with 2 mM glutamine (EMEM, Flow Laboratories, Woodcock Hill, Rickmansworth, Herts). CL measurements were performed with a Luminometer (LKB 1250, LKB Instruments Ltd, Selsdon, S. Croydon, Surrey) as follows; to each polypropylene cuvette was added 0.2 ml of 2.0×10^{-4} M luminol (Sigma), 0.1 ml of bacterial suspension (E_{470} 1.0), and 0.1 ml of modified EMEM. The bacteria, buffer and luminol were incubated at 37°C for 5 min before addition of 0.2 ml of PMN suspension (2.5×10^6 cells/ml). The ratio of bacteria to PMNs in the mixture was *c.* 60:1. CL measurements were made at 15-min intervals over a 60-min period during which the reaction mixture was maintained at 37°C.

Results

Fig. 1 shows the OM-protein profiles of *K. aerogenes* strain NCTC5055 and the (K^+O^+) and (K^+O^-) mutants. The only detectable difference is the absence of a 16 000 mol-wt protein in the K^+O^+ strain. We conclude that the major OM proteins do

Fig. 1. SDS-PAGE of the OM proteins of *K. aerogenes* K⁺O⁺ (A), K⁻O⁺ (B) and K⁻O⁻ (C). Mol. wts are shown

not vary in these strains and, because they are non-fimbriate, the differences in physicochemical surface properties reported below are due to the presence or absence of the major surface components, the K2 and O1 polysaccharide antigens.

Surface hydrophobicity

The differences in surface hydrophobicity measured by two different techniques are shown in figs. 2 and 3. Both HIC and BATH show that the K⁻O⁻ mutant was markedly more hydrophobic than the other two strains, which are indistinguishable by either method. Therefore, loss of the capsular polysaccharide alone did not render the organism more hydrophobic, whereas loss of both K and O antigens resulted in a substantial increase in surface hydrophobicity.

The BATH method was chosen to explore the

effect of opsonisation by unheated non-immune serum on surface hydrophobicity, because it enabled detection of increases in surface hydrophobicity of the K⁻O⁻ strain as well as the encapsulated parent (K⁺O⁺) and K⁻O⁺ mutant. Although similar results were obtained with octane or hexadecane in the unopsonised system, octane was chosen as the hydrocarbon phase for the complement studies because it provided a cleaner separation of phases after vortex mixing. Fig. 4 shows that incubation of the K⁺O⁺ and K⁻O⁺ strains with fresh non-immune guinea-pig serum had no detectable effect on the encapsulated strain but produced a marked increase in the surface hydrophobicity of the K⁻O⁺ strain. The hydrophobicity of the K⁻O⁺ strain treated with guinea-pig serum was far greater than that of the untreated K⁻O⁻ strain. These increases in surface hydrophobicity were not observed when the cells were incubated with heat-inactivated serum. A similar pattern of results was obtained when the same experiments were performed with human serum. Fig. 4 shows that the surface hydrophobicity of the relatively hydrophobic K⁻O⁻ strain was further increased by treatment with fresh but not by heat-inactivated guinea-pig serum.

Phagocytosis

Incubation of K⁺O⁺ and K⁻O⁺ strains with PMNs in the absence of serum produced no CL response. Pre-opsonisation of the cells with non-immune guinea-pig serum produced a significant CL response with K⁻O⁺ but not with K⁺O⁺ (fig. 5). The K⁻O⁻ strain produced a significant CL response when pre-opsonised with either fresh or heat-inactivated non-immune sera (fig. 5). However, pre-absorption of the heat-inactivated serum with the K⁻O⁻ strain reduced the CL response of this strain to that observed with unopsonised cells (fig. 5).

Antimicrobial susceptibility

The table shows that sensitivity of the three *Klebsiella* strains to a wide range of antimicrobials, including antibiotics, disinfectants and preservatives was hardly affected by the loss of either or both major polysaccharide surface antigens.

Discussion

The initial stage of phagocytosis is a surface phenomenon that can be described in physicochemical terms (Van Oss, 1978; Stendahl, 1983).

Fig. 2. Surface hydrophobicity of *K. aerogenes* measured by HIC on octyl-sepharose (A) and phenyl-sepharose (B) gels expressed as percentage of the initial absorbance (E_{470}) bound to the gels.

Fig. 3. Surface hydrophobicity of $K^+ O^+$ (○), $K^- O^+$ (▲) and $K^- O^-$ (□) measured by adherence to octane (A) and hexadecane (B). Results are expressed as percentage of the initial absorbance (E_{470}) of the aqueous suspension as a function of hydrocarbon volume.

Fig. 4. Effect of complement on surface hydrophobicity measured by adherence to octane: (A) shows the effect of fresh (●,▲) and heat inactivated (○,△) non-immune guinea-pig serum on K^+O^+ (●,○) and K^-O^+ (▲,△) respectively; (B) shows the effect of fresh (■) and heat-inactivated serum (□) on K^-O^- .

Fig. 5. CL response of PMNs to *K. aerogenes*: (A) shows the CL response of PMNs to K^+O^+ (●,○) and K^-O^+ (▲,△) after pre-incubation with fresh (●,▲) or heat inactivated (○,△) serum respectively; (B) shows the effect of fresh (■—■), heat inactivated (□---□) and heat inactivated and absorbed (■---■) serum on the response of PMNs to K^-O^- ; (□—□) shows the response to K^-O^- in the absence of serum pre-treatment.

Table. Influence of the O and K antigens of *Klebsiella* on susceptibility to antimicrobial agents

Antimicrobial agents	Antimicrobial concentration (mg/L) required to reduce the number of cfu by 90%, for strains:		
	K ⁺ O ⁺	K ⁻ O ⁺	K ⁻ O ⁻
Cefuroxime	2.7	2.4	2.2
Azlocillin	5.8	5.7	7.1
Gentamicin	0.06	0.08	0.04
Polymyxin	1.1	1.1	1.1
Tetracycline	1.5	1.2	1.7
Rifampicin	6.2	5.8	7.6
Chlorhexidine	0.02	0.02	0.02
Cetrimide	0.09	0.09	0.09
Benzalkonium chloride	0.05	0.05	0.05

Bacterial surface hydrophobicity plays an important role in non-specific attachment to phagocytes and to mammalian epithelial cells (Smyth *et al.*, 1978; van Oss, 1978; Stendahl 1983).

Envelope components that influence bacterial surface properties of gram-negative bacteria include OM proteins, acidic capsular polysaccharides, the polysaccharide O chain of LPS and fimbriae. We have studied the influence of K and O antigens upon surface hydrophobicity and phagocytosis in *Klebsiella* using a non-fimbriate encapsulated strain and mutants derived from it which lack the K or both the O and K antigens. The encapsulated strain was hydrophilic; hydrophobicity was increased by loss of both the K and O antigens but not by loss of the K antigen alone. This is presumably due to surface exposure of hydrophobic OM components such as phospholipids and proteins in the K⁻O⁻ strain. A similar increase in surface hydrophobicity of *Escherichia coli* and *Salmonella typhimurium* following loss of the O side chain and core polysaccharides of LPS has been reported (Stendahl, 1983; Xiu *et al.*, 1983).

After brief exposure of the strains to non-immune serum, only the non-encapsulated strains showed a marked increase in hydrophobicity. Because heat inactivation of the serum abolished this effect we conclude that complement was responsible for the enhanced hydrophobicity. The ability of complement to increase the surface hydrophobicity of the non-encapsulated but smooth (K⁻O⁺) *klebsiella* mutant is in contrast to its effect on smooth (O⁺) *Salmonella* strains (Stendahl *et al.*, 1977). Complement had little effect on smooth salmonellae in the absence of specific IgG although complement was able to enhance further the increase in surface

hydrophobicity brought about by IgG (Stendahl *et al.*, 1977; Stendahl, 1983).

There are two possible explanations for the failure of complement to alter the surface hydrophobicity of the encapsulated parent strain. Either the capsule blocks activation and covalent binding of the C3b component of complement to surface components or complement is activated and bound but is buried within the capsular polysaccharide matrix (Joiner *et al.*, 1984). In our earlier studies (Williams *et al.*, 1983) on the sensitivity of these strains to phagocytosis in non-immune whole blood, we were unable to distinguish the factors that influenced ingestion by phagocytes. Hydrophobicity of the bacterial cell surface, activation and deposition of complement and binding of antibody to the surface all contribute to the attachment to phagocytes (Stendahl, 1983). To study the effect of non-immune serum opsonins and surface hydrophobicity on phagocytosis we chose to use washed PMNs in a luminol-enhanced chemiluminescence system (Allen, 1977; Robinson *et al.*, 1984). In the absence of serum neither the K⁺O⁺ nor the K⁻O⁺ strains produced a detectable CL response, and this is consistent with their hydrophilic surface. Pre-incubation of these strains with fresh non-immune serum produced a marked increase in CL with the K⁻O⁺ strain but not with the K⁺O⁺ strain. This response correlates well with the pattern of susceptibility to phagocytosis observed previously in the whole-blood assay (Williams *et al.*, 1983) and with the changes in surface hydrophobicity brought about by complement. An increase in the CL response with the K⁻O⁻ strain was observed after pre-opsonisation in fresh or heat-inactivated serum. Because only fresh serum increased the hydrophobicity of this strain, we conclude that other heat-stable serum components can opsonise this strain without further increasing its hydrophobicity. These opsonising factors could be removed by absorption of the heat-inactivated serum with whole cells.

The resistance of encapsulated *klebsiellae* to phagocytosis in non-immune serum appears to result from the protective function of the capsule in interfering with the ability of complement to opsonise. For effective phagocytosis, specific antibody is required (Ehrenworth and Baer, 1956; Williams *et al.*, 1983). We have shown that anti-O antibodies alone can opsonise the K⁺O⁺ strain for phagocytosis (Williams *et al.*, 1983) and increase surface hydrophobicity (unpublished observation). It therefore appears that antibodies bound to the O antigen are accessible to phagocyte receptors. This implies that failure of complement to opsonise the

encapsulated strain is due to lack of complement activation rather than occlusion by the capsule.

The presence of a complete O antigen on the LPS of *E. coli* and *Salmonella* spp. increases resistance to hydrophobic antimicrobials (Nikaido and Nakae, 1979; Leive *et al.*, 1984). However, the role of acidic capsular polysaccharides as a barrier to antibiotic penetration is unclear (Slack and Nichols, 1982; Tannenbaum *et al.*, 1984). We were unable to distinguish any major differences in the susceptibility of the three *Klebsiella* strains to a range of hydrophobic, hydrophilic and cationic antimicrobials. Therefore, despite the marked changes in susceptibility to serum killing (Williams *et al.*, 1983) and phagocytosis accompanying loss of the O and K antigens, the lack of these major envelope components had no general effect on antimicrobial sensitivity. The alginate exopolysaccharide of *Pseudomonas* spp. does not bind significant amounts of aminoglycoside antibiotics under physiological

conditions and does not influence sensitivity to these antibiotics (Tannenbaum *et al.*, 1984).

Concentrations of antibiotics below the minimum inhibitory concentrations (sub-MICs) influence the susceptibility of bacteria to phagocytosis (Lorian and Atkinson, 1984). In the case of *Klebsiella* spp., a reduction in capsule production occurs in the presence of several cephalosporins with an accompanying increase in surface hydrophobicity (Kadurugamuwa *et al.*, 1985). Sub-MICs of azlocillin and mezlocillin have also been reported to render bacteria including *Klebsiella* spp. more susceptible to the antimicrobial action of leukocytes (Dalhoff and Stubner, 1985). As antibiotics may not always achieve MIC levels in tissues, sub-MIC effects of this kind might explain the clinical efficacy of some cephalosporins.

This work was assisted by grants from the Cystic Fibrosis Research Trust and the Medical Research Council.

REFERENCES

- Allen R C 1977 Evaluation of serum opsonic capacity by quantitating the initial chemiluminescent response from phagocytizing polymorphonuclear leukocytes. *Infection and Immunity* **15**:828–833.
- Casewell M W 1982 The role of multiply resistant coliforms in hospital-acquired infection. In: Reeves D S, Geddes A M (eds) *Recent Advances in Infection*, vol 2. Churchill Livingstone, Edinburgh, pp 31–50.
- Costerton J W, Marrie T J 1983 The role of the bacterial glycocalyx in resistance to antimicrobial agents. In: Easmon C S F, Jeljaszewicz J, Brown M R W, Lambert P A (eds) *Medical Microbiology* vol 3. Academic Press, London, pp 63–85.
- Cryz S J 1983 Progress in immunization against *Klebsiella* infections. *European Journal of Clinical Microbiology* **2**: 523–528.
- Dalhoff A, Stubner G 1985. Comparative analysis of the antimicrobial action of polymorphonuclear leukocytes *in vitro*, *ex vivo* and *in vivo*. *Journal of Antimicrobial Chemotherapy* **15** Suppl A:283–291.
- Duguid J P 1959 Fimbriae and adhesive properties in *Klebsiella* strains. *Journal of General Microbiology* **21**:271–286.
- Easmon C S F, Cole P J, Williams A J, Hastings M 1980 The measurement of opsonic and phagocytic function by luminol-dependent chemiluminescence. *Immunology* **41**:67–74.
- Ehrenworth L, Baer H 1956 The pathogenicity of *Klebsiella pneumoniae* for mice: the relationship to the quantity and rate of production of type-specific capsular polysaccharide. *Journal of Bacteriology* **72**:713–717.
- Filip C, Fletcher G, Wulff, J L, 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.
- Joiner K A, Brown E J, Frank M M 1984 Complement and bacteria: chemistry and biology in host defense. *Annual Reviews of Immunology* **2**:461–491.
- Jones R J, Roe E A 1984 Vaccination against 77 capsular types of *Klebsiella aerogenes* with polyvalent *klebsiella* vaccines. *Journal of Medical Microbiology* **18**:413–421.
- Kadurugamuwa J L, Anwar H, Brown M R W, Zak O 1985 Effect of subinhibitory concentrations of cephalosporins on surface properties and siderophore production in iron-depleted *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy* **27**:220–223.
- Leive L, Telesetsky S, Coleman W G, Carr D 1984 Tetracyclines of various hydrophobicities as a probe for permeability of *Escherichia coli* outer membranes. *Antimicrobial Agents and Chemotherapy* **25**:539–544.
- Lorian V, Atkinson B A 1984 Bactericidal effect of polymorphonuclear neutrophils on antibiotic-induced filaments of gram-negative bacilli. *Journal of Infectious Diseases* **149**: 719–727.
- Lugtenberg B, Meijers J, Peters R, van der Hoek P, van Alphen L 1975 Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K12 into 4 bands. *FEBS Letters* **58**:254–258.
- Mizuta K, Ohta M, Mori M, Hasegawa T, Nakashima I, Kato N 1983 Virulence for mice of *Klebsiella* strains belonging to the O1 group: relationship to their capsular (K) types. *Infection and Immunity* **40**:56–61.
- Montgomery J Z 1979 Epidemiology of *Klebsiella* and hospital-associated infections. *Reviews of Infectious Diseases* **1**:736–753.
- Nikaido H, Nakae T 1979 The outer membrane of gram-negative bacteria. *Advances in Microbial Physiology* **20**:163–250.
- Poxton I R, Sutherland I W 1976 Isolation of rough mutants of *Klebsiella aerogenes* and their synthesis of polysaccharides. *Journal of General Microbiology* **96**:195–202.
- Robbins J B, Schneerson, R, Egan, W B, Vann W, Liu, D T 1980 Virulence properties of bacterial capsular polysaccharides—unanswered questions. In: Smith H, Skehel J J, Turner M J (eds) *The Molecular Basis of Microbial Pathogenicity*, Verlag Chemie, Weinheim, pp 115–132.
- Robinson P, Wakefield D, Breit S N, Easter J F, Penny R 1984 Chemiluminescent response to pathogenic organisms: normal human polymorphonuclear leukocytes. *Infection and Immunity* **43**:744–752.
- Rosenberg M 1984 Bacterial adherence to hydrocarbons: a useful technique for studying cell surface hydrophobicity. *FEMS Microbiology Letters* **22**:289–295.

- Rosenberg M, Gutnick D, Rosenberg E 1980 Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiology Letters* **9**: 29–33.
- Slack M P E, Nichols W W 1982 Antibiotic penetration through bacterial capsules and exopolysaccharides. *Journal of Antimicrobial Chemotherapy* **10**: 368–372.
- Smyth C J, Jonsson P, Olsson E, Soderlind O, Rosengren J, Hjerten S, Wadstrom T 1978 Differences in hydrophobic surface characteristics of porcine enteropathogenic *Escherichia coli* with or without K88 antigen as revealed by hydrophobic interaction chromatography. *Infection and Immunity* **22**: 462–472.
- Stendahl O 1983 The physicochemical basis of surface interaction between bacteria and phagocytic cells. In: Easmon C S F, Jeljaszewicz J, Brown M R W, Lambert P A (eds) *Medical Microbiology* vol 3. Academic Press, London pp 137–152.
- Stendahl O, Tagesson C, Magnusson K E, Edebo L 1977 Physico-chemical consequences of opsonization of *Salmonella typhimurium* with hyperimmune IgG and complement. *Immunology* **32**: 11–18.
- Sutherland I W 1977 Bacterial exopolysaccharides—their nature and production. In: Sutherland I W (ed) *Surface Carbohydrates of the Prokaryotic Cell*, Academic Press, London, pp 27–96.
- Tannenbaum C S, Hastie A T, Higgins M L, Kueppers, F, Weinbaum G 1984 Inability of purified *Pseudomonas aeruginosa* exopolysaccharide to bind selected antibiotics. *Antimicrobial Agents and Chemotherapy* **25**: 673–675.
- Umsawasdi T, Middleman, E A, Luna M, Bodey G P 1973 *Klebsiella* bacteremia in cancer patients. *American Journal of Medical Sciences* **265**: 473–482.
- Van Oss C J 1978 Phagocytosis as a surface phenomenon. *Annual Reviews of Microbiology* **32**: 19–39.
- Wicken A J, Knox K W 1980 Bacterial cell surface amphiphiles. *Biochimica et Biophysica Acta* **604**: 1–26.
- Williams P, Lambert P A, Brown M R W, Jones R J 1983 The role of the O and K antigens in determining the resistance of *Klebsiella aerogenes* to serum killing and phagocytosis. *Journal of General Microbiology* **129**: 2181–2191.
- Xiu J H, Magnusson K E, Stendahl O, Edebo K 1983 Physico-chemical surface properties and phagocytosis by polymorphonuclear leucocytes of different serogroups of salmonella. *Journal of General Microbiology* **129**: 3075–3084.
- Young S E J 1982 Bacteraemia 1975–1980: A survey of cases reported to the PHLS Communicable Disease Surveillance Centre. *Journal of Infection* **5**: 19–26.