Influence of Erythrocyte Concentration and Cation Levels on the Hemolytic Action of Staphylococcal α-Hemolysin

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It is generally assumed that the hemolytic action of staphylococcal α-hemolysin (α-toxin; kindly supplied in lyophilized form by Wellcome Research Laboratories, Beckenham, England) is dependent on toxin concentration, duration of incubation, temperature, pH of the incubation medium, and species of the erythrocytes. No attention appears to have been paid to the cation levels of the incubation medium. Furthermore, no uniform opinion exists about the importance of the erythrocyte concentration. B. Wiegershagen (Arch. Intern. Pharmacodyn. 136:174, 1962) found some influence of cell concentration in the case of the rabbit, cat, and rat, but none in the case of sheep and cattle. A. A. Marucci (J. Bacteriol. 86:1182, 1963) found no influence of the number of rabbit cells originally present in the reaction mixture. From his curves, it is seen that he used relatively high α-toxin concentrations.

In our studies of the mechanism of lysis by α-toxin, we found a definite influence of cell concentration (Fig. 1), when using pooled human erythrocytes. The concentration of dried α-toxin
was about 305 hemolytic units/100 mg. Medium, erythrocytes, and α-toxin were kept ice-cold and were pipetted into test tubes, and each experiment was made in triplicate. The tubes were placed in a 37°C water bath. Samples were removed at appropriate intervals and were centrifuged immediately. All experiments were repeated at least once. The results of representative experiments are indicated in Fig. 1 and 2. At low cell concentrations (or at a relatively high α-toxin concentration), there was rapid and complete lysis, but at higher cell concentrations lysis by the same amount of toxin was less. Thus, it seems likely that the ratio of α-toxin to cell number is important.

To study the effect of medium composition, the following media were prepared (all concentrations in millimoles per liter): I = NaCl, 102.7; Na-lactate, 45.3; KCl, 5.4; CaCl₂, 0.9; Ca-lactate, 1.6; and MgCl₂, 1.0. II = KCl, 53.6; NaCl, 42.8; and MgCl₂, 1.0. III = tris(hydroxymethyl)-aminomethane (Tris), 169.0; H₃PO₄, 25.0; HCl, 105.0; and MgCl₂, 1.0. IV = NaCl, 110.0; Na₂HPO₄, 25.0; and MgCl₂, 1.0. V = KCl, 93.9; KH₂PO₄, 7.4; and MgCl₂, 1.0. In each case, the final pH was adjusted to 7.4 with solid Tris, whereupon the osmolarity was adjusted to 315 milliosmols with solid glucose.

The lysis of human erythrocytes was tested at 37°C in these media. Figure 2 shows the influence of different cationic concentrations on the hemolytic action of α-toxin. The different amounts of glucose added to the various diluents could not account for the results. In tests with erythrocytes from several species, we found lysis decreasing in the order indicated. Cavia: I, II, V, IV, III. Chicken: II, V, IV, I, III. Hamster: I, IV, V, II, III. Human: V, II, I, IV, III. Mouse: IV, V, II, I, III. Rabbit: II, IV, V, I, III. Rat: I, IV, V, II, III. Sheep: II, V, I, IV, III. It is remarkable that in each case lysis was minimal in the medium without sodium and potassium (III).

From these findings, it seems desirable for achieving maximal sensitivity in the detection of α-toxin to use very few cells and to use the medium in which α-toxin has the strongest hemolytic power for the species of erythrocytes used.