THE AGGLUTINATION OF RED BLOOD CELLS.

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The agglutination of a number of suspensions has been found to be closely related to the potential difference between the particle and the surrounding liquid as measured by the migration in an electric field.[1] If the potential is higher than a certain value—the critical potential—the particles remain separate, if it is lower than this value, they adhere or agglutinate. It follows that agglutination may be caused in two ways, (1) by decreasing the actual potential, or (2) by increasing the critical potential. In general, electrolytes do not affect the value of the critical potential so that in most cases agglutination occurs whenever sufficient electrolyte has been added to depress the potential below a certain definite value. This has been found in many cases to be about 14 millivolts. The value is usually independent of the nature of the electrolyte and even of the nature of the particle. It was found in the study of agglutination of *Bacillus typhosus*,[2] however, that in this case high concentrations of electrolyte decrease the critical potential as well as the actual potential, so that no agglutination occurred in strong NaCl although the potential was practically zero. In confirmation of this, it was found that the force with which two smears of the organism stuck together was also decreased by concentrated salt. Bacteria which had been sensitized with immune serum do not show this peculiarity and agglutination occurs whenever the potential is reduced below about 14 millivolts.[3]

The effect of the antiserum is, therefore, primarily on the critical potential whereas the effect of electrolytes is primarily on the poten-

[1] A general summary of the agglutination of colloidal particles can be found in Bogue, R. H., Theory of colloidal behavior, New York and London, 1924, chapter on the flocculation and stability of colloidal suspensions.

[2] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, iv, 639.

[3] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, iv, 655.

tial itself. The agglutination of red cells is essentially similar to that of bacteria and it has already been shown by Mines[4] and especially Coulter[5] that the acid agglutination point coincides with a very low potential. The present paper contains the result of experiments in which the effect of electrolytes, serum, ricin, colloidal stannic hydroxide, and paraffin oil on the potential and agglutination of red blood cells has been studied. It has been found that agglutination of unsensitized cells occurs if the potential is depressed below about 4 millivolts whereas cells which have been previously treated with immune serum, ricin, colloidal stannic hydroxide, or paraffin oil agglutinate when the potential is below about 12 millivolts.

Experimental Procedure.—The blood was collected in citrate, centrifuged, and the cells washed with 4 per cent dextrose containing M/10,000 trisodium citrate, in order to prevent agglutination by the CO_2. The suspension was then diluted to twenty times the original volume of the blood and 1 cc. of this added to 50 cc. of the various salt solutions. The potential was measured as described in a previous publication by noting the rate of migration in an electric field. The potential was calculated by means of the formula P.D. in millivolts $= \frac{13 \times \mu \text{ per second}}{\text{volt per centimeter}}$. There is considerable uncertainty as to the correctness of this formula and the results could be equally well expressed as rate of migration instead of potential difference. The tubes were allowed to stand about 18 hours at 10°C. and the readings made after this time. C was recorded when the suspension had settled, leaving a perfectly clear supernatant liquid. In some cases the suspension was filtered through a coarse grade of filter paper. Those tubes read as C gave a clear filtrate containing no cells.

Agglutination by Electrolytes.

Cells suspended in sugar alone frequently showed agglutination due to CO_2 adsorption and subsequent reduction of the pH to the acid agglutination range.[5] In order to avoid this difficulty, a very low concentration of trisodium citrate[6] (10^{-4} M) was added to the

[4] Mines, G. R., *Kolloidchem. Beihefte*, 1911–12, iii, 191.

[5] Coulter, C. B., *J. Gen. Physiol.*, 1920–21, iii, 309.

[6] Atkin, E. E., *Z. Immunitätsforsch., Orig.*, 1908–09, i, 387.

suspension. This salt confers a negative charge on the cells and renders the suspension stable. The variations in the potential of the control tubes are due to variations in the amount of citrate present.

The effect of electrolytes is shown in Table I. It is evident that whenever the potential is less than about 6 millivolts the cells agglutinate, except in the case of $MgCl_2$ and $CaCl_2$. In these two salts no agglutination occurs even though there is no potential. The same peculiarity was found in regard to bacteria and is due to a decrease in the critical potential by these salts. It could be shown in the case of bacteria that this decrease was paralleled by a decrease in the force required to tear apart two films of the organisms.[2] In the presence of concentrated Ca or Mg salts, therefore, there is no "cohesive force" between the particles and so no potential difference is required to keep them apart. That this is not due to an error in the P.D. measurement may be shown by adding increasing amounts of HCl to a suspension in 0.10 M $MgCl_2$. Under these conditions the potential will vary from very slightly negative to slightly positive and must therefore be zero in some intermediate tube even though there is a large error in the measurement. No agglutination occurs, however.

As usual the concentration required to agglutinate is less the higher the valence of the oppositely charged ion (cation), and salts of heavy metals are more efficient than alkaline earth salts. This agglutinating effect of salts had been noted by Eisner and Friedemann[7] and by Schürmann and Baumgärtel.[8] The present experiment shows that it is due to the reduction of the potential below the critical value. Excess of polyvalent cations or hydrogen ions confers a positive charge and the suspension may again become stable.

Agglutination by Immune Serum.

Table II shows the relation between the potential and agglutination by immune serum. In NaCl under the ordinary conditions of experiment the serum causes agglutination both by decreasing the

[7] Eisner, G., and Friedemann, U., *Z. Immunitätsforsch., Orig.*, 1914, xxi, 520. *Cf.* also Landsteiner and Jagić.[12]

[8] Schürmann, W., and Baumgärtel, T., *Z. Immunitätsforsch., Orig.*, 1921, xxxi, 151.

TABLE I.

Effect of Various Electrolytes on Potential and Agglutination of Sheep Cells in Isotonic Sugar.

1/1,000 red cells in isotonic solution containing 10^{-4} M Na_3 citrate.

Electrolyte.	NaCl		NH_4Cl		$MgCl_2$		$CaCl_2$		$CuSO_4$		Pb acetate.		HCl (0.001 citrate.)		$LaCl_3$		$Th(NO_3)_4$	
Concentration.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.
M	*mv.*		*mv.*		*mv.*		*mv.*		*mv.*		*mv.*		*mv.*		*mv.*		*mv.*	
—	17.0	—	−15.0	—	17.8	—	−12.2	—	−11.0	—	−16.3	—	−44.0	—	−16.0		−13.8	—
5×10^{-7}													−32.0	—			−14.0	—
3×10^{-6}																	−6.0	+
5×10^{-6}									8.0	—							0	C
10^{-5}											−17.0	—	−39.0	—			+4.7	C
3×10^{-5}									−4.2	+			−22.0	+	−12.6	—		
6×10^{-5}													−6.0	++			+12.0	—
10^{-4}									0	C	−8.0	—	0	C	−7.1	+		
3×10^{-4}									+3.0	C			0	C				
10^{-3}					−13.8	—	−12.2	+	+3.5	C	−1.0	C	+12.0	(Hemolysis.)	0	C		
3×10^{-3}			−13.0	—														
6×10^{-3}					−8.7	—	−9.2	+							+2.0	+++		
10^{-2}	15.0	—	−8.0	—	−2.5	—	8.4	+	+4.0	C	0	C						
3×10^{-2}	15.0	—			0	—	3.8	+							+3.0	+++		
6×10^{-2}	12.0	—	−8.4	—	0	—	2.5	+										
10^{-1}	11.0	—			0	—					0	C						

potential and increasing the critical potential. Normal serum has no effect on either value. In $MgCl_2$ there is no change in the actual potential and yet agglutination occurs. This experiment shows that the effect of the immune serum is primarily to raise the critical potential. This effect is shown more clearly if the cells are first sensitized with antiserum in sugar and then treated with increasing salt concentrations. A large excess of inactivated antiserum, equivalent to ten agglutinating doses, was added to a suspension of sheep cells in citrated sugar. The suspension was centrifuged and the cells washed with sugar. 1 cc. of this suspension was then added to the 50 cc. of the salt solutions. The results are shown in Table III.

TABLE II.

Effect of Immune Serum on Agglutination and Potential of Sheep Cells Suspended in NaCl or $MgCl_2$.

Concentration of rabbit anti-sheep serum. (Cc. serum in 50 cc. of suspension.)	Sheep cells in 0.85 per cent NaCl.		Sheep cells in M/15 $MgCl_2$.	
	P.D.	Agglutination.	P.D.	Agglutination.
M	mv.		mv.	
—	−13.0	—	—	—
10^{-4}	−11.8	+	—	++
10^{-3}	−11.0	+	—	+++
10^{-2}	−6.7	+++	—	C
5×10^{-2}	−3.0	C	—	C
10^{-1}	−2.0	C	—	C
Normal rabbit serum.				
10^{-1}	−12.7	—	—	—

Agglutination now occurs whenever the potential is less than about 12 millivolts instead of 6, as was the case with unsensitized cells, and the zone of agglutination is therefore broadened. This is the value that was found for sensitized *Bacillus typhosus*[3] and also for collodion particles and particles of denatured egg albumin.[9]

Agglutination by Ricin.

It was found by Stillmark[10] that rabbit cells are agglutinated by ricin and that the reaction was very similar to immune agglutination.

[9] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 479.

[10] Stillmark, H., *Arb. pharmacol. Inst. zu Dorpat*, 1889, iii, 59.

TABLE III.

Effect of Electrolytes on P.D. *and Agglutination of Sensitized Red Cells.*

Sheep cells washed in sugar + M/10,000 citrate. Three agglutinating doses of rabbit anti-sheep serum added. Centrifuged, washed, and resuspended in sugar + M/10,000 citrate.

Concentration, mols. per liter.	NaCl		NH_4Cl		$MgCl_2$		$CuSO_4$		HCl (0.0002 citrate.)		$Th(NO_3)_4$	
	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.
	mv.		mv.		mv.		mv.		mv.		mv.	
–	−23.0	–	14.0	–	−22.0	–	−14.0	–	−35.0	–	−16.0	–
3×10^{-6}							−12.0	+++				
10^{-5}											−12.0	+
3×10^{-5}							−8.0	C	−25.0	–	−10.0	C
6×10^{-5}									−17.0	+++	0	C
10^{-4}							−5.0	C	−6.0	C		
3×10^{-4}					−12.0	+++	–	C	+9.0	C	+29.0	–
6×10^{-4}									+25.0	+		
									(Hemolysis.)			
10^{-3}	−20.0	–	−13.0	–								
3×10^{-3}			−11.0	C	−6.0	C	–	C				
10^{-2}	−17.0	+++	−8.0	C	0	C						
3×10^{-2}	−11.3	C			0	C						
6×10^{-2}	−9.0	C	7.0	C	0	C						
10^{-1}	−7.0	C			0	C						
5×10^{-1}	−6.0	C			0	C						

Ricin does not combine with sheep cells and therefore does not agglutinate them. The "specific" reaction is again the combination rather than the effect. This combination is probably not connected with any electrical properties since ricin will agglutinate cells which have been given a positive charge by the addition of $Th(NO_3)_4$. The same experiment may be performed with antiserum.[11] The specific part of the reaction is evidently the combination since, as will be

TABLE IV.

Effect of Electrolytes on P.D. *and Agglutination of Rabbit Cells Sensitized with Ricin.*

Rabbit cells in dextrose containing 0.0001 M Na_3 citrate + > two agglutinating doses of ricin. Centrifuged, resuspended in citrate-sugar solution. Electrolyte added.

Concentration.	NaCl		HCl		$CuSO_4$	
	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.
M	*mv.*		*mv.*		*mv.*	
—	−26.0	0	−22.0	0	−22.0	—
4×10^{-5}			−18.0	—		
10^{-4}					−19.0	+
2×10^{-4}			11.7	+++	−15.0	++
4×10^{-4}			10.0	C	−14.0	C
10^{-3}			0	C	−6.5	C
2×10^{-3}	−17.0	++				
4×10^{-3}	−12.0	C				
10^{-2}	−9.0	C				
2×10^{-2}	−2.5	C				
10^{-1}	2.0	C				

seen below, the effect of antibody on the cells does not differ from that of several other substances. Table IV contains the results of experiments with rabbit cells sensitized with ricin. The critical potential is again about 12 millivolts as was the case with sensitized sheep cells.

[11] Similar experiments can be made with bacteria. *Cf.* De Kruif, P. H., and Northrop, J. H., *J. Gen. Physiol.*, 1922–23, v, 127.

Agglutination with Colloidal Stannic Acid.

Landsteiner and Jagić[12] showed that typical hemagglutination could be caused by colloidal solutions of various acids and metal hydroxides. Table V shows the effect of colloidal stannic hydroxide on the agglutination and P.D. of sheep cells. In NaCl complete agglutination occurs even in high dilution of stannic hydroxide without any effect on the potential. The critical potential is evidently raised. No agglutination occurs in sucrose because the potential is higher than that of the critical value even after the latter has been raised by the stannic

TABLE V.

Agglutination by Stannic Acid.

Stannic acid prepared by precipitation of $SnCl_4$ with NaOH, precipitate washed and resuspended by NH_4OH. Excess NH_4OH boiled out.

Dilution of stannic acid solution.	Sheep cells in 0.9 per cent NaCl.	
	P.D.	Agglutination.
	mv.	
–	−12	–
1/3,200	−12	–
1/1,600	−12	+
1/800	−12	C
1/400	−12	C
1/100	−12	C
	Cells in isotonic sucrose + citrate.	
–	−41	–
1/80	−40	–
1/10	−50	–

hydroxide. It has been found in a number of instances that colloidal particles assume the characteristics of other substances added to the solution. Bacteria in the presence of proteins, for instance, assume the isoelectric point of the protein,[3] as do collodion particles. A clear cut case was described by Loeb,[9] who found that collodion particles treated with gelatin were agglutinated by the same concentration of salt which was required to salt out solutions of gelatin. It might be expected, therefore, that the same concentration of salt which agglutinated cells sensitized with stannic hydroxide would also coagulate

[12] Landsteiner, K., and Jagić, N., *Wien. klin. Woch.*, 1904, xvii, 63.

the stannic hydroxide itself. That this is the case is shown in Table VI. This relation does not always hold, however, as it was found by Landsteiner that colloidal silicic acid sensitized cells to the action of

TABLE VI.

Effect of Electrolytes on P.D. *and Agglutination of Cells Sensitized with Colloidal Stannic Acid, and on Precipitation of Stannic Acid Solution.*

Concentration.	NaCl — red cells.		NaCl agglutinates stannic acid alone.
	P.D.	Aggl.	
M	mv.		
—	52.0	—	—
0.007	30.0	—	—
0.015	24.0	+	—
0.03	20.0	+	—
0.06	11.7	C	C
0.12	8.0	C	C
0.25	<8.0	C	C
0.50	<8.0	C	C

TABLE VII.

Effect of Electrolytes on P.D. *and Agglutination of Sheep Cells Sensitized with Stannic Acid.*

Electrolyte concentration.	NaCl		HCl		$MgCl_2$	
	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl
M	mv.		mv.		mv.	
—	−52	—	−35	—	−38.0	—
10^{-4}			−18	—		
2×10^{-4}			−12	C		
4×10^{-4}			−8	C		
2×10^{-3}					−14.0	++
4×10^{-3}					−10.6	C
10^{-2}	−30	—			−9.0	C
2×10^{-1}	−24	—			−8.0	C
4×10^{-1}	−20	++				
10^{-1}	−12	C			<−8.0	C
2×10^{-1}	−6	C				
5×10^{-1}	<−6	C				

electrolytes without being itself precipitated. The effect of electrolytes on the potential and agglutination of cells sensitized with stannic hydroxide is shown in Table VII.

Agglutination of Cells Sensitized with Paraffin Oil.

It was shown by Powis[13] that oil drops have a very high critical potential since they agglutinate when the potential is less than about 30 millivolts. It would be expected, therefore, that if the cells could be coated with a film of oil that they would become also very unstable. This is partially true, as is shown in Table VIII. The critical potential is raised to about 12 millivolts as was the case with antiserum, ricin, or stannic hydroxide.

TABLE VIII.

Effect of Electrolytes on P.D. *and Agglutination of Sheep Cells Sensitized with Paraffin Oil.*

Suspension of sheep cells in citrated sugar shaken with a large excess of heavy paraffin oil (Nujol). The excess oil removed, suspension washed twice in sugar, and noted electrolytes added to the cell suspension.

Concentration of electrolyte.	NaCl		$MgCl_2$		HCl	
	P.D.	Aggl.	P.D.	Aggl.	P.D.	Aggl.
M	mv.		mv.		mv.	
—	−38.0	−	−49.0	−	−49.0	−
10^{-4}					−17.0	−
10^{-3}					+12.0	C
2×10^{-3}			−15.0	−		
4×10^{-3}			−14.0	+		
10^{-2}			−11.0	C		
2×10^{-2}	−22.0	−	−6.3	C		
4×10^{-2}	17.0	++	<5.0	C		
10^{-1}	13.0	C	<5.0	C		
2×10^{-1}	11.0	C	<5.0	C		

The effect of all these substances is therefore similar in that they increase the critical potential of the cells. This effect is evidently closely analogous to the more general one of "protective colloids." In this latter case the critical potential is decreased instead of increased and the suspension thereby rendered less sensitive to electrolytes instead of more sensitive.

[13] Powis, F., *Z. physik. Chem.*, 1914–15, lxxxix, 179, 186.

SUMMARY.

1. Unsensitized sheep cells suspended in sugar solutions are agglutinated by electrolytes whenever the potential is depressed to 6 millivolts or less, except in the case of $MgCl_2$ or $CaCl_2$.

2. With these salts no agglutination occurs although there is practically no potential. The presence of these salts prevents acid agglutination. This is presumably due to a decrease in the "cohesion" between the cells.

3. Cells which have been sensitized with specific antibody, ricin, colloidal stannic hydroxide, or paraffin oil, are agglutinated whenever the potential is decreased below about 12 millivolts.

4. The agglutination by electrolytes is therefore primarily due to a decrease in the potential whereas agglutination by immune serum, ricin, etc., is due primarily to an increase in the critical potential.