More uniform distributions give $F_0/F \ll 2$. This result suggests that recombination becomes progressively easier as the adjacent sites are filled, so that the full quartet is achieved when possible.

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The salt-sensitive step in immune hemolysis

The hemolysis of red blood cells (E) by antibody (A) and complement (C′) is believed to result from the following sequence of reactions[1]:

$$E + A \rightleftharpoons EA \quad (1)$$

$$EA + C'_{1,4} \xrightarrow{Ca^{++}} EAC'_{1,4} \quad (2)$$

$$EAC'_{1,4} + C'_2 \xrightarrow{Mg^{++}} EAC'_{1,4,2} \xrightarrow[37^\circ]{\text{rapid}} EAC_{1,4} \quad (3a)$$

$$EAC'_{1,4,2} + C'_3 \longrightarrow E^* \quad (4)$$

$$E^* \longrightarrow \text{ghost} + \text{hemoglobin} \quad (5)$$

where C'_1, C_2, C_3, C_4 refer to the components of complement and E^* refers to a damaged cell which spontaneously hemolyzes. The overall reaction of sensitized cell (EA) with C′ has been shown to be very sensitive to the concentration of NaCl in the reaction mixture[2]. In studying the precise steps at which various inhibitors of the immune hemolytic reaction operate it was observed that reaction step 4, $EAC'_{1,4,2} + C'_3 \longrightarrow E^*$, is extremely sensitive to the effect of NaCl concentration, and, in fact, the effect of NaCl concentration on the overall hemolytic reaction can be quantitatively accounted for by its effect on step 4.

Sheep erythrocytes, rabbit hemolysin and triethanolamine-buffered saline were prepared as described previously[3]. In determining the effect of salt, varying quantities of fresh guinea serum diluted 1:100, buffered saline and either distilled water or 0.3 *M* NaCl were added to give a volume of 1.2 ml. The amounts of distilled water or 0.3 *M* NaCl were chosen so as to give NaCl concentrations in the final reaction mixture varying from 0.130 *M* to 0.188 *M*. To the 1.2 ml were added $1.5 \cdot 10^8$ sensitized cells in a volume of 0.3 ml. Exactly the same procedure was used in studying the step, $EAC'_{1,4,2} + C'_3 \longrightarrow$ hemolysis, except $EAC'_{1,4,2}$ was used in place of EA and 0.005 *M* EDTA was also added to the reaction mixture. By binding Ca++ and Mg++ EDTA prevented reactions 2 and 3. In all instances the reaction mixtures were incubated for 30 min at 37°; at the end of this time, 0.5 ml buffered saline was added, the tubes were centrifuged and the amount of hemolysis determined by reading the supernatants in a Junior Coleman spectrophotometer at 550 mμ. The volume of complement required for 50% hemolysis of EA and $EAC'_{1,4,2}$ was determined in

Abbreviation: EDTA, ethylenediaminetetraacetic acid.

this way for each of the series of NaCl concentrations. For purposes of comparison, the volume of complement required for 50 % hemolysis when the reactions were run in 0.15 *M* NaCl was termed 100 %, and the amounts of complement required for the other NaCl concentrations were calculated as percentages of this value.

Fig. 1 shows that the effect of salt on the overall reaction and on the reaction, $EAC'_{1,4,2} + C'_3$ was essentially the same. Included for comparison are the recalculated data of MAYER *et al.*[2].

As carried out, the reaction with $EAC'_{1,4,2}$ and C′ in the presence of EDTA involves both step 4 and step 5; however, MAYER *et al.*[4] have shown that concentrations of NaCl up to 0.19 *M* have no effect on step 5 and this was confirmed in this work. Although it has been shown that increased concentrations of NaCl will decrease the binding of C'_1, the concentrations required are greater than used here. The effect of Na_2SO_4 on step 4 is even greater than NaCl and considerable greater than can be accounted for by the increased contribution of Na_2SO_4 to the ionic strength.

Dr. MYRON LEON has informed us that he has independently found that increased NaCl concentrations inhibited step 4 when human C′ is used.

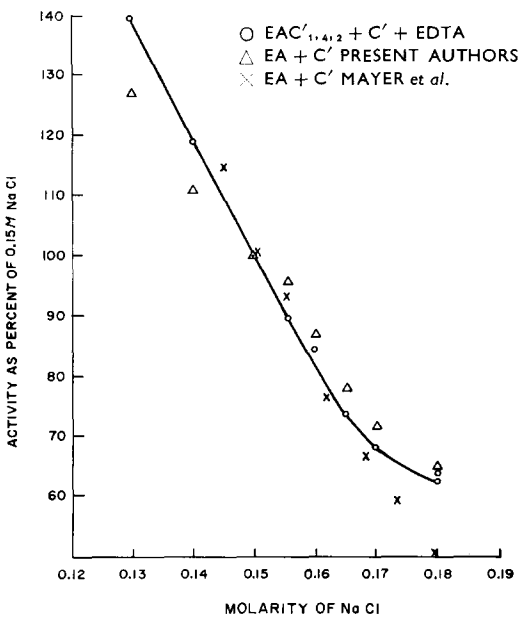

Fig. 1. Effect of NaCl concentration on overall hemolytic reaction and on $EAC'_{1,4,2} + C'_3$ reaction step.

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