MECHANISM OF HEMOLYSIS BY COMPLEMENT.

I. Complement Fixation as an Essential Preliminary to Hemolysis.

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Despite an enormous literature which has accumulated since the discovery of the bactericidal, bacteriolytic, and hemolytic properties of fresh serum, the terms alexin and complement still denote unexplained properties of serum rather than a chemical entity. The hemolysis of sensitized cells, as the most clearly defined of these immunologic properties, has been that most studied, and many theories have been suggested as to its mechanism. The analogy drawn by Noguchi (16) between complement and certain hemolytic lipoids has been found by Liefmann and Cohn (10) to be unwarranted. Dick (3), finding increased amino-acids after hemolysis, was inclined to identify complement as a proteolytic ferment; Olsen and Goette find a suggestive analogy between complement and serum lipase (18); while, more recently, it has been interpreted as an intracellular catalyst allowing the release of hemoglobin by the amboceptor ferment (9). The statement, which we have since found to be erroneous, that complement is not consumed during hemolysis (1), (11), seemed to support the theory of its enzymatic (catalytic) action. At best, the evidence is inconclusive, and the rôle of the preliminary sensitization with immune serum remains unexplained.

I.

Complement Fixation by Sensitized Cells.

It has already been shown (5) that sensitized cells "fix" complement, and that this fixation is exactly similar to that by agglutinated bacteria, or by the precipitate formed when, *e.g.*, sheep serum is added

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to a rabbit anti-sheep serum.   Because this fixation is obviously a
surface reaction, with a very low temperature coefficient, and because
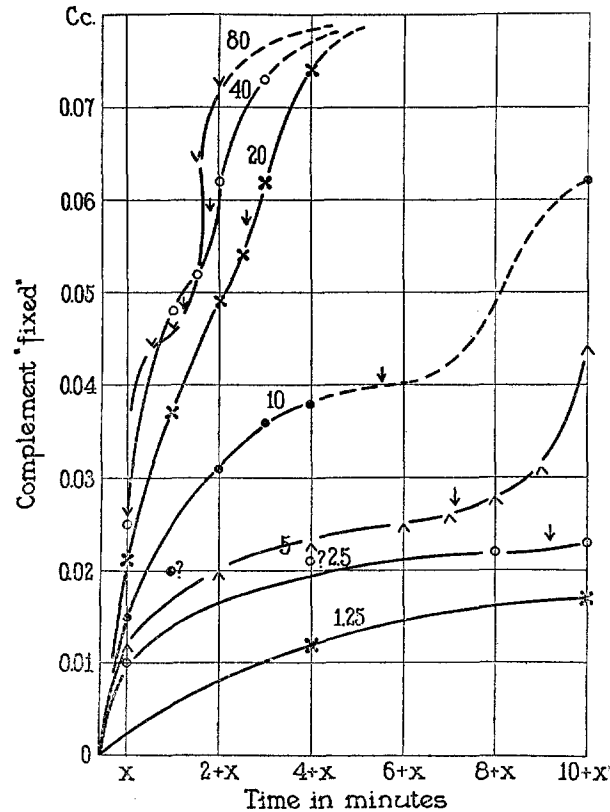

FIG. 1. Effect of degree of sensitization upon complement adsorption by sen-
sitized cells.

To 3.5 cc. of a washed 10 per cent suspension of cells are added immune
serum and NaCl N/7 to a total volume of 4 cc.   The numbers on the curves are
1000 times the volume of immune serum used in each experiment.   After 2 hours
at room temperature, 0.4 cc. of the sensitized cell suspension is added to each of
10 tubes and the supernatant fluid removed by *slow* centrifugation.   0.08 cc. com-
plement in 2 cc. NaCl N/7 is added to the sedimented cells at X minutes before
centrifugation.   This second centrifugation is performed at high speed, for ex-
actly 1 minute, the clear supernatant carefully decanted into a tube containing
0.1 cc. of a standard test suspension, and the residual complement determined by
a method already described (4) from the time required for complete hemolysis.

the quantitative relationships conform with reasonable accuracy to the empirical adsorption isotherm of Freundlich, it has been characterized as an adsorption process. The adsorbate is, of course, the labile substance in fresh serum called complement; and it was suggested that the adsorbent is *the same in all the in vitro immune reactions*: the immune-serum protein which adheres on the surface of the antigen during sensitization, agglutination, or precipitation. In the latter, it is visible as a precipitate consisting almost entirely of immune-
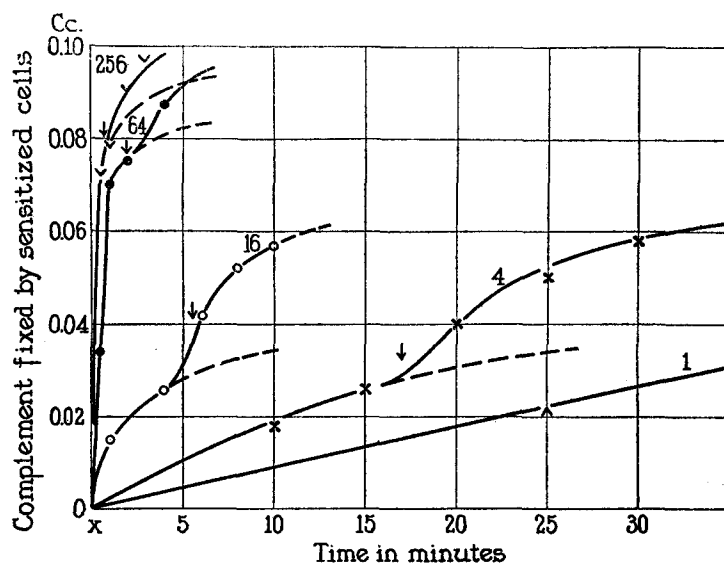

FIG. 2. Effect of degree of sensitization upon complement fixation.

serum globulins, but containing also antigenic protein. In the former it can be demonstrated immunologically, and by changes in the surface properties of the antigenic cell (cataphoresis; agglutination; effect of electrolytes).

The following experiment indicates that this hypothesis is fundamentally correct: using a constant quantity of cells, the velocity and degree of complement fixation is seen to increase enormously with the degree of sensitization, *i.e.*, with the amount of immune serum protein taken up by the cell[1] (Figs. 1 and 2).

[1] Whether or not the immune body is protein, it is always associated with the serum globulin. Increased sensitization results, not only in greater fixation of

It remains to show that this fixation is an essential preliminary to hemolysis; that complement is entirely inactive until it has been so adsorbed by the sensitized cell.

II.

The Adsorption of Complement by the Sensitized Cell as a Determining Factor in Hemolysis.

By definition, complement fixation is the removal (adsorption) from fresh serum of a substance (or substances) essential in immune hemolysis. Since there is always a fixation of complement by the sensitized cell *before* hemolysis (5), it is logical to assume that such a fixation is an essential feature of the reaction. The proof for this assumption is given by the following experiments.

(*a*) In Figs. 1 and 2 the arrows indicate the approximate time at which hemolysis began. There is obviously some significant relationship between the velocity of fixation and the velocity of hemolysis. Although the nature of the experiment does not justify a quantitative treatment, it is seen that the areas under all the curves from time 0 to the time of beginning hemolysis is *approximately* a constant, *i.e.*, the velocity of the hemolysis reaction at any moment is proportional to the total complement already adsorbed.[2] The fact that the same result is obtained when we vary, not the degree of sensitization,

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complement, but in a more and more pronounced tendency for the cell to assume the surface characteristics of a particle of denatured globulin.

[2] Let $C_A = f(t)$ = Complement adsorbed by cell

$\dfrac{dh}{dt}$ = Velocity of the complement-cell reaction, and

H = Amount of this reaction required for hemolysis, obviously a constant for any one suspension

t_h = Time required for hemolysis

Then $\displaystyle\int_0^{t_h} C_A dt = k = KH = K \int_0^{t_h} \dfrac{dh}{dt}$ in all the curves.

And $\dfrac{dh}{dt} = KC_A$: the velocity of hemolysis at any time is proportional to the total complement already adsorbed. This statement is an approximation only of the true relationships, and becomes quite inaccurate at high concentrations of complement. The kinetics of hemolysis will be discussed in a later paper.

but the amount of complement (Fig. 3) indicates a true causal relationship.

(b) The marked influence of electrolyte concentration and the reaction of the solution upon hemolysis is due to the concomitant inhibition of complement fixation.
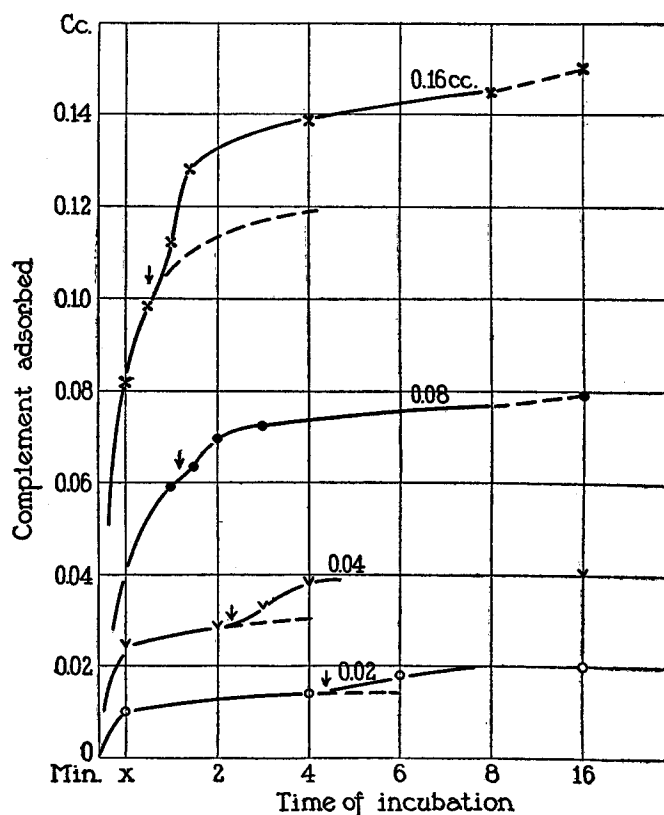

FIG. 3. Effect of (complement) upon velocity and degree of complement adsorption.

One of the earliest observations concerning complement was that it is destroyed by dilution with glucose or water, and that hemolysis does not occur in the absence of electrolytes (7), (13), (19). Manwaring (12) noted the inhibitory effect of electrolytes in excess, and suggested

as its cause the formation of a loosely bound salt-complement compound, from which active complement could be liberated by precipitation of the salt. Noguchi (17) commented on the strong inhibition by salts of Ca or Ba. More recently Wright and MacCallum (23) have tested the effects of many salts in hemolysis, and have arranged the ions in the order of their inhibitory activity in a series analogous to the Hofmeister ion series. Thus

$$K > Na > NH_4 > Ca > Ba$$
$$Acetate > Cl > NO_3 > I > SO_4$$
$$Na_2 HPO_4 > Na_3 PO_4$$
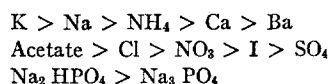
No attempt was made to preserve a constant pH, and as will be seen, this is essential for a proper comparison of the ion effects.

It is also known that the optimum reaction for hemolysis lies at about pH 7.0 to 7.5 (14), and that complement is irreversibly inactivated by acids and alkalis (6). More recently, Brooks (2) has found the destruction to begin at pH 5.3. This is apparently at variance with the previous observation of Michaelis and Skwirsky (14) that if sensitized cells are incubated with complement at pH ±5.6 (phosphate buffers 1/16) hemolysis does not occur; but if the optimum reaction is subsequently restored by adding alkaline phosphates, the cells are hemolyzed, *i.e.*, the complement was not irreversibly inactivated in acid reaction, but inhibited.

Obviously, the facts require re-investigation. Of interest, however, are not the data so obtained, but their interpretation. For *it will be shown that these changes in the efficiency of hemolysis are due to variations in the efficiency of complement adsorption.* The degree of hemolysis is determined solely by the amount of complement adsorbed, and bears no relation to the total quantity added. And failing adsorption, multiple units of active complement have no effect upon sensitized cells.

1. Methods.

(a) *Hemolysis.*—The rabbit anti-sheep cell system was used throughout. Large quantities of sensitized cells were prepared as follows: To a known volume of sheep's blood were added 10 units of hemolytic serum and several volumes of 0.14 N NaCl (0.85 per cent). After 1 hour's incubation at 37°C., the cells were sedimented by slow

centrifugation, washed once in 20 to 30 volumes of saline and re-suspended to 10 times the original volume of blood, making a stock 10 per cent suspension of sensitized cells (as compared to whole blood).

In order to secure comparable results, a standard method must be used, in which all variables save that studied are kept constant.

Volume.—All tubes are made up to total volume of 2 cc.

Cells.—0.5 cc. of the 10 per cent stock suspension of sensitized cells.

Diluents.—Both the 0.14 N NaCl and 5 per cent glucose used for dilution were brought to pH 7.4.

Incubation.—1 hour at 37°C., followed by at least 2 hours in ice box, or 1 hour at room temperature.

Reading.—

    0 = No observable hemolysis.
    ± = Doubtful.
    1 = Distinct partial hemolysis.
    2 = Approximately half of cells hemolyzed.
    3 = Distinct residual opacity of non-hemolyzed cells.
    4 = Complete hemolysis.

The hemolytic unit of complement is arbitrarily defined as the minimal quantity which will completely hemolyze 0.5 cc. of sensitized cells at pH 7.4 in 1/7 N NaCl under the above conditions of time and temperature.

(*b*) *Complement Fixation as Described in a Preceding Paper* (5).—As in the case of the hemolysis tests a uniform volume pH, tonicity, and time of incubation must be rigidly adhered to for comparable results.

(*c*) *Electrolytes.*—

NaCl	Na_2 HPO_4	$CaCl_2$
KNO_3	Na_2 SO_4	$BaCl_2$
$LiNo_3$	Na_2 Citrate[3]	$MgCl_2$
KCNS	Na_2 Tartrate	$MgSO_4$
LiCL	Na_2 Succinate	
	K_2 Oxalate[3]	

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[3] Neither K_2 oxalate nor Na_2 citrate could be used. The former, in concentration > 0.2 N, causes a slow hemolysis accompanied by a change in tint, in the absence of complement; the anti-complementary action of the latter is well known, and, as yet, unexplained.

All the salts were prepared in 1 N solution and brought to pH 7.4 by addition of HCl (NaOH) before making the final dilution.

(d) *Hydrogen Ion Concentration.*—This was determined colorimetrically, and checked occasionally by hydrogen electrode. The figures as given are accurate only to 0.1 pH. The shift caused by the addition of sensitized cells, significant even in strongly buffered solutions, was determined by centrifuging out the cells after sufficient time had been allowed for equilibrium to become established.
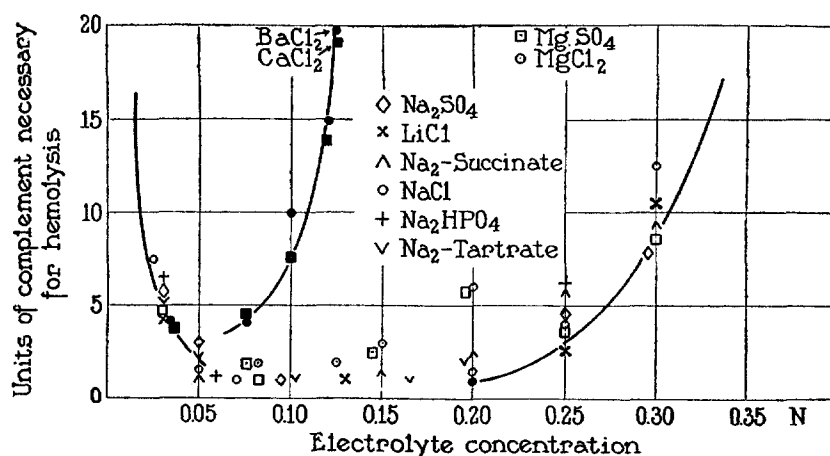

Fig. 4. Effect of electrolyte concentration upon hemolysis by complement.

2. The Effect of Electrolytes.

To a series of tubes containing increasing quantities of electrolytes were added 0.5 cc. of sensitized cells, washed and suspended in 5 per cent glucose, and 5 per cent glucose to a total volume of 2 cc. The minimal quantity of complement necessary for complete hemolysis was then determined for each concentration.[4]

[4] These experiments were carried out before there was available a rapid method of complement titration. That used here is cumbersome and relatively less precise than either the method of Brooks (2) or that used in experiments already described (4).

Table I gives the data for NaCl.   To simplify comparison, the results obtained with the other salts are combined in Fig. 4, in which abscissae represent electrolyte concentration and ordinates the number of complement units necessary for hemolysis.

By varying the electrolyte concentration, three distinct effects are produced upon the hemolytic activity of complement. Below a concentration of 0.025 to 0.03 N, even 20 units of complement fail to cause hemolysis, provided of course, the electrolyte in complement is

TABLE I.

Influence of [NaCl] upon Hemolysis of Sensitized Cells by Complement.

		ISOTONIC							HYPERTONIC			
NaCl N/1, cc		0.025	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5	0.6	0.8
H_2O, cc		0.15	0.3	0.6	0.9	1.2	1.25	1.2	1.1	1.0	0.9	0.7
Glucose 5 per cent, cc	1.5	1.3	1.15	0.8	0.45	0.1	0	0	0	0	0	0
Sensitized cells in glucose 5 per cent	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Final [NaCl] N		0.012	0.025	0.05	0.07	0.1	0.12	0.15	0.2	0.25	0.3	0.4
Complement												
cc. / units												
0.015 / 3/4	0	0	±	3	4–	4–	4–	4–	2	0	0	0
0.02 / 1	0	0	1	4–	4	4	4	4	3	1	0	0
0.025 / 1¼	0	0	1	4					4–	1	0	0
0.03 / 1½	0	1	2	4					4	2	0	0
0.04 / 2	1	2	2							3	1	0
0.05 / 2½	2	0?	2							3	2	0
0.08 / 4	2	2	3							4	2	0
0.1 / 5	2	2	4–								4–	1
0.2 / 10	2	4–	4								4–	2
0.4 / 20	2	4	4								4	2
Minimal units complement necessary for hemolysis	>20	10–20	5–10	1–2	1	1	1	1	1½	4	10–20	>20

included in the calculation of the final salt concentration. There follows a zone of optimal concentration, whose upper limit for monovalent salts seems to be serum tonicity. Beyond this, increasing electrolyte concentration necessitates more and more complement, until, in 0.4 N NaCl the inhibition is absolute and even 20 units of complement fail to cause hemolysis.[5]

[5] The puzzling observation of Muir and Browning (15) that hypertonic salt allows complement to pass through a Berkefeld filter, by which it is usually re-

Neither of these two zones of inhibition is due to a destruction of complement. From hypertonic solutions, active complement may be recovered quantitatively by making the proper dilution (Table II). It is true that in hypotonic solution there is an irreversible inactivation of complement function. But in the presence of sensitized red cells this destruction is only partial. Here also, sufficient complement

TABLE II.

Survival of Complement in Hypertonic Solution.

To 0.5 cc. complement are added 0.5 cc. of N/1 electrolyte. After 1 hour at 37°C., the minimal hemolytic quantity of each mixture is determined, using sensitized cells in glucose to avoid hypertonicity.

Mixture of complement and electrolyte	NaCl	LiCl	BaCl$_2$	Control (NaCl N/7)
cc.				
0.02	2	3	2	3
0.03	4—	4—	3	4—
0.04	4	4	4	4
0.05	4	4	4	4

remains to completely hemolyze the cells, were it not for an as yet un explained inhibiting factor; for when the proper tonicity is restored, hemolysis occurs (Table III).

But the most striking feature of the curves plotted in Fig. 4 is the influence of cation valence upon the second zone of inhibition, becoming even more manifest if we prevent the partial hemolysis caused by Ca and Mg salts (Fig. 5). The valence of the anion seems to play no rôle—but with increasing cation valence there is an astonishingly greater inhibitory action. The zone of optimal concentration is narrowed, and the succeeding inhibition much more pronounced. At

tained, is explained by these findings. Complement fails to pass the filter, not because of its molecular dimensions, but because it is adsorbed by the finely particulate material of which the filter is made. Hypertonic salt inhibiting such adsorption, the complement passes.

Similarly, the fact that after repeated passages of fresh serum, a filter finally becomes permeable to complement, is due to the saturation of the filter adsorbent with the complement adsorbate.

TABLE III.

Inhibition of Hemolysis in Hypotonic Solution not Entirely Due to Destruction of Complement.

Complement		Inhibition of hemolysis		Destruction in absence of cells	Control
		(a)	(b)		
		Cells 0.5 cc. Glucose 1.3 cc. Complement 37° 1 hour	NaCl N/1 −0.2 cc. added after incubation	Glucose 1.3 cc. Complement After 1 hour at 37° add Cells 0.5 cc. NaCl N/1 0.2 cc.	Glucose 1.3 cc. Cells 0.5 cc. NaCl N/1 0.2 cc.
cc.	units				
0.015	$\frac{3}{4}$	0	2	0	3
0.02	1	0	3	1	4
0.04	2	0	4	3	4
0.05	$2\frac{1}{2}$	0	4	4−	4

(A)-Concentration of each electrolyte
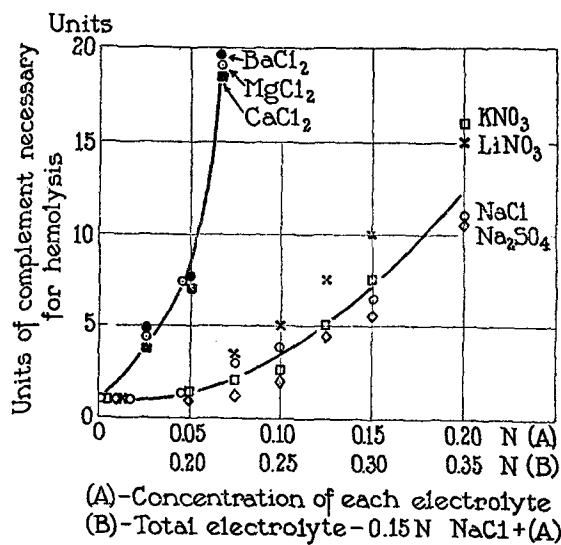(B)-Total electrolyte-0.15N NaCl+(A)

FIG. 5. Effect of electrolytes upon hemolysis (non-specific hemolysis by salts prevented in hypertonic solution).

To 0.5 cc. of sensitized cells in 0.15 N NaCl are added X cc. of N/1 electrolytes, each containing, in addition to the individual salt, 0.85 per cent NaCl (0.15 N). 0.15 N NaCl is added to a total volume of 2 cc., and the minimal hemolytic quantity of complement determined at each concentration.

The hemolysis caused by the salts themselves is almost completely inhibited in such hypertonic solutions.

TABLE IV.

Effect of Electrolyte Concentration upon Fixation of Complement by an Immune Globulin Suspension.

Complement is incubated with globulin at varying concentrations of NaCl. After 1 hour at 37°, to Y cc. of each tube (figures in left hand column), are added 0.5 cc. sensitized cells, and NaCl $N/7$ to a total volume of 2 cc. The figures in the body of the table represent degrees of hemolysis.

Globulin suspension, cc.	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Complement, cc.		0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
NaCl $N/7$, cc.		0.25	0.25	0.5	1.5	1.45	1.4	1.35	1.3	1.25
NaCl $N/1$, cc.						0.05	0.1	0.15	0.2	0.25
Glucose 5 per cent, cc.	1.7	1.5	1.25	1.0						
Final NaCl normality	0.014	0.035	0.055	0.07	0.14	0.16	0.18	0.21	0.23	0.25
cc.										
0.1	±	3	1	0	0	±	1	2	3	4−
0.2	3	4	4	0	2	3	3	3	4−	4
0.3	4	4	4	0	3	4−	4	4	4	4
0.5			4	2	4	4				
0.6				2						
1.0				3						
Total units complement remaining free	7	10	10	<2	4	4–6	7	7	7–10	10–20
Control without globulin **cc.** 0.1		3	4	4	4	4				
0.2	3	4	4	4	4					
0.3	4	4	4	4						
Units complement remaining in control	7	10	20	20	20	20				
Units complement adsorbed by globulin	0	0	10	>18	16	14–16	13	13	10–13	0–10
Per cent complement adsorbed	0	0	50	>90	80	70–80	65	65	50–65	0–50
Amount of complement from which one unit would be adsorbed	>20	>20	2	1+	1+	1+	1+	1+	1–2	2–20

0.07 N $BaCl_2$ it required 20 times as much complement to cause hemolysis as it does at 0.07 N NaCl. The limiting concentration for hemolysis where even 20 units fail to have any effect is 0.35 M KNO_3, but only 0.07 M $CaCl_2$.

As compared with such a pronounced valence effect, the differences between individual ions of the same valence are negligible, and do not justify their arrangement into an ionic series.

If we now carry out a similar series of experiments to determine the effects of electrolytes upon complement fixation, we obtain a series of
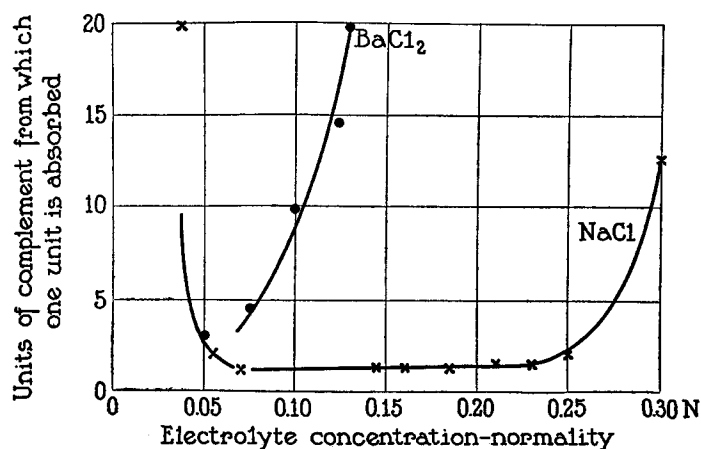

FIG. 6. Effect of electrolytes upon complement fixation.

With the more accurate velocity method of complement titration, one obtains a somewhat more sharply defined optimum zone for fixation. The limiting electrolyte concentrations, however, remain the same.

curves which are, within the limits of experimental error, exact duplicates of the curves for hemolysis. There is the same inhibition in hypotonic solution, the same optimum zone, and the same succeeding inhibition in hypertonic solution, again determined by the cation (Table IV and Fig. 6).

Knowing as we do that sensitized cells adsorb complement, the correlation between adsorption and hemolysis proves that the inhibition of hemolysis with changing tonicity is due to inhibited adsorption that adsorption is an essential preliminary to hemolysis.

3. The Effect of Hydrogen Ion Concentration.

The effect of changing pH upon hemolysis is summarized in Fig. 7. The irreversible destruction of complement in alkaline reaction is seen to begin at pH 8.5. Its causes are unknown, and need not concern us here. In the acid range, the hemolytic titer of complement begins to decrease at pH 5.9 as compared with 5.3 under the conditions used by Brooks. But it should be noted that sensitized cells in some
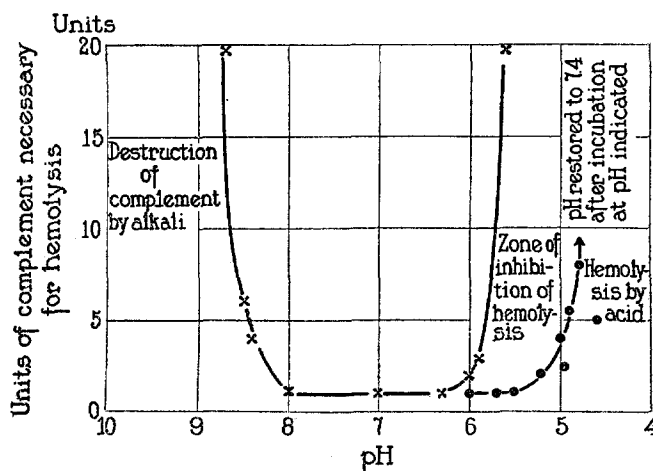
FIG. 7. Effect of hydrogen ion concentration upon hemolysis.

Complement is brought to the desired pH by the addition of NaOH N/7 (HCl). To X cc. of this mixture are added 0.5 cc. of sensitized cells and NaCl N/7 to a total volume of 2 cc. A significant change in reaction occurs when cells are added, and this must be determined for each quantity of complement used.

way prevent the destruction of complement between pH 5.9 to 5.0. When complement is brought to this pH and sensitized cells added at once, hemolysis does not occur, but not because the complement is destroyed, as it would have been in the absence of cells. When the optimum pH is subsequently restored by the addition of NaOH, hemolysis occurs, just as Michaelis and Skwirsky showed. The mechanism of this protective action is intimately related to the problem of midpiece and endpiece. Of interest in the present discussion

TABLE V.

Effect of pH upon Complement Fixation by Immune Globulin Suspension.

20 units of complement (0.4 cc.) are brought to the desired pH by acetate buffer, as indicated in the table, and incubated at 37°C. for 1 hour. To Y cc. of each tube (figures in left hand column), are then added 1), the neutralizing quantity of NaOH, 2), sensitized cells, and 3), NaCl N/7 to make 2 cc. The figures in body of table indicate degrees of hemolysis. The units remaining after incu-

$$\text{bation obviously} = \frac{2}{\text{minimal hemolytic quantity}}.$$

HAc N/7 (in NaCl), cc.		0.05	0.07	0.1	0.15	0.22	0.3
NaAc N/7, cc.		0.3	0.3	0.3	0.3	0.3	0.3
Complement, cc.	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Globulin in suspension, cc.	0.1	0.1	0.1	0.1	0.1	0.1	0.1
NaCl N/7, cc.	1.5	1.15	1.1	1.1	1.05	1.0	0.9
Final pH	7.4	6.5	6.2	6.0	5.7	5.4	5.1
cc.							
0.05	0	0	0	0	3	2	1
0.1	0	0	0	1	4	4	4
0.2	0	0	2	4	4		
0.5	0	0	2	4			
1.0	0	0	3				
Total units remaining free	0	0	<2	10	20	20	20
Control without globulin { *cc.* 0.05	3	3	3	3	3	2	1
0.1	4	4	4	4	4	4	3
0.2	4	4	4	4	4	4	4
Units remaining in control	20	20	20	20	20	20	10*
Per cent complement adsorbed by globulin suspension	100	100	>90	50	0	0	0
Amount of complement from which one unit would be adsorbed	1	1	1+	2	>20	>20	>20

* Beginning destruction of complement by acid reaction.

is the fact that there is a true zone of inhibition at pH 6.0 to 5.0, in which active complement fails to produce hemolysis.

Here also, the puzzling effects upon hemolysis are completely explained by the corresponding changes in complement adsorption by a suspension of globulin particles (and of red cells).

Table V is the protocol illustrating the method used to arrive at the data charted in Fig. 8. The inhibition of complement fixation at pH 5.9 quantitatively parallels and explains the inhibition of hemolysis.
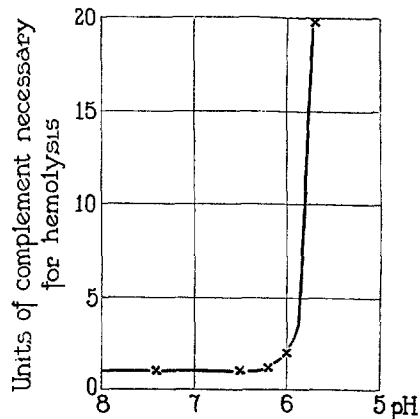

Fig. 8. Effect of pH upon complement fixation by a suspension of denatured immune globulins.

SUMMARY AND DISCUSSION.

1. Sensitization confers upon the red cell the property of adsorbing complement from solution. The submicroscopic film of immune serum protein deposited upon the cell surface during sensitization, and completely analogous to the precipitate formed in a soluble antigen-antibody reaction (e.g., sheep serum vs. rabbit anti-sheep serum) acts as absorbent, the degree of sensitization (size of the film) determining the amount of complement "fixed" (adsorbed).

2. This adsorption of complement by the sensitized cell is an essential preliminary to hemolysis, and when inhibited, even large quantities of demonstrably active complement have no hemolytic action.

The marked influence of electrolytes and of the hydrogen ion concentration upon hemolysis is due primarily to corresponding effects upon the fixation of complement by the sensitized cell. In the case of salts with monovalent cations, complement fixation (and hemolysis) is completely inhibited at any concentration < 0.02 M or > 0.35 M. Electrolytes with bivalent cations are much more inhibitory, and in low as concentration 0.07 M completely prevent fixation (and hemolysis).

The optimal reaction for complement fixation (and hemolysis) is pH 6.5 to 8.0. In slightly more acid range both are *inhibited*. But at a reaction pH 5.3, and in the alkaline range, there is an irreversible inactivation of complement, complete at pH 4.8 and 8.8 respectively.

It is perhaps more than a coincidence that complement fixation, and therefore, hemolysis, are prevented by just those factors which suppress the ionization of serum proteins, and lead to an increased aggregation state. Between a suspension of macroscopically visible particles of euglobulin in distilled water, and a solution is physiological saline, there is certainly a gradual transition, manifested at low electrolyte concentrations by the opacity of the solution. At pH 7.4, globulin would ionize as a Na-salt, an ionization inhibited as the isoelectric point (5.3) is approached, with a coincident greater tendency of the globulin to separate from solution. And the cataphoretic velocity of particles of globulin, as well as all the other properties which are a function of its ionization (viscosity, osmotic pressure, etc.), are suppressed by electrolytes, the degree of suppression being determined by the concentration and valence of the cation (on the alkaline side of the isoelectric point). The analogy with complement fixation is too complete to be dismissed as fortuitous.

3. The fact that the degree of complement "fixation" increases with the degree of sensitization explains one of the most puzzling phenomena in hemolysis,—that immune serum and complement are, to a certain extent, interchangeable, a decrease in either factor being compensated by an increase in the other (8), (20), (22). The explanation is evident from Figs. 1, 2, and 3. The exact quantitative relationships involved will be developed in a later paper. With increasing sensitization there is an enormously more complete and more rapid fixation of complement, and correspondingly more rapid hemolysis, exactly

the effect produced by increasing the quantity of complement instead of amboceptor (Fig. 3). All other variables being constant, the velocity of hemolysis is determined by the amount of complement adsorbed. With more amboceptor, a greater proportion is "fixed" by the cell; with more complement, a smaller proportion, but a larger absolute amount. The result is the same: more complement adsorbed, and a corresponding acceleration of hemolysis.

If this mobilization of complement is the sole function of immune-serum (and there is as yet no reason to assume any other), then the accepted terminology, in which amboceptor, immune body, and hemolysin are used synonymously, is erroneous. The immune body would function only as an "amboceptor," mobilizing the effective hemolysin, complement, upon the surface of the cell.

Nothing has been said of the multiple components into which complement may be split. *A priori*, it would be expected that the adsorption demonstrated is of the so called midpiece fraction.

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