# THE REQUIREMENT OF ELECTROLYTES FOR THE ADSORPTION OF ESCHERICHIA COLI ANTIGEN BY RED BLOOD CELLS[1]

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Like certain viruses, various bacterial antigens, including those of *Salmonella typhosa, Escherichia coli, Hemophilus influenzae, Pasteurella pestis, Mycobacterium tuberculosis*, are adsorbed by red blood cells. The erythrocytes thus modified by the bacterial antigen become specifically agglutinable by homologous bacterial antibodies. This indirect bacterial hemagglutination test and its hemolytic modification can be used for the demonstration and titration of either bacterial antigens or bacterial antibodies. Lowell and Buckingham (1948), Davenport and Horsfall (1948), Flick, Sanford, and Mudd (1949), Puck, Garen, and Cline (1951), and Burnet and Edney (1952) have shown that electrolytes are necessary for the adsorption of animal viruses (influenza virus, pneumonia virus of mice) by red blood cells and of bacteriophage by bacteria and inorganic adsorbants. The present study was undertaken to determine whether the adsorption of bacterial (*Escherichia coli*) antigens, too, is dependent upon the presence of electrolytes and to elucidate the effect of variation in concentration of certain electrolytes on this reaction. *E. coli* (serogroups 0111, 055, 026) hemagglutination and hemolysis have been described in detail in reports from this laboratory (Neter *et al.*, 1952*b*, *c*).

## MATERIAL AND METHODS

Suspensions of *E. coli*, serogroups 0111, 055, and 026, harvested from Kolle flasks which had been incubated for 24 hours at 37 C, were used as antigens. These serogroups are of particular interest since they have been found to be associated with infantile gastroenteritis (*cf* Neter *et al.*, 1951). The suspensions were heated in boiling water for 2½ hours in order to procure active erythrocyte modifying antigens as described in detail previously (Neter *et al.*, 1952*b*, *c*). A hemolytic unit of these antigens is defined as the smallest amount which just modifies sheep red blood cells for gross lysis by optimal amounts of homologous *E. coli* antiserum and guinea pig complement.

Suspensions of red blood cells (2.5 per cent) were prepared from defibrinated or oxalated blood of man, sheep, rabbit, duck, and chicken. The erythrocytes were washed three times in the diluents mentioned below. Stable suspensions in 5 per cent glucose solution were obtained with red blood cells of sheep and rabbit but not with those of man, chicken, and duck. Agglutination of the latter was avoided by the use of 5 per cent glucose solution containing 0.0025 M $Na_2HPO_4$, as suggested by Burnet and Edney (1952).

As diluents the following isotonic solutions were used: (1) 0.9 per cent (0.15 M) sodium chloride solution, (2) 5 per cent glucose solution, (3) 5 per cent sucrose solution, (4) 1.5 per cent (0.1 M) calcium chloride solution, (5) 1.1 per cent (0.67 M) potassium chloride solution, and (6) 3 per cent (0.1 M) sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) solution. All solutions were prepared from reagents in triple distilled water. Mixtures of electrolyte solution and 5 per cent glucose solution were employed in various proportions. Various solutions were used as diluent only during the first (antigen-adsorption) phase of the reaction; the diluent for the second (hemagglutination and hemolysis) phase was physiological saline (0.15 M) solution. Mixtures of red blood cells and bacterial antigen in various diluents were kept at 37 C for 30 minutes. The treated erythrocytes then were washed three times in physiological saline solution and mixed with equal amounts (0.2 ml) of serially diluted *E. coli* antiserum. In the hemagglutination experiments the mixtures of treated red blood cells and antiserum were incubated at 37 C for 30 minutes, and agglutination was read grossly after centrifugalization at 2,000 rpm for one minute. In

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the hemolysis tests, guinea pig complement[2] (0.1 ml of a 1:10 dilution) was added to the mixtures of red blood cells and *E. coli* antiserum. Lysis was read in the gross after incubation at 37 C for 30 minutes. The specificity of the resulting hemagglutination and hemolysis was checked by lack of hemagglutination and hemolysis in the presence of heterologous *E. coli* antisera.

## RESULTS

The first experiment was designed to determine whether *E. coli* antigen is adsorbed by red blood cells in the absence of electrolytes and to ascertain the effects of various concentrations of sodium chloride on this reaction. Approximately 50 minimal hemolytic units of *E. coli* 026 antigen (equivalent to a 1:100 dilution) were mixed with thrice washed sheep red blood cells; the following diluents were used: (1) 5 per cent glucose solution, (2) 5 per cent sucrose solution, and (3) varying concentrations (0.15 to 0.006 M) of NaCl in distilled water, made isotonic with 5 per cent glucose. The treated red blood cells were washed with physiological saline solution and mixed with homologous *E. coli* antiserum and complement, physiological saline solution serving as diluent. The resulting hemolysis was recorded. The data obtained in this experiment are summarized in table 1.

It can be seen that sheep red blood cells were not modified in the absence of electrolytes, as evidenced by the lack of hemolysis when glucose or sucrose was used as diluent.[3] In contrast, the homologous *E. coli* antiserum in high dilution caused lysis of the erythrocytes which had been modified by the antigen in physiological saline solution (0.15 M). It should be mentioned that suspending sheep red blood cells in 5 per cent glucose solution did not affect subsequent modification with *E. coli* antigen in physiological saline solution. It can be seen, furthermore, that as little as 0.03 M sodium chloride sufficed for optimal modification of the erythrocytes and that with smaller concentrations of NaCl the resulting hemolysis was either distinctly decreased (0.015 M) or completely absent (0.006 M).

The conclusion that adsorption of *E. coli* antigen required the presence of electrolytes finds additional support by the observato n that red blood cells of other species, i.e., sheep, man, rabbit, chicken, and duck, treated with *E. coli* 026 antigen in either 5 per cent glucose or 5 per cent sucrose solution were not agglutinated by *E. coli* 026 antiserum, whereas erythrocytes treated with the same antigen in physiological saline solution were agglutinated. Identical results were obtained with two other *E. coli* antigens (0111 and 055).

If, as suggested by the results of these experiments, adsorption of *E. coli* antigen does not take place with 5 per cent glucose solution as diluent, then it should be possible to demonstrate the antigen in the supernatant fluid after treatment of the red blood cells. This was found to be the case as shown by the results of the following experiment. *E. coli* antigen was mixed with the sediment of sheep red blood cell suspension in 5 per cent glucose solution and incubated for 30 minutes at 37 C. This procedure was repeated twice. The supernatant fluid to which appropriate amounts of NaCl had been added was used for treatment of sheep red blood cells. It was found that this supernate modified sheep red blood cells for hemolysis equally well as did unadsorbed *E. coli* antigen in identical concentration. On the other hand, when the adsorption experiment was carried out in physiological saline solution

TABLE 1

*The effect of electrolytes on adsorption of Escherichia coli antigen by red blood cells*

| ESCHERICHIA COLI 026 ANTISERUM IN DILUTIONS OF | LYSIS BY ESCHERICHIA COLI 026 ANTISERUM AND COMPLEMENT OF SHEEP RED BLOOD CELLS MODIFIED BY E. COLI 026 ANTIGEN IN | | | | | |
|---|---|---|---|---|---|---|
| | 5 per cent glucose | 5 per cent sucrose | 0.15 M NaCl | 0.03 M NaCl* | 0.015 M NaCl* | 0.006 M NaCl* |
| 1:100 | 0 | 0 | 4 | 4 | 4 | 0 |
| 1:200 | 0 | 0 | 4 | 4 | 3 | 0 |
| 1:400 | 0 | 0 | 4 | 4 | 2 | 0 |
| 1:800 | 0 | 0 | 4 | 4 | 1 | 0 |
| 1:1,600 | 0 | 0 | 4 | 4 | 0 | 0 |
| 1:3,200 | 0 | 0 | 3 | 3 | 0 | 0 |
| 1:6,400 | 0 | 0 | 2 | 1 | 0 | 0 |
| 1:12,800 | 0 | 0 | 1 | 1 | 0 | 0 |
| 1:25,600 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |

1 to 4 = various degrees of hemolysis.
0 = no hemolysis.
* = made isotonic with 5 per cent glucose.

[2] Carworth Farms, Inc., Rockland County, New City, New York.

[3] The electrolytes present in the red blood cells are excluded from consideration.

under otherwise identical conditions, the supernatant fluid failed to modify sheep red blood cells; the erythrocytes were not lysed in the presence of *E. coli* antiserum and complement.

On the basis of these observations it became of interest to ascertain whether other salts can replace sodium chloride. The minimal concentrations of calcium chloride, potassium chloride, and sodium citrate which make possible slight and optimal modification of sheep red blood cells were determined. Using 50 units of *E. coli* 026 antigen and hemolysis as indicator for modification of sheep red blood cells, the results recorded in table 2 were obtained.

It can be seen from this table that adsorption of the bacterial antigen took place in the presence of any one of the four electrolyte solutions. It is interesting to point out that calcium chloride in concentrations up to 0.1 M was the only salt which did not make possible optimal modification of red blood cells although partial modification of red blood cells occurred with lower concentrations of calcium chloride than with those of the other salts. The data suggest, furthermore, that no one particular ion is indispensable for the adsorption of the bacterial antigen by red blood cells.

TABLE 2

*The minimal and optimal concentrations of various electrolytes for adsorption of Escherichia coli antigen by sheep red blood cells*

| MODIFICATION OF RED BLOOD CELLS | ELECTROLYTE CONCENTRATION (M)* | | | |
|---|---|---|---|---|
| | NaCl | $CaCl_2$ | KCl | Sodium citrate |
| Optimal | 0.03–0.15 | not optimal up to 0.1 | 0.03–0.6 | 0.017–0.1 |
| Minimal | 0.015 | 0.00025 | 0.0015 | 0.004–0.005 |
| Absent | 0.006 | 0.00006 | 0.0007 | 0.001–0.002 |

* = made isotonic with 5 per cent glucose.

Additional experiments revealed that with lesser amounts of antigen higher concentrations of the salt are required to effect modification. For example, 0.015 M sodium chloride solution sufficed to allow adsorption of enough antigen to be demonstrable by specific hemolysis when 50 units but not when 10 units were used. Since smaller amounts of *E. coli* antigen can be detected on red blood cells by means of the hemolysis test as compared to the hemagglutination test, it is not surprising to find that higher concentrations of the various electrolytes were required for minimal modification of red blood cells for agglutination than for lysis. For example, with 50 hemolytic units of antigen an 8 to 10-fold greater concentration of KCl and sodium citrate was needed to allow modification of sheep red blood cells for specific hemagglutination than for hemolysis.

## DISCUSSION

The present study revealed that electrolytes are necessary for the adsorption of *E. coli* (serogroups 026, 055, and 0111) antigens by red blood cells. This is borne out by the fact that modification of sheep red blood cells does not take place in 5 per cent glucose or 5 per cent sucrose solutions. On the other hand, in the presence of the electrolytes, namely, sodium chloride, potassium chloride, calcium chloride, or sodium citrate, sheep red blood cells adsorb these antigens and thus become specifically agglutinable (and lyzable) in the presence of homologous bacterial antibody (and complement). Erythrocytes of other animal species (man, rabbit, chicken, duck) likewise do not adsorb *E. coli* antigens in the absence of the electrolytes. Whether electrolytes are needed for the adsorption on red blood cells of other bacterial antigens which are known to modify erythrocytes, such as antigenic components or products of *S. typhosa* (Vi and O antigens), *M. tuberculosis*, *P. pestis*, and others, remains to be determined.

The findings reported in this communication parallel observations on the role of electrolytes in the adsorption of certain viruses. Davenport and Horsfall (1948) reported that the combination between the pneumonia virus of mice and red blood cells is inhibited at low electrolyte concentrations and that the virus can be dissociated from lung particles and erythrocytes in solutions of low electrolyte concentration. Lowell and Buckingham (1948) observed that the adsorption by red blood cells of influenza A virus (PR8) failed to take place at a sodium chloride concentration of approximately 0.003 M. It is interesting to note that the adsorption of *E. coli* antigen does not occur at approximately the same NaCl (0.006 M) concentration. Flick, Sanford, and Mudd (1949) found that influenza A virus is not adsorbed at very low salt concentrations and that the virus adsorbed to cells in

the presence of salt can be eluted quickly by salt-free water at 4 C. Experiments are now in progress to determine whether *E. coli* antigen, too, can be recovered from red blood cells.

Extensive studies on the role of electrolytes in the adsorption of bacteriophage by bacterial host cells were carried out by Puck, Garen, and Cline (1951). These authors found that T1 virus does not attach to its host cell, *E. coli*, strain B, in distilled water and that by the proper addition of salts the rate of attachment can be adjusted to any desired value up to the maximum limit set by the diffusion rate of the virus. Interestingly, the virus can be attached also to glass filters, and virus thus adsorbed can be recovered almost quantitatively by washing the filter with a solution in which the attachment reaction does not occur. It remains to be seen whether *E. coli* antigen can be adsorbed on, and recovered from, glass filters and other adsorbants. Such a procedure may aid materially in the purification of the *E. coli* antigens operative in hemagglutination and hemolysis.

Burnet and Edney (1952) who confirmed the part played by electrolytes in the adsorption of influenza virus by red blood cells found that absence of electrolytes also interferes with the reaction between virus and virus-inhibitor. Since, as shown from this laboratory (Neter *et al.*, 1952*a*), certain materials (normal serum; human plasma fractions IV-1, IV-7, and albumin; egg yolk; lecithin; cholesterol) inhibit *E. coli* hemagglutination and hemolysis, presumably, in part at least, by interference with adsorption of *E. coli* antigen, it will be interesting to determine whether electrolytes are needed for the action of the inhibitors.

In the present study the adsorption of *E. coli* antigens by red blood cells was considered optimal when the process was carried out in physiological saline solution. It is conceivable that solutions of electrolytes may be found which facilitate the adsorption of bacterial antigens to a greater degree than does 0.9 per cent NaCl solution. If this be so, such diluents may be useful in indirect bacterial hemagglutination and hemolysis tests carried out for diagnostic purposes.

## SUMMARY AND CONCLUSIONS

The role of electrolytes in the adsorption of *Escherichia coli* serogroups 026, 055, and 0111 antigens was determined, and the following results were obtained.

*E. coli* antigens are not adsorbed by red blood cells of sheep, man, rabbit, chicken, and duck in the absence of electrolytes, i.e., when 5 per cent glucose or 5 per cent sucrose solutions are used as diluent. Lack of adsorption is evidenced by the fact that erythrocytes thus treated are not agglutinated specifically by homologous bacterial antiserum and, in the case of sheep cells, are neither agglutinated nor lysed. The supernatant fluid contains the unadsorbed antigen as shown by the observation that, on addition of sodium chloride, it modifies red blood cells for specific bacterial hemagglutination and hemolysis.

Adsorption of the *E. coli* antigens by red blood cells takes place in the presence of suitable concentrations of NaCl, KCl, $CaCl_2$, and sodium citrate. The lowest concentrations of these electrolytes required to make possible adsorption of the antigens for both minimal and optimal modification of red blood cells and thus for subsequent hemagglutination and hemolysis were determined. It was found, furthermore, that within certain limits the less antigen is used for treatment of erythrocytes the higher must be the concentration of the electrolytes.

The requirement of electrolytes for adsorption by red blood cells of bacterial (*E. coli*) antigens parallels similar findings with certain viruses.

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