INFECTION AND IMMUNITY, Mar. 1983, p. 1136–1141
0019-9567/83/031136-06$02.00/0

Vol. 39, No. 3

Influence of Growth Temperature of *Escherichia coli* on K1 Capsular Antigen Production and Resistance to Opsonization

ROBERT BORTOLUSSI,[1]† PATRICIA FERRIERI,[2] AND PAUL G. QUIE[2]

Department of Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada,[1] and Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455[2]

Received 5 October 1982/Accepted 16 December 1982

When *Escherichia coli* strains that produce K1 capsular polysaccharide antigen at 37°C were grown at 22°C, K1 antigen was not detected in the supernatant or washed-cell fraction of broth cultures. Significant amounts of K1 polysaccharide were detected only when the organism was grown at temperatures of 30°C or higher. Rabbits immunized with an *E. coli* K1 strain (serotype O18ac:K1:H7) grown at 37°C produced agglutinating antibody to somatic antigen and precipitating and agglutinating antibody to capsular K1 antigen; those immunized with this strain grown at 22°C produced antibody to somatic antigen, but not to K1 antigen. Antibody to somatic antigen was markedly reduced by adsorption with the organism grown at 22°C, while antibody to capsular antigen was not. *E. coli* K1 strains grown at 37°C (K1 present) resisted phagocytosis and killing if they were opsonized solely by the alternative complement pathway (ACP) using magnesium ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid-chelated serum. When these strains were grown at 22°C (K1 absent), they were opsonized efficiently by the ACP (28 versus 94% killing, respectively; $P < 0.001$). In addition, a non-K1 mutant of an *E. coli* K1 strain was opsonized efficiently by the ACP although its encapsulated K1 parent was not. Sensitivity of *E. coli* strains to the bactericidal activity of serum was observed in strains with and without K1 capsular antigen. These studies demonstrated that production of K1 polysaccharide antigen was regulated by environmental temperature and that K1 capsule plays an essential role in rendering the organism resistant to opsonization by the ACP.

In 1974, it was recognized that *Escherichia coli* strains that are isolated from cerebrospinal fluid are much more likely to possess K1 capsular polysaccharide antigen than would be predicted by their frequency of isolation from the stools of normal neonates (14). This suggested that *E. coli* strains with this antigen are more virulent in human newborn infants than are non-K1 strains. We and others have found this relationship to be true in newborn rats as well (2, 9).

K1 capsular polysaccharide is a polymer of sialic acid virtually identical to the capsular polysaccharide of *Neisseria meningitidis* group B (11). The presence of K1 capsular polysaccharide antigen on *E. coli* strains is not associated with serum resistance (1). However, most *E. coli* K1 strains resist opsonization by the alternative complement pathway (ACP) (1, 18, 20). The ability to resist opsonization by this pathway is associated with increased virulence (1).

It is possible that the resistance to opsonization by the ACP demonstrated in these studies with several *E. coli* strains may be due to another bacterial factor found more frequently among K1 strains. To directly implicate K1 antigen as the critical factor determining the opsonic requirements of these strains, we attempted to isolate mutant strains which had lost their K1 capsular antigen. During these attempts, we observed that *E. coli* K1 strains grown at 22°C failed to produce capsular antigen yet appeared to be identical in other respects to strains grown at 37°C.

The relationship between the temperature of incubation and *E. coli* K1 capsular antigen production and the behavior of these organisms in serum sensitivity and phagocytic assays was studied. The observations on opsonic requirements of *E. coli* K1 strains made previously, namely, that they resist opsonization by the ACP, were supported by experiments with K1 strains grown at different temperatures and with a spontaneous non-K1 mutant of a K1 strain.

† Present address: Izaak Walton Killam Hospital for Children, Halifax, Nova Scotia, Canada B3J 3G9.

MATERIALS AND METHODS

Bacteria and growth conditions. The nine *E. coli* strains studied are listed in Table 1. Strains were stored in 20% glycerol at −20°C until needed. Serotyping was performed by Fritz Ørskov. To compare the K1 content of bacteria grown at different temperatures, 10^4 CFU were inoculated into 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and agitated for 18 to 24 h at a selected temperature. The number of CFU was estimated spectrophotometrically with a Coleman Junior II spectrophotometer (Coleman Instruments Division, Oak Brook, Ill.). To determine if specific nutrient requirements were necessary for capsular K1 antigen production, strain no. 3 was inoculated into nutrient broth (Difco Laboratories) or Davis minimal essential medium with 0.1% glucose (3).

E. coli K1 strains were identified by the agarose halo technique with equine immune serum to *N. meningitidis* group B (15); colonies surrounded by a precipitin halo are K1 antigen positive. Eighteen standard biochemical tests were performed after 24 to 48 h at 22 or 37°C using a miniaturized version of conventional procedures (API 20E; Analytab Products, Inc., Plainview, N.Y.).

Quantitation of K1 polysaccharide. After incubation, the number of viable bacteria was estimated by the pour plate technique with triplicate samples of the suspension. The remaining suspension was centrifuged at 2,000 × *g* for 30 min at 4°C. After centrifugation, the bacterial pellet was washed twice in 0.85% NaCl and resuspended in 1 ml of 0.85% NaCl. The pellet and a portion of the supernatant were stored at −20°C. Before quantitation of K1 antigen, the bacterial pellet was thawed and refrozen six times to disrupt the cells.

K1 polysaccharide was quantitated by a rocket immunoelectrophoresis technique with agarose plates incorporated with 12% equine immune serum to *N. meningitidis* group B (21). Equine immune serum and purified K1 polysaccharide, used as the standard for quantitation, were kindly provided by John B. Robbins (Division of Bacterial Products, Bureau of Biologics, Food and Drug Administration, Bethesda, Md).

Opsonic assay and serum sensitivity. Opsonic studies used bacteria that had been incubated at one of several temperatures. Organisms were washed three times in phosphate-buffered saline at pH 7.4 and adjusted to a concentration of 5×10^7 CFU/ml in phosphate-buffered saline. Human blood was collected in heparin, and leukocytes were separated on dextran as described previously (1). Pooled human serum (PHS) from three normal donors kept in 0.5-ml equal portions at −70°C was thawed immediately before use. A phagocytic mixture consisted of 0.5 ml of polymorphonuclear leukocytes (PMN) (5×10^6 PMN per ml of mixture), 0.4 ml of diluted serum (final concentration, 20%), and 0.1 ml of bacterial suspension (5×10^6 CFU per ml of mixture). The opsonic assay was always performed at 37°C as described previously (1). Samples obtained from the phagocytic mixture at 0 and 60 min were diluted in distilled water, and the number of viable bacteria was estimated by the pour plate technique. Bacterial killing was expressed as a percentage of the original inoculum. Opsonic studies were performed at least twice. Opsonization by the ACP was evaluated in serum containing 0.01 M ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetic acid (EGTA; Sigma Chemical Co., St. Louis, Mo.) with 0.01 M $MgCl_2$ (MgEGTA) (7). Controls with 20% serum without PMN were included. A reduction in the viable bacterial count of less than 20% was considered resistant to phagocytosis.

TABLE 1. *E. coli* strains used

Strain	Serotype	Isolated from	Reference
1	O7:K1:NM	Neonatal cerebrospinal fluid	1
2	O?:K1	Stool from uninfected infant	
3	O18ac:K1:H7	Neonatal cerebrospinal fluid	1
4	O?:K1	Stool from uninfected infant	
5	O1:K1	Stool from uninfected infant	
6	R:K1	Stool from uninfected infant	
7	O?:K1	Stool from uninfected infant	
699	O1:K1:H7	Stool	13
703	O1:K⁻:H7	Mutant of 699	

Sensitivity of *E. coli* to the bactericidal activity of serum was determined with 40% PHS without PMN in the mixture. Strains that showed less than a 20% decrease in the viable bacterial count were considered resistant to the bactericidal activity of serum.

Immunization. Pathogen-free San Juan rabbits (ca. 2 kg) were obtained from Bioscience Animal Services, Edmonton, Canada. After preimmunization sera were tested for preexisting antibody, rabbits were injected by the ear vein with Formalin-killed *E. coli* strain no. 3 that had been grown overnight on brain heart infusion agar at 37°C (two rabbits) or at 22°C (two rabbits). Three more intravenous injections of increasing concentrations of Formalin-killed organisms were given over the next 2 weeks followed by two injections of live organisms 5 and 10 days later. Post-immunization serum was obtained 10 days after the final injection of bacteria.

Agglutination studies were done in U-bottom microagglutination plates (Dynatech Laboratories, Inc., Alexandria, Va.) with serial twofold dilutions of heat-inactivated serum (56°C for 30 min) and a washed suspension of strain no. 3 diluted in phosphate-buffered saline (optical density of 0.7 at 620 nm).

For adsorption of serum, three parts serum were added to one part packed bacteria. After vortexing to resuspend the bacteria, the mixture was tumbled end-over-end for 60 min at 4°C. Bacteria were sedimented, and adsorbed serum was decanted. The procedure was repeated twice to ensure that serum was adequately adsorbed.

Precipitating antibody was assessed by countercurrent immunoelectrophoresis with 100 μg of purified K1 antigen on the cathodal side and rabbit serum on the anodal side of agarose plates (15).

Statistical methods. Data were expressed in the text and figures as the mean ± the standard error. Analysis

of the difference between means used a paired *t*-test. *P* values of 0.05 or less were accepted as significant.

RESULTS

Effect of temperature and duration of incubation on K1 production. When *E. coli* strains 1, 2, and 3 were incubated at room temperature (22°C), 18 to 24 h as required before there was visible growth. For all other temperatures, visible growth was detected at 16 to 18 h, and the organisms were studied at these times. No strain incubated at 22°C had detectable antigen in the pellet or supernatant (Fig. 1). At 25°C, strain no. 3 had a trace of K1 antigen detected in the bacterial sediment (but not in the supernatant). At 30°C and above, all three strains had detectable amounts of K1 antigen in the pellet and supernatant. Since the number of viable organisms in the broth culture grown at 25°C (1.6×10^9 to 3.9×10^9 CFU) was similar to the number at 30°C (1.5×10^9 to 1.9×10^9 CFU), the presence of K1 polysaccharide at 30°C could not be attributed to the number of organisms present. The concentration of K1 antigen in the supernatant increased steadily from 23 μg/ml per 10^{10} CFU at 30°C to 294 μg/ml per 10^{10} CFU at 40°C and decreased slightly to 260 μg/ml per 10^{10} CFU at 42°C. Proportionally more of the total detectable antigen was present in the cell fraction of 30°C (37%) than at the higher temperatures (16% at 35°C and 2.8% at 42°C, Fig. 1).

Because the doubling time of the organism is longer at lower temperatures, we considered the possibility that the lower cell-associated concentration of K1 antigen at 37°C was related to the number of cell divisions which had occurred. Therefore, the kinetics of K1 polysaccharide production at various times in the growth cycle were studied with strain no. 3 grown at 37°C. At 4 h of incubation, antigen was present exclusively in the cell fraction; thereafter, it was present predominantly in the supernatant (Fig. 2).

The 18 biochemical reactions for the three strains were not altered by the change in temperature. Carbohydrate fermentation was seen at the lower temperature after 48 h.

Nutrient requirements for K1 production by *E. coli*. K1 antigen was present in the supernatant and whole-cell fraction when strain no. 3 was grown in all three media, brain heart infusion broth, nutrient broth, and Davis minimal essential medium with 0.1% glucose.

Although growth on antibody-agarose plates gives a qualitative rather than a quantitative estimate of K1 antigen production, we confirmed, using antibody-agarose plates, that K1 capsular antigen was produced when bacteria were incubated at 37°C and was not produced when they were incubated at 22°C. Plates inoculated with K1 *E. coli* and incubated at 22°C showed no precipitin halo around individual colonies. When these plates were reincubated at 37°C overnight, these colonies (now larger) had become precipitin halo positive, indicating that they produced K1 capsular polysaccharide at 37°C.

Opsonic and serum sensitivity of K1 *E. coli* grown at different temperatures. Seven *E. coli*

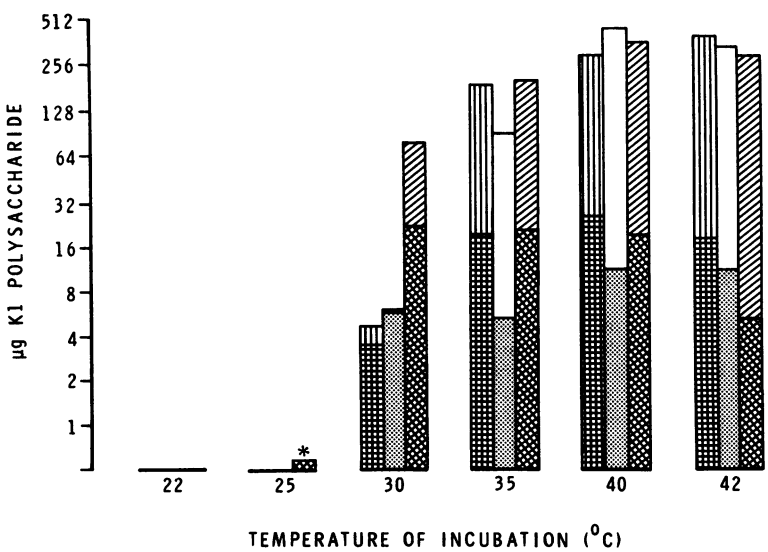

FIG. 1. Concentrations of K1 polysaccharide antigen (micrograms per 10^{10} CFU per milliliter) in the supernatant and washed-cell fraction measured by rocket immunoelectrophoresis and indicated for three strains of K1 *E. coli* grown at various temperatures. Symbols: strain 1, ▥, ▦; strain 2, □, ▩; and strain 3, ▨, ■ (supernatant and washed-cell fractions, respectively). The asterisk indicates trace amount of antigen.

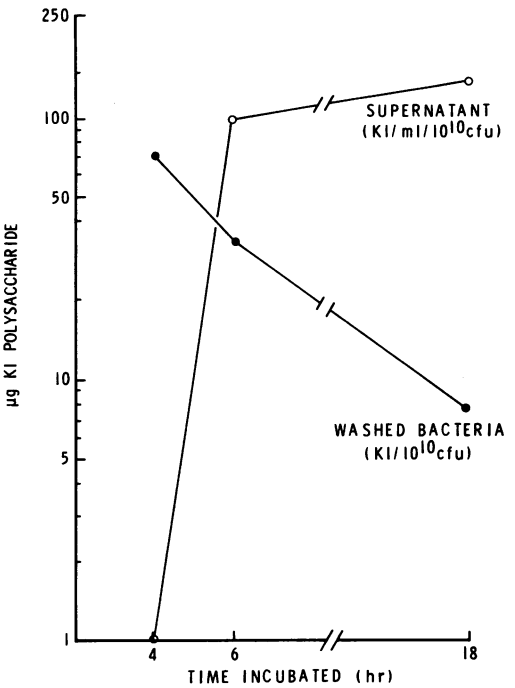

FIG. 2. Concentration of K1 polysaccharide antigen (micrograms per 10^{10} CFU per milliliter) in the supernatant and washed-cell fraction measured by rocket immunoelectrophoresis and indicated for strain no. 3 at different times in the growth cycle after inoculation into brain heart infusion broth and incubation at 37°C.

K1 strains including the three K1 strains described earlier were studied in opsonic and serum bactericidal assays (Table 2). Regardless of incubation temperature, all of the strains were opsonized well in 20% unchelated PHS. However, a marked difference in sensitivity to opsonization was noted when the strains were studied with 20% PHS with MgEGTA. Strains incubated overnight at 22°C were opsonized and killed efficiently (81 to 98% killing; mean, 94%); however, five of the seven strains incubated at 37°C overnight were highly resistant to opsonization and killing (0 to 7% killing). The two strains (no. 6 and 7) that were opsonized efficiently in MgEGTA serum were found to have little K1 antigen in the washed-cell fraction (ca. 1 and <0.5 μg/10^{10} CFU), while the five strains that were not opsonized in this serum had higher concentrations of K1 antigen (6 to 17 μg/10^{10} CFU).

For each of the seven strains, the sensitivity to the bactericidal activity of serum was the same whether the organism was grown at 22 or 37°C: sensitive strains (no. 1, 2, 6, and 7) remained sensitive and resistant strains (no. 3, 4, and 5) remained resistant.

E. coli strain 699 and strain 703, a spontaneous mutant without K1 capsular antigen, were assessed also in opsonic and serum bactericidal assays. Both strains agglutinated with antiserum to somatic antigen and had identical biochemical reactions. With MgEGTA-chelated serum, only 6% of strain 699, but 61% of the mutant strain, was opsonized and killed after 60 min. Both strains were resistant to the bactericidal activity of serum.

Immunization studies. Agglutinating and precipitating antibody titers are shown in Table 3. Strain no. 3 and strain no. 1 were used in the agglutination studies. High agglutinating titers against both somatic (O18ac) antigen and capsular (K1) antigen were observed with sera from animals immunized with strain no. 3 grown at 37°C. Adsorption of immune serum with the strain grown at 22°C significantly reduced the agglutination titer of this serum to somatic antigen (from 1,024 to 16 and 32) but not to capsular antigen. Serum from animals immunized with *E. coli* grown at 22°C developed high agglutinating antibodies to somatic (10,000) but not to capsular antigen. The absence of precipitating antibody in sera from these animals offered further support that K1 antigen was not produced when strain no. 3 was grown at 22°C. In contrast, a precipitating antibody titer of 1:8 developed in rabbits immunized with strain no. 3 grown at 37°C.

DISCUSSION

Most bacteria that are virulent in man have a polysaccharide capsule. They usually resist phagocytosis and killing in the absence of specific antibody. To assess the role that K1 capsular polysaccharide plays in making *E. coli* resistant

TABLE 2. Opsonization in human serum and in chelated human serum of *E. coli* K1 strains grown at 22 or 37°C

Strain no.	% Reduction in viable bacteria after 60 min			
	Opsonized with 20% PHS		Opsonized with 20% PHS with MgEGTA	
	22°C	37°C	22°C	37°C
1	94	98	81	7
2	97	98	94	0
3	98	93	98	0
4	99	98	96	0
5	98	93	98	0
6	64	80	98	94
7	100	100	97	92
Mean ± SE	94 ± 4.8[a]	94 ± 2.6[a]	94 ± 2.3[b]	28 ± 17[b]

[a] Difference between means was not significant.
[b] Difference between means was significant, $P < 0.001$.

TABLE 3. Agglutinating and precipitating antibody to K1 *E. coli* strains grown in brain heart infusion broth for 18 h at 22 or 37°C

Immunizing organism	Serum adsorption with	Agglutinating titer[a] against:			Precipitating titer[b] to K1 antigen
		Strain no. 3		Strain no. 1	
		22°C	37°C	37°C	
Strain no. 3 (O18ac:K1:H7) grown at 37°C	Nil	1,024	1,024	32	8
	Strain no. 3, 22°C	16	32	32	NA[c]
	Strain no. 3, 37°C, boiled[d]	16	16	16	NA
	Purified K1 (100 μg/ml)	512	512	8	2
Strain no. 3 (O18ac:K1:H7) grown at 22°C	Nil	10,000	10,000	4	0
	Strain no. 3, 22°C	512	32	NA	NA
	Strain no. 3, 37°C	128	256	NA	NA

[a] Reciprocal of agglutinating titer using strain no. 3 (O18ac:K1:H7) grown at 22 or 37°C or strain no. 1 (O7:K1:H unknown) grown at 37°C.

[b] Reciprocal of precipitating titer to K1 antigen using countercurrent immunoelectrophoresis.

[c] Information not available.

[d] Adsorption was done using strain no. 3 which had been grown at 37°C and then boiled for 1 h.

to opsonization, we studied colonies which appeared to be identical except for the presence or absence of K1 antigen. When grown at 22°C, *E. coli* K1 organisms did not produce K1 antigen that could be detected in the cell sediment or supernatant. Antibody to K1 antigen did not develop in rabbits immunized with an *E. coli* K1 strain grown at 22°C. In addition, a strain that was grown at this temperature adsorbed somatic antibody, but not K1 antibody, from immune sera, while the strain grown at 37°C adsorbed antibodies to both antigens. All of these observations suggested that immunoadsorbing and antigenic K1 capsular polysaccharide antigen was not produced when the organism was grown at 22°C, while somatic antigen was produced. Since the biochemical reactions of the organisms and their sensitivity to the bactericidal activity of serum were the same regardless of the temperature at which the bacteria grew, we concluded that these other properties were not modulated by K1 capsular polysaccharide.

Other investigators have found that fatty acid synthesis (8), oxygen metabolism (12), and protein synthesis (22) can be modulated in specific strains of *E. coli* by varying the temperatures of incubation. In addition, temperatures under 22°C may affect the size of *E. coli* cells (17) and their growth potential (16). It is of interest that earlier studies on the effect of temperature on capsular polysaccharide production with *Salmonella, Aerobacter*, and *Escherichia* species concluded that capsule production was enhanced or unaffected at temperatures below 37°C (4). *E. coli* K1 strains were not examined in these experiments. In contrast to these studies, our investigations indicated that K1 polysaccharide was not produced at 22°C and appeared as the incubating temperature increased from 25 to 30°C.

Resistance to opsonization and killing in the presence of 20% MgEGTA-chelated serum were markedly altered if the organism was grown at different temperatures. All strains tested were susceptible to opsonophagocytosis in chelated serum when grown at 22°C (81 to 98% killing), but five were resistant when grown at 37°C (0 to 7% killing). These strains produced no K1 capsular polysaccharide antigen at 22°C but did produce large amounts (>6 μg/10^{10} CFU) of cell-associated K1 antigen at 37°C. Both strains of K1 *E. coli* that were susceptible to opsonophagocytosis, when grown at 37°C, produced less than 2 μg of cell-associated K1 antigen per 10^{10} CFU at this temperature. We have reported previously that *E. coli* strains with low concentrations of K1 polysaccharide in the washed-cell fraction were significantly more likely to be opsonized efficiently in chelated serum than strains with higher amounts (1). In the present experiments, the change observed in susceptibility to opsonophagocytosis using MgEGTA-chelated serum seen with strains grown at 22°C compared with 37°C was highly significant ($P < 0.001$). Effective opsonophagocytosis was observed in nonchelated normal PHS when the organisms were grown at these two temperatures.

It therefore appeared that polysaccharide on the bacterial surface inhibited opsonophagocytosis of *E. coli* when MgEGTA-chelated serum was the opsonic source. Supporting these conclusions was the observation that a non-K1 mutant (O1:K^-:H7) of an *E. coli* K1 strain

(O1:K1:H7) was more susceptible to opsonophagocytosis in chelated serum than was its parent. Since MgEGTA blocks the classical complement pathway, allowing only the ACP to be activated (7), these experimental results suggested that K1 polysaccharide causes resistance to opsonization by inhibiting effective activation of the ACP. Similar conclusions have been drawn by others (10, 18, 20) comparing encapsulated *E. coli* strains with non-encapsulated *E. coli* strains.

A possible explanation for the role of K1 polysaccharide in opsonization may be related to its effect on activated C3, C3b. When C3b is fixed to the surface of a particle containing sialic acid, it is thought that H (formerly designated β1H [19]) is bound with almost 100-fold-greater affinity than factor B (6). Binding of H to C3b effectively blocks the amplification loop of the ACP by increasing the cleavage of C3b by C3b inactivator, yielding iC3b, which is an inactive form of C3b (6). Another bacterial pathogen for newborn infants, type III group B *Streptococcus*, may resist opsonization by this mechanism as well (5). Our experiments have shown that for *E. coli*, K1 polysaccharide acted as an inhibitor of bacterial opsonization when the classical complement pathway of serum was blocked.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance provided by Hattie Grey, JoAnne Cunningham, and Rosemary Sampson.

Funds for this project were provided in part from Medical Research Council grant no. 210 and by a grant-in-aid from the Graduate School, University of Minnesota. R.B. is supported by a grant from the Medical Research Council of Canada, no. 209.

LITERATURE CITED

1. **Bortolussi, R., P. Ferrieri, B. Björkstén, and P. G. Quie.** 1979. Capsular K1 polysaccharide of *Escherichia coli*: relationship to virulence in newborn rats and resistance to phagocytosis. Infect. Immun. **25**:293–298.
2. **Bortolussi, R., P. Ferrieri, and L. W. Wannamaker.** 1978. Dynamics of *Escherichia coli* infection and meningitis in infant rats. Infect. Immun. **22**:480–485.
3. **Difco Laboratories.** 1968. Difco supplementary literature (supplement to Difco Manual, 9th ed.), p. 96–99. Difco Laboratories, Detroit, Mich.
4. **Duguid, J. P., and J. F. Wilkinson.** 1953. The influence of cultural conditions on polysaccharide production by *Aerobacter aerogenes*. J. Gen. Microbiol. **9**:174–189.
5. **Edwards, M. S., A. Nicholson-Weller, C. J. Baker, and D. L. Kasper.** 1980. The role of specific antibody in alternative complement pathway-mediated opsonophagocytosis of type III, group B *Streptococcus*. J. Exp. Med. **151**:1275–1287.
6. **Fearon, D. T., and K. F. Austen.** 1980. The alternative pathway of complement—a system for host resistance to microbial infection. N. Engl. J. Med. **303**:259–263.
7. **Forsgren, A., R. H. McLean, A. F. Michael, and P. G. Quie.** 1975. Studies of the alternate pathway in chelated serum. J. Lab. Clin. Med. **85**:904–912.
8. **Garwin, J. L., and J. E. Cronan, Jr.** 1980. Thermal modulation of fatty acid synthesis in *Escherichia coli* does not involve de novo enzyme synthesis. J. Bacteriol. **141**:1457–1459.
9. **Glode, M. P., A. Sutton, E. R. Moxon, and J. B. Robbins.** 1977. Pathogenesis of neonatal *Escherichia coli* meningitis: induction of bacteremia and meningitis in infant rats fed *Escherichia coli* K1. Infect. Immun. **16**:75–80.
10. **Horwitz, M. A., and S. C. Silverstein.** 1980. Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. J. Clin. Invest. **65**:82–94.
11. **Kasper, D. L., J. L. Winkelhake, W. D. Zollinger, B. L. Brandt, and M. S. Artenstein.** 1973. Immunochemical similarity between polysaccharide antigens of *Escherichia coli* O7:K1(L):NM and group B *Neisseria meningitidis*. J. Immunol. **110**:262–268.
12. **Mainzer, S. E., and W. P. Hempfling.** 1976. Effects of growth temperature on yield and maintenance during glucose-limited continuous culture of *Escherichia coli*. J. Bacteriol. **126**:251–256.
13. **Ørskov, F., I. Ørskov, A. Sutton, R. Schneerson, W. Lin, W. Egan, G. E. Hoff, and J. B. Robbins.** 1979. Form variation in *Escherichia coli* K1: determined by O-acetylation of the capsular polysaccharide. J. Exp. Med. **149**:669–685.
14. **Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Ørskov, I. Ørskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290**:1216–1220.
15. **Sarff, L. D., G. H. McCracken, Jr., M. S. Schiffer, M. P. Glode, J. B. Robbins, I. Ørskov, and F. Ørskov.** 1975. Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. Lancet **i**:1099–1104.
16. **Shaw, M. K., A. G. Marr, and J. L. Ingraham.** 1971. Determination of the minimal temperature for growth of *Escherichia coli*. J. Bacteriol. **105**:683–684.
17. **Shehata, T. E., and A. G. Marr.** 1975. Effect of temperature on the size of *Escherichia coli* cells. J. Bacteriol. **124**:857–862.
18. **Stevens, P., C. L. Chu, and L. S. Young.** 1980. K-1 antigen content and the presence of an additional sialic acid-containing antigen among bacteremic K-1 *Escherichia coli*: correlation with susceptibility to opsonophagocytosis. Infect. Immun. **29**:1055–1061.
19. **Subcommittee of the IUIS/WHO Nomenclature Committee.** 1982. Nomenclature of the alternative activating pathway of complement. Clin. Exp. Immunol. **47**:221–224.
20. **Van Dijk, W. C., H. A. Verbrugh, M. E. van der Tol, R. Peters, and J. Verhoef.** 1979. Role of *Escherichia coli* K1 capsular antigens during complement activation, C3 fixation, and opsonization. Infect. Immun. **25**:603–609.
21. **Weeke, B.** 1973. Rocket immunoelectrophoresis. Scand. J. Immunol. **2**(Suppl. 1):37–46.
22. **Yamamori, T., and T. Yura.** 1980. Temperature-induced synthesis of specific proteins in *Escherichia coli*: evidence for transcriptional control. J. Bacteriol. **142**:843–851.