JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1981, p. 301-308
0095-1137/81/02-0301/08$02.00/0

Vol. 13, No. 2

Analysis of Parameters Affecting the Hemagglutination Activity of *Escherichia coli* Possessing Colonization Factor Antigens: Improved Medium for Observing Erythrocyte Agglutination

DAVID M. SEDLOCK,† HENRY F. BARTUS, IHOR ZAJAC,* AND PAUL ACTOR

Smith Kline and French Laboratories, Philadelphia, Pennsylvania 19101

The hemagglutination (HA) activity of two strains of *Escherichia coli*, each possessing different colonization factor antigens (CFA), was examined under different test conditions. The effects of ionic strength, temperature, pH, cations, and reaction surface on erythrocyte (RBC) agglutination were analyzed. Strain H-10407 (CFA/I) caused the agglutination of human, bovine, and chicken RBC, whereas strain CL-9699 (CFA/II) agglutinated only bovine and chicken RBC. The HA activity of both strains increased with decreasing ionic strength, pH, and temperature, the effects of temperature being negligible at low ionic strength. When accounting for ionic strength, the presence of Ca^{2+}, Mg^{2+}, Fe^{2+}, or Fe^{3+} ions did not increase the HA activity of these bacteria. Optimum conditions for HA of reactive RBC by bacteria included low ionic strength (<50 mM) and slightly acidic pH (6.0 to 7.0). Use of a low-ionic-strength medium permitted application of microtitration methods to visualize the HA reactions. Storage of RBC in low-ionic-strength medium did not change their HA properties, and the use of this medium proved superior to saline in overcoming HA variation observed with different preparations of RBC.

A number of biological substances are known to cause the agglutination of erythrocytes (RBC), and the mechanism of hemagglutination (HA) in each case is governed by a complex variety of physicochemical parameters (1, 3, 12, 13, 14, 17, 21, 22). The conditions causing this reaction can be altered by changing any of a number of variables, including the RBC surface potential, surface charge, cell surface shape, length and charge of an adsorbing particle, and the potential, kinetic, or free energy levels of the system (18).

Recently, investigators have examined the various HA properties of diarrheagenic strains of *Escherichia coli* and have reported a correlation between certain serospecific strains found to be enterotoxigenic and their ability to hemagglutinate RBC in the presence of mannose (18, 24). Evans and co-workers (4–6) have described strains of enterotoxigenic *E. coli* that produce fimbrial structures which were termed colonization factor antigens (CFA). Two types of CFA have been described, CFA/I (6) and CFA/II (4), which are antigenically distinct and also vary in their HA properties.

Generally, to demonstrate the HA activity of these and other bacteria, a slide HA test has been used in which both reactants are suspended in saline at physiological pH. Using buffered saline, we have attempted to apply microtiter methods to examine bacterial agglutination of RBC with limited success. Consequently, this study was undertaken to examine HA parameters to understand better the bacterial HA reaction. This study led to the development of a standard microtiter procedure for the detection of HA caused by CFA/I- or CFA/II-positive bacteria.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. Two strains of *E. coli* isolated from separate outbreaks of gastroenteritis were studied. Strain H-10407 (O78:H11), originally isolated by Evans and Evans (7) and possessing CFA/I fimbriae (5, 6), was kindly supplied by Stanley Falkow. Strain CL-9699 (O6:H16), isolated from a diarrhea outbreak at Crater Lake (20), was kindly provided to us by P. Shipley, who characterized the culture as producing CFA/II but not CFA/I fimbriae (personal communication). From each strain, spontaneously derived CFA-negative variants were obtained in our laboratory. These cultures had lost the ability to agglutinate RBC in the presence of mannose and were used as negative controls during the course of this study.

The bacteria were maintained in a 1% Casamino

† Present address: Sterling-Winthrope Research Institute, Rensselaer, NY 12144.

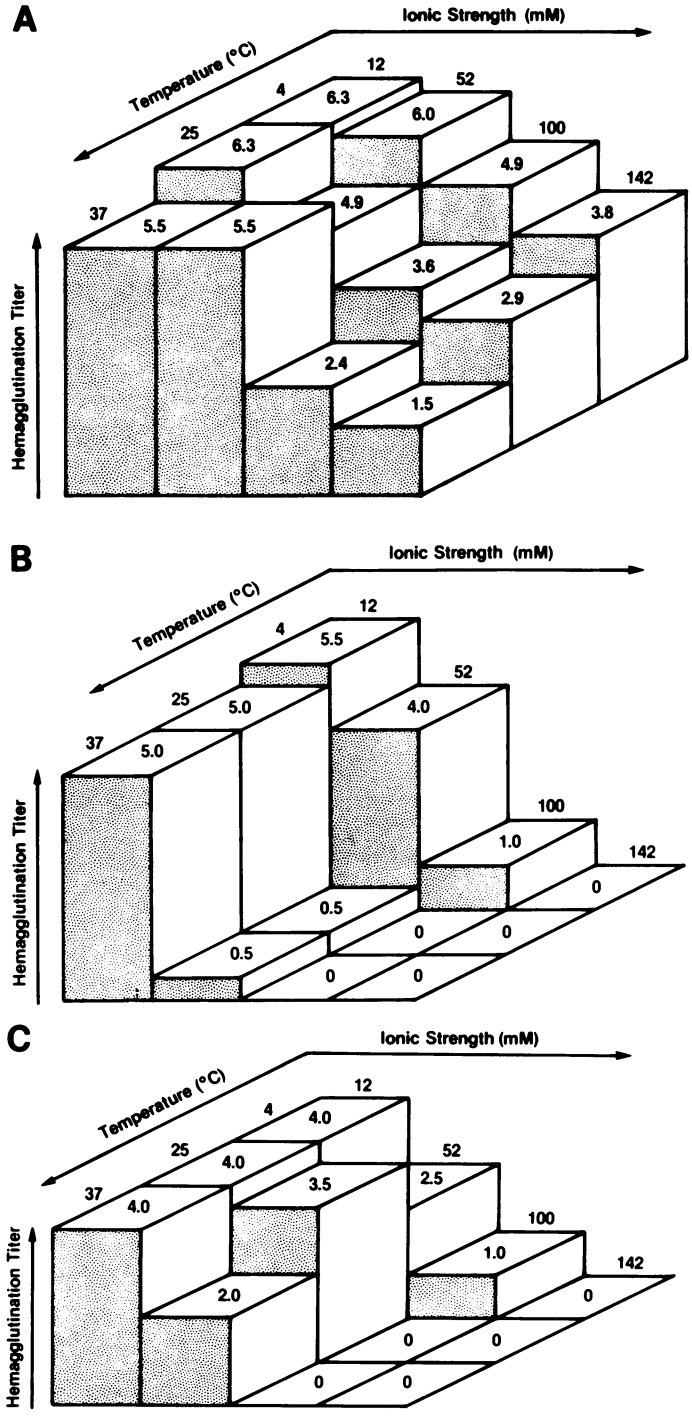

FIG. 1. *Effect of ionic strength and temperature on the HA reaction between (A) CFA/I-positive E. coli and human RBC, (B) CFA/I-positive E. coli and bovine RBC, and (C) CFA/II-positive E. coli and bovine RBC. The HA titers represent geometric mean values of eight tests with human RBC and two tests with bovine RBC. Tests were conducted in microtiter trays, and the titers were recorded after 1 h of incubation.*

TABLE 1. *Comparison of HA titers for CFA/I- and CFA/II-positive E. coli as observed with microtiter trays and glass slides*

HA system	HA titer[a] of RBC[b] at ionic strength (mM) of:															
	12				52				100				142			
	Hu	Bv	Ch	Gp	Hu	Bv	Ch	Gp	Hu	Bv	Ch	Gp	Hu	Bv	Ch	Gp
CFA/I																
Microtiter	6.3	5.3	5.8	0	5.5	2.3	1.0	0	4.3	0.5	0	0	3.6	0	0	0
Slide	5.9	4.5	3.5	1.0	4.5	3.5	2.8	0	4.5	2.3	1.0	0	3.7	0.8	0	0
CFA/II																
Microtiter	0	4.9	0	0	0	3.3	0	0	0	0.5	0	0	0	0	0	0
Slide	0	6.3	5.0	0	0	4.5	1.8	0	0	1.8	0.8	0	0	1.0	0	0

[a] Hemagglutination titer expressed as a composite geometric mean for tests at 4 and 25°C. The values are averages of eight tests with human RBC, two each for bovine and chicken RBC, and a single test with guinea pig RBC.

[b] RBC used were human (Hu), bovine (Bv), chicken (Ch), and guinea pig (Gp).

Acids medium described by Evans et al. (5) supplemented with 15% glycerol and stored at −70°C. Weekly, a vial of bacteria was opened, and CFA agar plates were inoculated and incubated overnight at 37°C. A cell suspension was prepared by harvesting the bacteria aseptically from agar plates and storing them at 4°C in 10 mM sodium phosphate (pH 7.2). The freshly harvested cells were monitored by HA activity of isolated colonies to insure the presence of greater than 70% CFA-positive bacteria. In addition, each bacterial preparation was monitored for HA activity. No change in HA activity was ever observed during a 1-week storage. The cells were diluted and suspended in the appropriate solutions before testing.

RBC preparations. The HA reactions were monitored with human, bovine, chicken, and guinea pig RBC. All human blood obtained was 4 to 5 days old. During the course of this investigation, a total of eight different units of citrated human RBC [eight group A, Rh (+); Interstate Blood Banking, Philadelphia, Pa., and Community Blood and Plasma, Folcroft, Pa.], four lots of defibrinated bovine RBC (Gibco Diagnostics), two lots of chicken RBC stored in Alsever solution (Gibco Diagnostics), and one lot of guinea pig RBC in Alsever solution (Flow Laboratories) were utilized. The blood was stored at 4°C.

Before testing, the RBC were washed at least twice and resuspended in the test solution to a final concentration of 2% (vol/vol).

HA assay. The bacterial agglutination of these RBC preparations was studied by using two methods of macroscopic observation and at three different temperatures. The bacteria were initially suspended to an absorbance of 4.0 units (λ = 640 nm) in the test solution and serially diluted (twofold). RBC were added as a 2% suspension prepared in the same test solution. In one set of experiments, microtiter plates (MIC-2000; Dynatech Laboratories) were used to observe the HA reaction. Fifty-microliter amounts each of the bacterial and RBC suspensions were mixed, and the sealed trays were incubated for 1 h at 4, 25, and 37°C. The HA reactions were enhanced by vibrating the plates for 15 s (Micro-Shaker II, Dynatech Laboratories). In an additional set of experiments, HA tests were performed with glass microscope slides. Ten-microliter amounts of each suspension were mixed, and the slides were incubated for 4 to 6 min at both 4 and 25°C in a humidified chamber. The HA reactions were enhanced by gently rocking the slides. For all tests, HA activity was recorded at each bacterial dilution, the highest dilution showing visible macroscopic agglutination of the RBC being the endpoint. A mean geometric HA titer was calculated for each test condition and the values were expressed as the $\log_2$ (reciprocal of the highest bacterial dilution).

Ionic strength measurement. Ionic strength (μ) for all experiments was determined with a Radiometer conductivity meter type CDM_{2e} and by measuring solution resistance, expressing μ as equivalents of NaCl concentration.

RESULTS

Effect of ionic strength and temperature on HA activities. Microtiter trays were used to monitor the HA profile of the CFA/I and CFA/II bacteria. Four test solutions were prepared, each containing varying levels of NaCl to control ionic strength, with D-sorbitol and D-mannose to maintain tonicity. Sodium chloride was used at concentrations of 145, 100, 50, and 10 mM, with sorbitol being included at concentrations of 0, 30, 130, and 210 mM, respectively. Mannose was included at a concentration of 50 mM in all four solutions. Sodium bicarbonate, prepared as a 1,000× stock solution, was used as a buffer at a concentration of 0.3 mM (pH 7.2 to 7.5). The pH was monitored with a pH meter.

CFA/I-positive *E. coli* caused agglutination of human RBC at any temperature or ionic strength tested; however a significant difference ($P < 0.05$; Wilcox ranking procedure) in the level of activity was observed when comparing the titers under the various conditions (Fig. 1A). The HA activity was markedly increased at $\mu <$ 52 mM at all three temperatures, whereas significant temperature effects were noted only between 4 and 37°C at the higher ionic strengths ($\mu >$ 100 mM). The HA reaction with bovine RBC by CFA/I-positive *E. coli* was also ionic strength and temperature dependent, because agglutination occurred optimally at low temper-

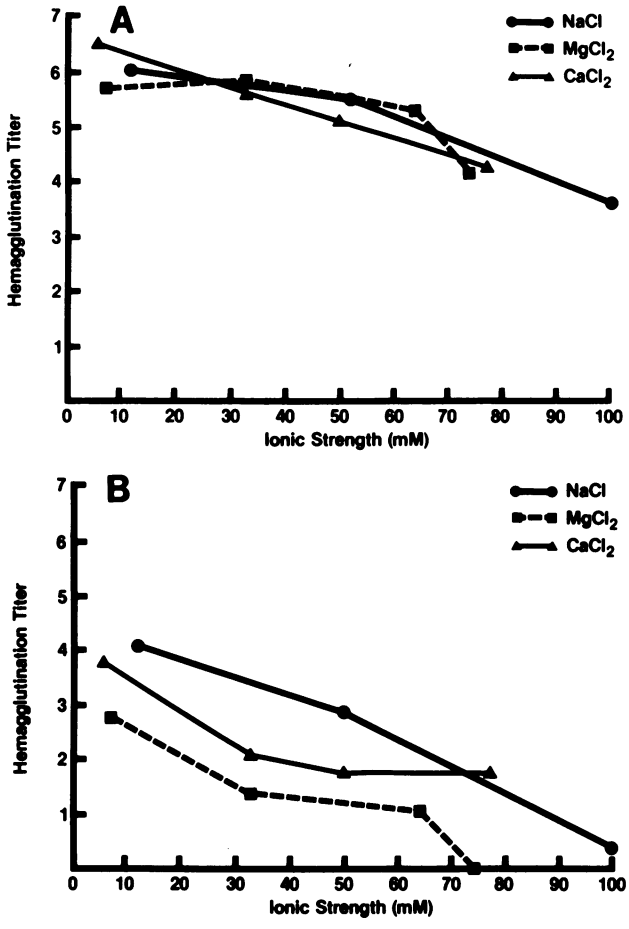

FIG. 2. *Effect of divalent cations on the HA reaction between (A) CFA/I-positive E. coli and human RBC and (B) CFA/II-positive E. coli and bovine RBC. The HA titers are expressed as a composite geometric mean for eight tests with human and two tests with bovine RBC conducted at 4, 25, and 37°C. All tests were conducted in microtiter trays, and the titers were recorded after 1 h of incubation.*

ature and low ionic strength (Fig. 1B). The bovine RBC were the only preparation agglutinated by CFA/II-positive *E. coli* when tested in microtiter trays, and the reaction was only apparent at $\mu \leq 52$ mM (Fig. 1C). The HA reactions were refractory to temperature, and HA titers were highest when a low-ionic-strength medium (LIM) was used.

The HA tests were examined routinely after 1 h of incubation; however, additional studies in our laboratory have revealed that maximum HA occurs very rapidly in LIM (<30 s for CFA/I-positive *E. coli* and <3 min for CFA/II-positive *E. coli*) and does not appreciably change with increased time. Consequently, lengthy incubation was not necessary to enhance the reaction.

Since mannose was incorporated in these testing solutions to monitor mannose-resistant HA, solutions were composed without mannose, substituting sorbitol in equimolar amounts. No effect was seen on HA activity by the presence or absence of mannose. To test further the effects of the carbohydrate used for tonicity balance, mannose and sucrose were completely substituted for sorbitol at different ionic strengths. No difference was observed in HA activity by either CFA/I- or CFA/II-positive bacteria. Sorbitol was chosen to maintain tonicity because of its chemical stability in solution compared with the other carbohydrates (9).

Slide microtiter HA. By using HA test solutions at four different ionic strengths (as described above), a comparison of the HA activity of CFA/I- and CFA/II-positive *E. coli* revealed the importance of the reaction surface or container. No real difference in HA titers was seen

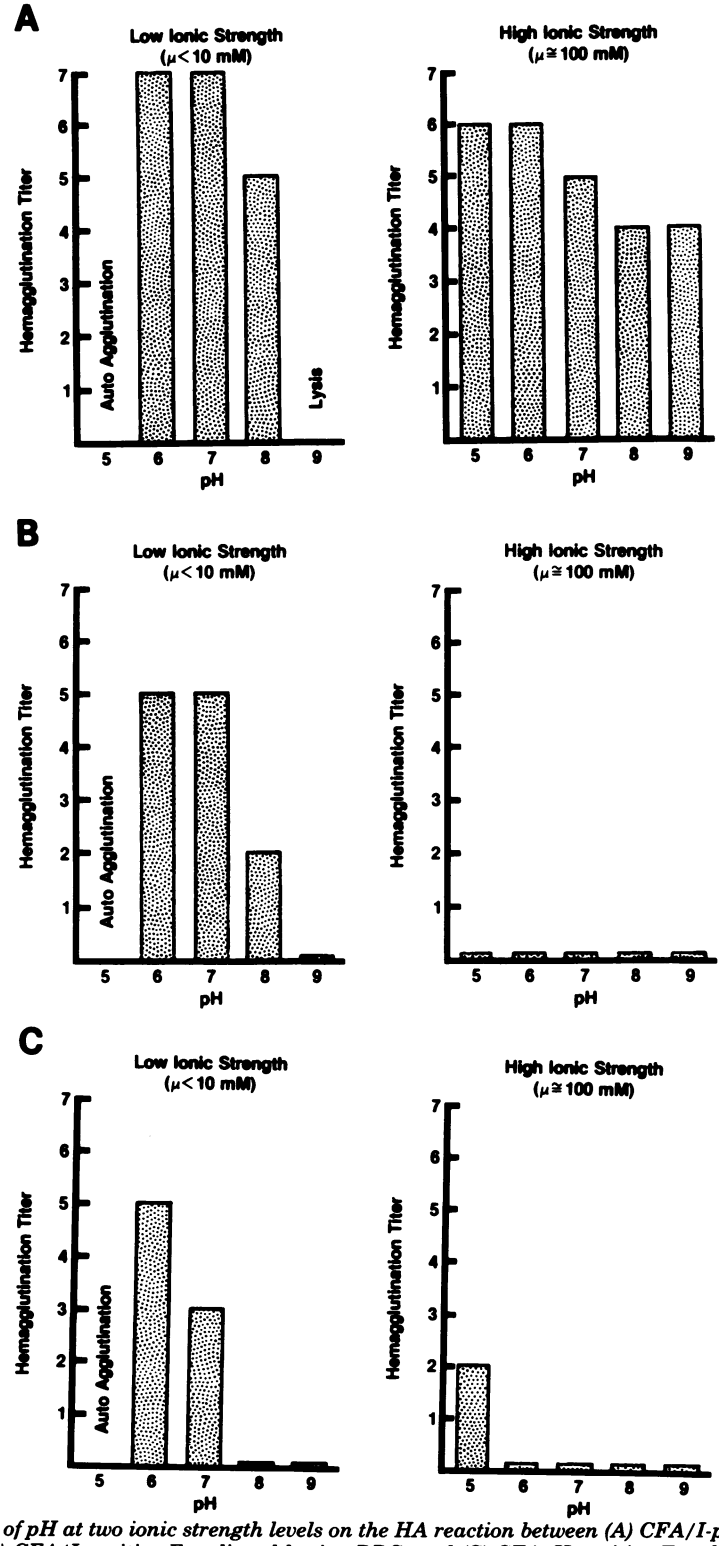

FIG. 3. *Effect of pH at two ionic strength levels on the HA reaction between (A) CFA/I-positive E. coli and human RBC, (B) CFA/I-positive E. coli and bovine RBC, and (C) CFA/II-positive E. coli and bovine RBC. Tests were conducted in microtiter trays at 25°C, and HA titers were recorded after 1 h of incubation.*

TABLE 2. *Effect of iron on HA activity*

Bacteria, RBC	Iron content	HA titer	
		LIM[a]	PBS[b]
CFA/I, Human	None	6.0	3.0
	0.1 mM Fe^{2+}	6.0	3.0
	1.0 mM Fe^{2+}	6.0	4.0
	0.1 mM Fe^{3+}	6.0	3.0
	1.0 mM Fe^{3+}	Autoagglutination	Weak autoagglutination
CFA/II, Bovine	None	4.0	0
	0.1 mM Fe^{2+}	4.0	0
	1.0 mM Fe^{2+}	4.0	0
	0.1 mM Fe^{3+}	4.0	0
	1.0 mM Fe^{3+}	Autoagglutination	Weak autoagglutination

[a] LIM as described in text; μ = 13 mM.
[b] Dulbecco phosphate-buffered saline containing 50 mM mannose; μ = 156 mM.

when using human RBC and CFA/I-positive *E. coli*; however, the other three types of blood cells exhibited a difference in HA reactivity when the two reaction methods were used (Table 1). With the exception of using CFA/I-positive bacteria at low ionic strength, bovine RBC were more reactive on glass slides than in microtiter trays, and CFA/II-positive *E. coli* agglutinated chicken RBC only when tested on slides. Also CFA/I-positive *E. coli* reacted weakly with guinea pig RBC only when tested on slides and only in LIM. When using either system, a decrease in HA activity was seen with increasing ionic strength. The use of LIM, however, enabled us to use microtiter trays to visualize HA reactions with human or bovine RBC.

Effect of divalent cations on HA activity. The effect of Ca^{2+} and Mg^{2+} ions on the HA reaction was tested using microtiter trays incubated at 4, 25, and 37°C. Ionic strength was adjusted by using the chloride salt of these cations at concentrations of 2.5, 12.5, 25, and 37.5 mM with sorbitol at respective concentrations of 220, 200, 175, and 160 mM. These solutions all contained mannose at 50 mM and $NaHCO_2$ buffer at 0.3 mM. When compared with the HA reaction which occurs with Na^+ at equivalent ionic strengths, the data showed that these cations did not enhance the HA activities of either strain of bacteria (Fig. 2A and B). When testing CFA/II-positive *E. coli* with bovine RBC, the HA reaction was consistently poorer in the presence of Mg^{2+} when compared with Na^+ at all ionic strengths tested (Fig. 2B).

Effect of pH on HA activity. The effect of pH was monitored at values from pH 5.0 to 9.0 both at low ($\mu < 10$ mM) and high ($\mu \cong 100$ mM) ionic strengths. Three organic buffers were used: (i) 2-(*N*-morpholino)ethanesulfonic acid (MES; Calbiochem), (ii) *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; Calbiochem), and (iii) tris(hydroxymethyl)aminomethane (Tris; Bio-Rad Laboratories). Low-ionic-strength solutions were composed of 210 mM sorbitol, 50 mM mannose, and 10 mM buffer (MES adjusted to pH 5.0 and 6.0; HEPES to pH 7.0; and Tris to pH 8.0 and 9.0). Ionic strength values ranged from 1.6 to 4.5 mM for these solutions. The high-ionic-strength solutions were composed of 10 mM sorbitol, 50 mM mannose, 100 mM NaCl, and 10 mM buffer (adjusted to the same pH values as above). The values for μ ranged from 100 to 110 mM for these solutions. The HA reaction with CFA/I-positive bacteria and human as well as bovine RBC and with CFA/II-positive bacteria and bovine RBC was examined with microtiter trays incubated for 1 h at 25°C.

HA titers were highest at acidic pH values and decreased with increasing pH (Fig. 3A to C). CFA/I-positive bacteria exhibited HA at all pH values tested, maximum levels being seen at pH 6.0 or 7.0 in LIM (Fig. 3A). The CFA/I reaction with bovine cells was observed only at low ionic strength, again optimum reactivity being seen only at nonalkaline pH values (Fig. 3B). The reaction between CFA/II bacteria and bovine RBC was detected at high ionic strength, but only at pH 5.0 (Fig. 3C). Optimum reactivity with these bacteria occurred in LIM at pH 6.0, whereas no HA reaction was seen at pH 8.0 or 9.0.

Effect of iron on HA activity. Both ferrous and ferric ions were examined for their ability to augment the HA reaction when tested in either a high-ionic-strength medium or LIM. The LIM contained 200 mM sorbitol, 50 mM mannose, and 25 mM MES buffer, pH 6.5 (μ = 13 mM).

The high-ionic-strength solution consisted of Dulbecco phosphate-buffered saline (Ca^{2+} and Mg^{2+} free; Flow Laboratories) with the addition of 50 mM mannose, $\mu = 156$ mM. Both solutions were modified by addition of 100× stock solutions of $FeCl_3$, $FeSO_4$, or $Fe_2(SO_4)_3$ to final concentrations of 0.10 and 1.0 mM. The iron salts were tested individually; a composite solution was not prepared. The effect of these ions both in LIM and phosphate-buffered saline was measured with microtiter trays incubated at 25°C. No change in activity was observed when compared with control values (i.e., no iron present) except when testing 1.0 mM Fe^{3+} (Table 2) which caused autoagglutination of both the RBC and bacteria.

DISCUSSION

The effect of ionic strength on particle attachment or HA varies with the systems tested. We found that HA caused by CFA-positive *E. coli* was optimum in a low-ionic-strength solution of slightly acidic pH. This is analogous to HA reactions mediated by immunoglobulin G antibody (16). In contrast, viral attachment (8, 19), polymer-induced HA (12), and lectin-mediated HA (17) have been enhanced by physiological or high-ionic-strength media.

In our studies changing the pH had an effect on the CFA HA activity in that the reaction was improved in nonalkaline solutions. Both bacteria and RBC show decreases in net surface charge and surface charge density as the pH is lowered (9, 23). This effectively lowers the cell surface potential, resulting in shorter distances of repulsion between interacting cells. In this way, acidic pH conditions enhance HA by enabling a closer approach between the RBC. When the carboxyl groups were blocked on the surface of nonpiliated *Neisseria gonorrhoeae*, Heckels et al. (10) observed that these bacteria attached to human tissue culture cells as abundantly as a piliated strain. The authors concluded that these bacterial cell surface antigens were mediating attachment by overcoming the electrostatic repulsion due to the negative cell surface charge. This also is the probable role of CFA with *E. coli* attachment or HA.

We observed no appreciable temperature effect on HA reactions tested in LIM; however, as the ionic strength of the test solution was increased, bacterial HA decreased, especially at 37°C. It should be noted that reactions which occurred optimally at 4°C remained visible when recording the results at 25°C. Poor bacterial attachment activity at elevated temperatures has been observed by other investigators (2–4, 13); however, the gonococci are reported to attach to cells optimally at 37°C (11).

The data in this study revealed no augmentation of RBC agglutination in the presence of Ca^{2+}, Mg^{2+}, Fe^{2+}, or Fe^{3+} ions. When CFA/II-positive bacteria were tested with bovine RBC, we did observe that Mg^{2+} and to some degree Ca^{2+} depressed the HA activity of these bacteria. In contrast to our work, other investigators have shown an effect by di- and trivalent cations in the attachment of bacteria to both solid (15) and cell (11) surfaces. In addition, using dextran polymers to agglutinate RBC Jan and Chien (12) found that divalent cations increased the aggregation of normal RBC but had no effect on neuraminidase-treated cells. The authors theorized that the enhanced agglutination in the presence of these ions resulted from a decrease in surface potential and concurrent shrinkage of the RBC double layer which decreased the electrostatic repulsion. Our data, however, indicate that, at least with CFA-mediated HA, overall net surface charge density might be more important than the size of the cell surface potential in promoting or preventing HA. This is indicated, since we could not increase the HA titers above those exhibited when using LIM containing no di- or trivalent cations.

The use of glass slides was found in some instances to be more sensitive than that of microtiter trays; however, the HA activity of both CFA/I and CFA/II bacteria was easily detectable by using the latter system. Also when using solutions at physiological ionic strength, we have observed considerable variation in HA activity among different type A units of blood received. This variation was minimized when the HA reaction was tested in LIM (unpublished data).

We concluded this study with evidence that solutions other than buffered saline could be used preferentially in bacterial HA tests. One formulation which we have used consistently, based on the results of these experiments, contained 200 mM sorbitol, 50 mM mannose, and 25 mM MES buffer. The pH was adjusted to 6.5 with 1 N NaOH, and the final ionic strength was 13 mM. This LIM did not contain NaCl or divalent cations and had provided us with a superior testing solution in our studies with bacteria exhibiting mannose-resistant HA properties.

The use of LIM to study HA caused by these *E. coli* exhibited several advantages over existing systems: (i) temperature sensitivities and overall time of incubation were reduced; (ii) variation in HA activity within a single blood type was minimized; (iii) a greater sensitivity in the HA reaction was achieved such that reactions which were not visible in saline were evi-

dent in LIM; and (iv) the use of microtiter trays increased the speed and convenience of testing.

ACKNOWLEDGMENTS

We thank Lisa Nurmi, Frank Giesa, and Glenn Hofmann for their excellent technical assistance and Linda Hampton for her preparation of this manuscript.

LITERATURE CITED

1. **Abramson, H. A., and L. S. Moyer.** 1935. The electrical charge of mammalian red blood cells. J. Gen. Physiol. **19**:601–607.
2. **Deneke, C. F., G. M. Thorne, and S. L. Gorbach.** 1979. Attachment pili from enterotoxigenic *Escherichia coli* pathogenic for humans. Infect. Immun. **26**:362–368.
3. **Duguid, J. P., S. Clegg, and M. I. Wilson.** 1979. The fimbrial and nonfimbrial haemagglutinins of *Escherichia coli*. J. Med. Microbiol. **12**:213–227.
4. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups O6 and O8. Infect. Immun. **21**:638–647.
5. **Evans, D. G., D. J. Evans, Jr., and W. Tjoa.** 1977. Hemagglutination of human group A erythrocytes by enterotoxigenic *Escherichia coli* isolated from adults with diarrhea: correlation with colonization factor. Infect. Immun. **18**:330–337.
6. **Evans, D. G., R. P. Silver, D. J. Evans, Jr., D. G. Chase, and S. L. Gorbach.** 1975. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. Infect. Immun. **12**:656–667.
7. **Evans, D. J., Jr., and D. G. Evans.** 1973. Three characteristics associated with enterotoxigenic *Escherichia coli* isolated from man. Infect. Immun. **8**:322–328.
8. **Hahon, N., J. A. Booth, and H. L. Eckert.** 1973. Cell attachment and penetration by influenza virus. Infect. Immun. **7**:341–351.
9. **Heard, D. H., and G. V. F. Seaman.** 1960. The influence of pH and ionic strength on the electrokinetic stability of the human erythrocyte membrane. J. Gen. Physiol. **43**:635–654.
10. **Heckels, J. E., B. Blackett, J. S. Everson, and M. E. Ward.** 1976. The influence of surface charge on the attachment of *Neisseria gonorrhoeae* to human cells. J. Gen. Microbiol. **96**:359–364.
11. **James, A. N., J. M. Knox, and R. P. Williams.** 1976. Attachment of gonococci to sperm, influence of physical and chemical factors. Br. J. Vener. Dis. **52**:128–135.
12. **Jan, K., and S. Chien.** 1973. Influence of the ionic composition of fluid medium on red cell aggregation. J. Gen. Physiol. **61**:655–668.
13. **Jones, G. W., and J. M. Rutter.** 1974. The association of K 88 antigen with haemagglutinating activity in porcine strains of *Escherichia coli*. J. Gen. Microbiol. **84**:135–144.
14. **Jørgensen, J., M. Nielson, C. B. Nielson, and J. Nørmark.** 1980. The influence of ionic strength, albumin and incubation time on the sensitivity of the indirect Coombs' test. Vox Sang. **36**:186–191.
15. **Marshall, K. C., R. Stout, and R. Mitchell.** 1971. Mechanism of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol. **68**:337–348.
16. **Moore, H. C., and P. L. Millison.** 1976. Use of a low-ionic-strength medium in manual tests for antibody detection. Transfusion **16**:291–296.
17. **Ochoa, J. L.** 1979. The mechanisms of lectin-mediated cell agglutination. Pathol. Biol. (Paris) **27**:103–113.
18. **Ørskov, I., and F. Ørskov.** 1977. Special O:K:H serotypes among enterotoxigenic *E. coli* strains from diarrhea in adults and children. Med. Microbiol. Immunol. **163**:99–110.
19. **Pierce, J. S., E. G. Strauss, and J. H. Strauss.** 1974. Effect of ionic strength on the binding of Sindbis virus to chick cells. J. Virol. **13**:1030–1036.
20. **Rosenberg, M. L., J. P. Koplan, K. Wachsmuth, J. G. Wells, E. J. Gangarosa, R. L. Guerrant, and D. A. Sack.** 1977. Epidemic diarrhea at Crater Lake from enterotoxigenic *Escherichia coli*. Ann. Intern. Med. **86**: 714–718.
21. **Rosenfield, R. E., S. H. Shaikh, F. Innella, Z. Kaczera, and S. Kochwa.** 1979. Augmentation of hemagglutination by low ionic conditions. Transfusion **19**:499–510.
22. **Schnebli, H. P., C. Roeder, and L. Tarcsay.** 1976. Reaction of lectins with human erythrocytes. III. Surface charge density and agglutination. Exp. Cell Res. **98**:273–276.
23. **Schott, H., and C. Y. Young.** 1972. Electrokinetic studies of bacteria. I. Effect of nature, ionic strength, and pH of buffer solutions on electrophoretic mobility of *Streptococcus faecalis* and *Escherichia coli*. J. Pharm. Sci. **61**: 182–187.
24. **Smyth, C. J., B. Kaijser, E. Bäck, A. Faris, R. Möllby, O. Söderlbind, G. Stintzing, and T. Wadström.** 1979. Occurrence of adhesins causing mannose-resistant hemagglutination of bovie erythrocytes in enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett. **5**:85–90.
25. **van Oss, C. J., J. F. Mohn, and R. K. Cunningham.** 1978. Influence of various physicochemical factors on heamagglutination. Vox Sang. **34**:351–361.