野口英世著　Journal of Experimental Medicine　所収論文

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A BIOCHEMICAL STUDY OF THE PHENOMENA KNOWN AS COMPLEMENT-SPLITTING.

FIRST PAPER: SPLITTING OF THE COMPLEMENT ASSOCIATED WITH GLOBULIN PRECIPITATION.*

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INTRODUCTION.

During the last few years much attention has been directed toward a phenomenon known as the splitting of complement. The conception is that complement, when treated according to certain procedures, can be decomposed into two definite portions each of which is by itself inactive but can act in combination with the other as complement. The two components of the complement differ in their behavior in hemolytic processes; one has the property of being bound by the sensitized erythrocytes, while the other possesses a lytic action upon sensitized cells that have been acted upon by the first component. Thus, according to certain investigators, complement is composed of an intermediary portion, called the mid-piece, and a lytic portion, called the end-piece. The terms "mid" and "end" are employed because the conception is that the mid-piece stands between the sensitized cells and the lytic portion of the complement, while the lytic portion when viewed graphically, becomes the end-piece.

In view of the high lability of complement it is natural to ask if the phenomenon of splitting brought about by certain procedures may not be due to some modifications of the complement, such as a reversible inactivation through changes in the reaction of the medium in which the complement is present. It appeared to us of interest to approach this question from a biochemical standpoint.

In our first paper we deal with the splitting phenomena which are

* Received for publication, April 1, 1912.

associated with the precipitation of the globulin fraction, and in the second with those which occur without visible change in the physical conditions of the serum proteids.

HISTORICAL.

In fresh blood serum Landois found a hemolytic action upon alien corpuscles, and Fodor, Nuttall, and others, a bactericidal property, but none of the early investigators made exhaustive studies upon the nature of the active principles.

It was Hans Buchner who first pursued the study in a systematic manner and threw much light on this interesting phenomenon. Buchner (1890) established the fact that the active principle causing bacteriolysis or hemolysis is very labile and can be made inactive by a temperature of 55° C., by dialysis, or by dilution with distilled water. The active principle was designated by him alexin.

Bordet (1899) subsequently found that the alexin of Buchner is composed of two distinct principles, one a sensitizing substance and the other a thermolabile alexin. Somewhat later Ehrlich and Morgenroth (1899) made a similar observation, but by Ehrlich the sensitizing portion was called the amboceptor, and the alexin the complement. In this study Ehrlich and Morgenroth mention that the complement becomes inactive when mixed with water. Markl (1902) observed that sodium chlorid in concentration above 3 per cent. suppresses the activity of the complement.

While the disappearance of the complement activity after heating the serum to 55° C. was recognized by all as due to the destruction of the active principle at this temperature, no one was able to explain why the complement should lose its action in distilled water. This question was difficult to approach inasmuch as in a hypotonic salt solution a hemolytic experiment could not be carried out. Ferrata (1907), however, utilized the well known fact (noted by Hamburger, Madsen, and Arrhenius), that the corpuscles can be kept intact when suspended in an isotonic solution of sugar, and he studied the action of the salt-free complement (dialysed) in a salt-free isotonic medium, and found the complement to be inactive in this medium. Ferrata found also that during dialysis the serum separates into two portions, one the serum globulin (precipitate) and the other the albumin fraction (in solution). When tested separately in an isotonic salt solution neither of them caused hemolysis, while when united the action of complement was restored. He affirms that the component of complement remaining in the albumin portion is inactivated at 55° C.

The finding of Ferrata was confirmed by Brand, who showed further that the component carried down with the globulin fraction attaches itself to the sensitized corpuscles and that the component in the albumin fraction will afterwards act upon the cells so altered, He introduced, therefore, the terms mid-piece (Mittelstück) for the former and end-piece (Endstück) for the latter.

According to Brand the mid-piece of complement remains active when kept as precipitate (globulin) in distilled water, but loses its property on standing in salt solution. Brand and Tsurusaki (1908) found the mid-piece as well as the end-piece inactivated at 55° C.

Hecker (1907) and Sachs and Bolkowska state that the binding of the mid-

piece takes place at 0° C. as well as at higher temperatures, while that of the end-piece occurs only at higher temperatures. Thus they claim that the selective absorption of the natural hemolysin by means of corpuscles at 0° C. effects not only the fixation of the amboceptor, but also the mid-piece of complement.

Sachs and Teruuchi (1907) observed that the complement loses its activity in distilled water or isotonic sugar solution when the degree of dilution with distilled water is about 1 : 10, but not in much higher dilution. They were inclined to believe that the inactivation of the complement was caused by a certain ferment of the serum capable of exerting an injurious action only in a salt-free medium. They state that the inactivation of complement in a salt-free medium does not take place at a low temperature or in the presence of an excess of amboceptor. Complement a few days old does not undergo similar alteration (inactivation with water, etc.).

Tsurusaki found that the so-called splitting of complement is quite inconstant and seems to depend upon the quality of the dialysing membranes, upon the variations in different individuals of the same species of animal, and upon the temperature. For instance, the unaltered complement of rabbit was found in the supernatant fluid when dialysed at 0° C.

Sachs, and Sachs and Altmann (1908) then introduced a new method of splitting the complement. They used a weak solution of hydrochloric acid by which a certain portion of serum is precipitated (globulin). The precipitate corresponds with the mid-piece, and the supernatant fluid with the end-piece obtained by dialysis.

Later Michaelis and Skwirsky found that when corpuscles, amboceptor, and complement were put together in a medium containing a certain quantity of NaH_2PO_4 and Na_2HPO_4 in proportions resulting in isotonicity, there was no hemolysis. But on further analysis, they found that in this mixture the corpuscles had fixed not only the amboceptor, but also the mid-piece of complement. The corpuscles so modified were called persensitized, because they readily underwent hemolysis when the neutralized supernatant fluid (containing the end-piece) was added. In other words, persensitization can take place in a medium containing an excess of NaH_2PO_4 which suppresses the action of the end-piece, while the activity of the end-piece can be restored by neutralizing the excess of the acid phosphate with Na_2HPO_4.

Liefmann and Cohn introduced carbon dioxid for splitting the complement, whereupon the mid-piece is precipitated and the end-piece remains in solution. This phenomenon occurs only in a serum diluted with a sufficient quantity of water. They studied the influence of certain acids and alkalis and concluded that carbon dioxid exerts a far more inactivating effect than the acids or alkalis studied by them. Cholesterin inactivates the mid-piece in a salt-free medium. From the fact that the persensitization of corpuscles is affected with a minute amount of the mid-piece, they were inclined to think that this component acted like a ferment, an assumption held also by Rusznyák.

Jacoby and Schutze state that the complement inactivated by shaking and also the precipitate that is formed may be reactivated either by the mid-piece or the end-piece. On the other hand, the supernatant fluid of the shaken complement becomes active only with the end-piece.

Complement is known to be carried down by certain colloidal substances

through an adsorption (Landsteiner). Skwirsky confirmed this and points out a very interesting difference existing between the colloidal adsorption and the specific complement fixation as it occurs in the Bordet-Gengou or the Wassermann reaction. According to this author, later confirmed by Amako, in the former instance the end-piece as well as the mid-piece is carried down from the solution, while in the complement fixation the mid-piece alone is absorbed, leaving the end-piece free in the supernatant fluid.

Landsteiner, Liefmann and Cohn, and Fränkel state that the mid-piece prepared by the hydrochloric acid or carbon dioxid method from sera of different animals can persensitize the corpuscles for the end-piece of guinea pig serum, but not *vice versa.* This phenomenon is ascribed by them to the presence of natural amboceptor in the fraction containing the mid-piece. Fränkel points out certain differences in the behaviors of mid-pieces derived from the sera of different animals when these are acted upon by cholesterin, or sodium chlorid, or inactivated by the temperature of 57° C.

Marks observed that the reactivation of the end-piece by the mid-piece occurs when the latter is employed in a smaller quantity than the ratio of 1:1. He found that the heated as well as the fresh serum can furnish an active mid-piece, but a prolonged heating of the mid-piece at 55° C. finally destroys its function.

Liefmann studied the relation of certain lipoids and soaps to the mid- and end-pieces of complement and found that they have no effect upon the end-piece, but that they destroy the mid-piece. He brought out also the fact that persensitization can not take place unless the amount of amboceptor reaches nearly fifty units.

That the active principle of complement undergoes an inactive modification in the presence of various acids, alkalis, and salts has been carefully studied by Noguchi, von Liebermann, Hektoen, and others. According to the experiments of Noguchi, the complement is permanently injured by a strong solution of these substances, especially by the acids and alkalis, but when used within a certain limit, the activity can be restored with but slight loss of power.

EXPERIMENTAL PART.

There are at least five different procedures by which it is claimed that complement may be split[1] into two components. Sachs employs hydrochloric acid; Liefmann, carbon dioxid gas; Ferrata, dialyzes against distilled water; Michaelis and Skwirsky use a mixture of acid and alkaline phosphates; the fifth method is that of splitting by complement fixation.

[1] "Complement-splitting" is the term generally adopted by English speaking bacteriologists. This is the reason for the employment of the term in this paper, although a better word might be substituted, and the following work almost necessitates its replacement by the term "inactivation," since this is what has really been proved to take place in almost all cases of so-called splitting.

In our first paper we shall consider only the first three methods. In these the globulin fraction of the serum is precipitated during the process of splitting.

COMPLEMENT-SPLITTING WITH HYDROCHLORIC ACID.

The procedure introduced by Sachs is as follows: One cubic centimeter of guinea pig serum (complement) is mixed with 8.2 cubic centimeters of N/250 hydrochloric acid solution (in distilled water), and after standing at room temperature for one hour the mixture is centrifugalized, whereupon the precipitate settles down to the bottom leaving a clear supernatant fluid above. The supernatant fluid is carefully separated from the deposit[2] and is mixed with 0.8 of a cubic centimeter of N/25 sodium hydroxid solution (in 10 per cent. sodium chlorid). The addition of alkali is intended to neutralize the acid introduced into the serum for splitting, while the resultant solution is made isotonic at the same time (about 0.9 per cent. sodium chlorid). The deposit is washed with water and finally collected by centrifugalization, and then dissolved in 10 cubic centimeters of 0.9 per cent. sodium chlorid solution. The supernatant fluid and the precipitate, both now in clear solution, represent the end- and mid-piece, respectively. Thus, the supernatant fluid has no action upon the sensitized corpuscles unless the precipitate portion is added, and *vice versa.* Apparently the complement is separated into two components.

Experimental evidence was also brought forward to show that the end-piece or supernatant portion carries the labile zymotoxic component, and the mid-piece, or precipitate portion, possesses the property of enabling the end-piece to act upon corpuscles sensitized by amboceptor, and that it is not destroyed by the temperature of 56° C.

The above experiments have been confirmed by various workers, but we have been able to corroborate the results in part only, inasmuch as the supernatant fluid was found to be active upon sensitized corpuscles in most of our experiments (table I, experiment II) and was very seldom inactive.

[2] Finding that the supernatant fluid was often active without the addition of mid-piece, we took the precaution of filtering the end-piece through a hardened filter paper in order to be certain of the absence of the mid-piece.

TABLE I.

Complement-Splitting by Hydrochloric Acid.

Experiment I (Ideal Case).[3]

Amounts taken.	Guinea pig serum 1 c.c. + N/250 HCl 8.2 c.c. (in distilled water). Centrifugalized after 1 hr. at room temperature.			Guinea pig serum 1 c.c. + 0.9 % NaCl 9 c.c.
	Supernatant fluid + 0.8 c.c. N/25 NaOH in 10 per cent. NaCl.	Deposit dissolved in 10 c.c. of 0.9 per cent. NaCl.	Equal amounts of both ingredients.	After standing 1 hr. at room temperature (control).
	2 units of amboceptor and 0.1 c.c. of a 10 % suspension of corpuscles and the total volume made up to 15 c.c. Results taken after 1 hr.			
1.0 c.c.	No H.[4]	No H.	——	
0.7 c.c.	No H.	No H.	——	
0.5 c.c.	No H.	No H.	C.H.	
0.4 c.c.			C.H.	
0.3 c.c.			C.H.	
0.2 c.c.			Sl.H.	C.H.
0.15 c.c.			Tr.H.	F.C.H.
0.1 c.c.			No H.	Tr.H.

Experiment II (Usual Splitting).

Amounts taken.	Guinea pig serum 1 c.c. + N/250 HCl 8.2 c.c. (in distilled water). Centrifugalized after 1 hr. at room temperature.						Guinea pig serum 1 c.c. + 0.9 % NaCl 9 c.c.	
	Supernatant fluid + 0.8 c.c. N/25 NaOH in 10% NaCl.		Deposit dissolved in 10 c.c. of 0.9 % NaCl.		Equal volumes of both ingredients.		After standing at room temperature for 1 hr. (control).	
	2 units of amboceptor and 0.1 c.c. of a 10 % suspension of corpuscles added to each tube and the total volume made up to 1.5 c.c. Results taken after 30 and 60 min.							
	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.	30 min.	60 min.
1.0 c.c.	C.H.		No H.	No H.				
0.7 c.c.	C.H.		No H.	No H.	C.H.	C.H.		
0.5 c.c.	F.C.H.	C.H.			C.H.	C.H.		
0.4 c.c.	Mch.H.	C.H.			C.H.	C.H.		
0.3 c.c.	Sl.H.	C.H.			C.H.	C.H.		
0.2 c.c.	No H.	Sl.H.			F.C.H.	C.H.	C.H.	C.H.
0.15 c.c.		No H.			No H.	Sl.H.	C.H.	C.H.
0.1 c.c.						No H.	Sl.H.	Mch.H.
0.07 c.c.							No H.	No H.

[3] Throughout this work 0.9 per cent. sodium chlorid solution was used instead of 0.85 per cent. usually employed by other workers.

[4] In this and the following tables, for the sake of brevity, certain symbols are employed to indicate the degree of hemolysis; thus C.H. = complete hemolysis; F.C.H. = fairly complete hemolysis; Sl.H. = slight hemolysis; Tr.H. = trace of hemolysis; No H. = no hemolysis; Mch.H. = much hemolysis.

TABLE II.

The Effect upon Components of Guinea Pig Serum of Heating for 30 Minutes at 56° C.

		Experiment I.[5]			*Experiment II.*			
Fresh guinea pig serum 1 c.c.+8.2 c.c. HCl N/250 (in distilled water).					Guinea pig serum heated at 56° C. for 30 minutes + 8.2 c.c. HCl N/250 (in distilled water).			
Kept at room temperature for 60 minutes, centrifugalized and supernatant fluid separated, neutralized, and made isotonic by 0.8 c.c. of N/25 NaOH in 10% NaCl.					Kept at room temperature for 60 minutes, centrifugalized and supernatant fluid separated, neutralized, and made isotonic by 0.8 c.c. of N/25 NaOH in 10% NaCl.			
0.15 c.c. of supernatant fluid, 2 units of amboceptor, and 0.1 c.c. of 10% suspension of corpuscles in each tube.					0.2 c.c. of supernatant fluid, 2 units of amboceptor, and 0.1 c.c. of 10% suspension of corpuscles in each tube.			
		Results taken after:			Results taken after:			
		30 min.	60 min.	90 min.	30 min.	60 min.	90 min.	0 control.
+0		No H.	Sl.H.	C.H.	No H.	No H.	No H.	
+Alanin 0.3 c.c. of 7.5% solution in 0.9% NaCl.		C.H.	C.H.	C.H.	No H.	No H.	No H.	No H.
+Deposit from splitting in experiment I dissolved in 10 c.c. 0.9% salt solution.	0.15 c.c.	C.H.	C.H.	C.H.	No H.	No H.	No H.	No H.
	0.07 c.c.	C. H.	C.H.	C.H.	No H.	No H.	No H.	
+Deposit from splitting in experiment II dissolved in 10 c.c. 0.9% salt solution.	0.2 c.c.	No H.	Sl.H.	C.H.	No H.	No H.	No H.	No H.
	0.1 c.c.	C.H.[6]	C.H.	C.H.	No H.	No. H	No H.	

While the phenomenon suggests a double constitution of the complement, yet it does not exclude the possibility of its being caused by an excess of acid or alkali. Guinea pig serum, when mixed with hydrochloric acid in the proportion prescribed in this procedure, becomes almost neutral or but faintly alkaline. This causes the precipitation of certain proteids, mainly the globulins. Thus the acid introduced is quickly taken up by certain alkaline salts of the serum and there is none left free. The precipitated globulins also bind a certain amount of the acid. The supernatant fluid which is almost neutral and contains chiefly the albumin fraction of the serum, is then mixed with sufficient free alkali to neutralize exactly

[5] The total volume was brought up to 1 c.c. by 0.9 per cent. salt solution in both experiments.

[6] This phenomenon does not occur constantly. With some specimens of complement (very few) the mid-piece is undoubtedly destroyed by heating the whole serum previous to the splitting, but it is easier to destroy the mid-piece after its separation.

the amount of acid added, but this amount of alkali is in excess when added to the serum mixture from which the globulin fraction was removed after its precipitation by hydrochloric acid. Thus it happens that in the serum treated with the acid and then neutralized with the alkali in the manner prescribed, in the presence of the globulin fraction, the complement action is restored. On the other hand, if the globulin fraction is first removed and the same amount of the alkali is added to the remaining supernatant fluid, the complement activity of the supernatant fluid is paralyzed. The exami-

TABLE III.

Demonstration of Complement Inactivation by Sodium Hydroxid in the Process of Complement-Splitting.

	Tubes.					
	1	2	3	4	5	6
Guinea pig serum	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.	1.0 c.c.
N/250 HCl	8.2 c.c.	8.2 c.c.	8.2 c.c.	8.2 c.c.	——	——
0.9% NaCl					9.0 c.c.	
Distilled water						8.2 c.c.
After 1 hour at room temperature all the tubes were centrifugalized. From tubes 1 and 2 only supernatant fluid was used; from the others the entire contents of the tubes were used.						
N/25 NaOH in 10% NaCl	0.8 c.c.	——	——	0.8 c.c.	——	——
10% NaCl	——	0.8 c.c.	0.8 c.c.	——	——	0.8 c.c.
	Supernatant fluid		The entire contents of the tubes			
Reaction of resulting fluids to alizarin-indigo[7] (calculated for the whole volume).	5.6 c.c. HCl N/250	2.5 c.c. NaOH N/250	2.5 c.c. NaOH N/250	5.6 c.c. HCl N/250	6.2 c.c. HCl N/250	6.2 c.c. HCl N/250
Complement activity of the resulting mixtures (with 0.1 c.c. of 10% suspension of corpuscles and 2 units of amboceptor.						
Amounts of mixture: 1.0 c.c.	C.H.					No H.
0.7 c.c.	Sl.H.					
0.5 c.c.	Tr.H.			C.H.		
0.3 c.c.	No H.	C.H.	C.H.	F.C.H.		
0.2 c.c.		F.C.H.	F.C.H.	Mch.H.	C.H.	
0.15 c.c.		Mch.H.	Sl.H.	Tr.H.	F.C.H.	
0.1 c.c.		No H.	No H.	No H.	Sl.H.	
0.07 c.c.					No H.	

[7] The use of this indicator, first introduced by Bernhardt, was suggested to us by Prof. W. J. Gies. The indicator is not noticeably influenced by the presence of proteids or carbon dioxid.

nation of the so-called neutralized supernatant fluid for alkalinity indicates a slight excess of alkali over that present in the same dilution of the untreated serum.

That this slight excess of alkalinity is not solely responsible for the loss of action of the untreated complement, but that the nature of the alkali employed has more to do in paralyzing the complement seems very probable. Thus, the free alkali in the form of sodium hydroxid fails to restore the original alkaline salts, and exerts an injurious effect upon the active complement constituents. That this is the case is easily shown by the experiments in table III.

These experiments show that the supernatant fluid from which the native alkalinity has been almost completely removed by hydrochloric acid still has nearly two thirds of the original complement power of the serum. But by adding gradually increasing amounts of sodium hydroxid, the activity is steadily reduced (table V) until in some cases it disappears completely (table VI), when the amount of sodium hydroxid reaches the point supposed to be necessary to restore the original alkalinity of the treated serum. The completeness of the inactivation of the complement action of the supernatant fluid is also enhanced by a slight excess of the free sodium hydroxid toward which the complement shows a great susceptibility. It may be assumed that the salts that cause the native alkalinity, principally the carbonates and phosphates, are split by hydrochloric acid and form sodium chlorid and the acids, but the greater part of carbon dioxid leaves the fluid, thus causing the free alkali introduced to act directly upon the complement.

It is interesting, however, that, when the serum was acted on by hydrochloric acid and sodium hydroxid was subsequently added, without removing the globulin fraction, much if not all of the complement activity was restored (table IV, A).

The reason for sodium hydroxid not causing the same injurious effect upon the complement in this instance (table IV, A) must be due to the presence of the globulin fraction which seems to have a greater affinity for the free sodium hydroxid than the albumin fraction in which the active principle of the complement seems to reside.

That the globulin fraction has a greater affinity for sodium

TABLE IV.

Comparison of Complement Inactivation by Different Alkalis in the Presence and Absence of Globulin Fractions.

Amounts of neutralized dilution used.	To 1 c.c. of guinea pig complement 8.2 c.c. of N/250 HCl were added and kept at room temperature for 1 hr.								1 c.c. complement + 8.2 c.c. HCl N/250 in 0.9% NaCl.		1 c.c. complement + 9 c.c. 0.9% NaCl.	1 c.c. complement + 8.2 c.c. distilled water.
	After 1 hr. corresponding amounts of alkali were added to neutralize the acidity and made isotonic.						Made isotonic without neutralization (control).		Neutralized with NaOH.	Non-neutralized.		Made isotonic.
	NaOH		$NaHCO_3$		Na_2HPO_4							
	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Supernatant fluid alone.	Whole serum.	Whole serum.		
1.0 c.c.		C.H.							Mch.H.			No H.
0.7 c.c.		Sl.H.		C.H.					Sl.H.			
0.5 c.c.		No H.		Mch.H.		C.H.		C.H.	No H.			
0.3 c.c.				Sl.H.	C.H.	Sl.H.		F.C.H.				
0.2 c.c.	C.H.		C.H.	No H.	Sl.H.	No H.	C.H.	No H.		C.H.		
0.15 c.c.	No H.		F.C.H.		No H.		F.C.H.			Mch.H.	C.H.	
0.1 c.c.			Sl.H.				No H.			Sl.H.	Sl.H.	
0.07 c.c.			No H.							No H.	No H.	
	A		B		C		D		E		F	G

TABLE V.

The Effect of the Excess of Hydrochloric Acid and Sodium Hydroxid upon the Serum from Which the Globulin Fraction Is Removed.

1 c.c. of complement + 8.2 c.c. of N/250 HCl at room temperature for 1 hour, then centrifugalized and the globulin fraction removed. Only the supernatant fluid was used after it was made isotonic by 10 per cent. NaCl.

Amounts of mixtures.	1 c.c. of supernatant fluid in all tubes, then different amounts of HCl or NaOH were added and the resultant mixtures tested in varying amounts.									Native complement + 1 c.c. 0.9% NaCl 9 c.c.
	N/250 HCl				Original isotonized supernatant fluid.	N/250 NaOH.				
	0.8 c.c.	0.6 c.c.	0.4 c.c.	0.2 c.c.	0 (non-neutralized.)	0.2 c.c.	0.4 c.c.	0.6 c.c.	0.8 c.c.	
0.5 c.c.	No H	Mch.H.							Sl.H.(?)	
0.3 c.c.	No H.	F.C.H.	C.H.				C.H.	C.H.	C.H.	
0.2 c.c.	Sl.H.	C.H.	F.C.H.(?)	C.H.			F.C.H.	F.C.H.	F.C.H.	
0.1 c.c.	Sl.H.	C.H.	F.C.H.(?)	F.C.H.(?)			F.C.H.	F.C.H.	F.C.H.	
0.07 c.c.	Tr.H.	Sl.H.	F.C.H.(?)	F.C.H.(?)	C.H.	C.H.	F.C.H.	Sl.H.	Sl.H.	
0.05 c.c.	Tr.H.(?)	Tr.H.	Mch.H.	F.C.H.(?)	F.C.H.(?)	F.C.H.	Sl.H.	Sl.H.	Sl.H.	
0.03 c.c.	No H.	No H.	Sl.H.	Sl.H.	F.C.H.(?)	Sl.H.	Sl.H.	Sl.H.	No H.[8]	
0.02 c.c.			No H.	No H.	Sl.H.	Sl.H.	Sl.H.	Sl.H.	No H.	C.H.
0.01 c.c.					No H.	No H.	Tr.H.(?)	Sl.H.	No H.	F.C.H.
0.005 c.c.							No H.	No H.		No H.

[8] So-called "Endstück."

hydroxid than the albumin fraction is easily demonstrated by experiments described later.

In order to ascertain whether the loss of complement action in the albumin fraction, the so-called end-piece, is really due to a reversible inactivation through the action of sodium hydroxid, such as had been observed by Noguchi in his earlier experiments, and not due to the removal of one of the interdependent components, the mid-piece, it was necessary to make a series of reverse experiments with an acid.

The following experiments show that the complement activity of the inactive end-piece can be restored to a great extent by means of neutralization of the alkali with adequate amounts of hydrochloric acid (table VII).

We now come to consider the mechanism of the restoration of the complement activity of the end-piece by the globulin fraction known as the mid-piece. From the reactivation experiments with hydrochloric acid, it is not improbable that the combination of the complement and sodium hydroxid can also be split by means of an amphoteric compound. Among the amphoteric substances we find various proteids, proteoses, and many non-coagulable synthetic amino acids. The serum globulins are undoubtedly amphoteric. In the present experiments we have also used egg-white, alanin, deuteroalbumose, and peptone.[9]

These experiments (table VII) demonstrate that the complement action of the so-called end-piece can be readily restored by adding adequate quantities of the amphoteric substances herein employed. One may wonder how such a weak acid as the amino acid, which does not form a salt at ordinary temperature, can reverse the combination of the alkali and the complement fraction. But, it must be remembered that the affinity between the complement and alkali cannot be stronger than that which exists between any other amphoteric bodies. Moreover, the direction of the reversion is dependent upon the total number of acid radicals possessed by a given substance. Thus, a greater amount of the substance is

[9] We are indebted to Dr. F. J. Birchard, of The Rockefeller Institute for Medical Research, for the albumose and peptone preparations. The albumose was prepared from fibrin according to Pick's method, and the peptone from Witte's peptone according to Sigfried's method.

TABLE VI.

Comparative Action of Sodium Hydroxid upon the Whole Complement and upon the Albumin Fraction Alone.

	NaOH N/25 in c.c.	Amounts of 0.9 % NaCl and 3 % alanin in c.c.	Supernatant fluid (after centrifugalization) of the mixture. 1 c.c. guinea pig serum + 8.2 c.c. HCl N/250 for 1 hour at room temperature.							
			1 c.c.	0.7 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.
Amounts of NaOH N/25 in 10% NaCl added to each 1 c.c. of the solutions examined.	0	Salt 0.3			C.H.	F.C.H.	Sl.H.	Sl.H.	No H.	
		Alanin 0.3					C.H.	C.H.	Mch.H.	No H.
	0.01	Salt 0.3					C.H.	Sl.H.	No H.	
		Alanin 0.3						C.H.	No H.	
	0.015	Salt 0.3				C.H.	Mch.H.	No H		
		Alanin 0.3					C.H.	F.C.H.	F.C.H.	No H.
	0.02	Salt 0.3				C.H.	F.C.H.	Tr.H.	No H.	
		Alanin 0.3					C.H.	Tr.H.	No H.	
	0.03	Salt 0.3				C.H.	Tr.H.	No H.		
		Alanin 0.3				C.H.	F.C.H.	No H.		
	0.04	Salt 0.3	C.H.	F.C.H.	No. H.					
		Alanin 0.3			C.H.	F.C.H.	F.C.H.	No H.		
	0.05	Salt 0.3	C.H.	F.C.H.	No H.					
		Alanin 0.3			C.H.	F.C.H.	Sl.H.	No H.		
	0.07[9]	Salt 0.3	No H.							
		Alanin 0.3				C.H.	Tr.H.	No H.		
	0.1	Salt 0.3	No H.							
		Alanin 0.3			C.H.	F.C.H.	Sl.H.	No H.		

	NaOH N/25 in c.c.	Amounts of 0.9 % NaCl and 3 % alanin in c.c.	Guinea pig complement 1 c.c. + 9 c.c. 0.9% NaCl for 1 hour at room temperature (control).								
			1 c.c.	0.7 c.c.	0.5 c.c.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.15 c.c.	0.1 c.c.	0.05 c.c.
Amounts of NaOH N/25 in 10% NaCl added to each 1 c.c. of the solutions examined.	0	Salt 0.3							C.H.	F.C.H.	F.C.H.
		Alanin 0.3									
	0.01	Salt 0.3									C.H.
		Alanin 0.3									C.H.
	0.015	Salt 0.3									F.C.H.
		Alanin 0.3									C.H.
	0.02	Salt 0.3								F.C.H.	Sl.H.
		Alanin 0.3								C.H.	C.H.
	0.03	Salt 0.3							Sl.H.	No H.	
		Alanin 0.3							C.H.	Tr.H.	No H.
	0.04	Salt 0.3				C.H.	F.C.H.	Mch.H.	Sl.H.	No H.	
		Alanin 0.3					C.H.	Mch.H.	Sl.H.	No H.	
	0.05	Salt 0.3			C.H.	F.C.H.	No H.				
		Alanin 0.3				C.H.	F.C.H.	No H.			
	0.07[10]	Salt 0.3	C.H.	Sl.H.	No H.						
		Alanin 0.3			C.H.	F.C.H.	No H.				
	0.1	Salt 0.3	No. H.								
		Alanin 0.3			C.H.	F.C.H.	Tr.H.(?)	No H.			

In this experiment sheep corpuscles were used (0.5 c.c. of 5 per cent. suspension) and 50 units of amboceptor.

[10] The amount of sodium hydroxid used in the original complement-splitting experiments corresponds to 0.08 c.c. in this series.

TABLE VII.

Reactivation of the End-Piece of the Complement Split by Hydrochloric Acid.

	Alanin (5%), 0.075 c.c.	a peptone (2%), 0.1 c.c.	Deutero-albumose B (2%), 0.05 c.c.	Mid-piece from HCl-splitting, 0.05 c.c.	Mid-piece + NaOH N/250 (1 c.c. + 0.25 c.c.), 0.05 c.c.	Mid-piece heated 30 min. at 56° C., 0.05 c.c.	Sheep serum diluted 1:10 heated 56° C., 0.05 c.c.	Mid-piece from dialysis, 0.05 c.c.	Mid-piece from dialysis + HCl (3:1), 0.05 c.c.	HCl N/250	0 control.
Native complement 0.03 c.c. inactivated by 0.6 c.c. NaOH N/250	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	0.005 c.c. C.H.	No H.
End-piece[11] non-neutralized, 0.02 c.c.	No H.	No H.	Sl.H.	Sl.H.	F.C.H.	F.C.H.	Mch.H.	Mch.H.	Tr.H.	0.005 c.c. No H.	Tr.H.(?)
End-piece[12] 9.2 c.c. + 0.2 NaOH N/25 in NaCl, 0.05 c.c.	Sl.H.	Tr.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Tr.H.(?) Sl.H.(?)
End-piece[12] 9.2 c.c. + 0.4 c.c. NaOH N/25 in NaCl, 0.05 c.c.	Mch.H.	F.C.H.	C.H.	F.C.H.	F.C.H.	Sl.H.	Tr.H.	F.C.H.	F.C.H.	0.02 c.c. F.C.H.	
End-piece[12] 9.2 c.c. + 0.8 c.c. NaOH N/25 in NaCl, 0.05 c.c.	Mch.H.	F.C.H.	C.H.	Sl.H.	Mch.H.	F.C.H.	Tr.H.	Sl.H.	Sl.H.	0.04 c.c. C.H.	Tr.H.
End-piece[12] 9.2 c.c. + 0.4 c.c. HCl N/25, 0.03 c.c.	Sl.H.	Sl.H.	Sl.H.	Tr.H.	Tr.H.	Tr.H.	Mch.H.	Tr.H.	Tr.H.	0.005 c.c. Tr.H.	Tr.H.
Control 0	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	No H.	

[11] The end-piece resulted from mixing 1 c.c. of complement + 8.2 c.c. N/250 HCl at room temperature for 60 minutes. Then the mixture was centrifugalized and the supernatant fluid made isotonic and diluted to 10 c.c.

[12] All the mixtures, beginning with the third end-piece, before being made isotonic were neutralized (partly) by the amounts of NaOH shown, then made isotonic and, if necessary, diluted to 10 c.c.

required if it possesses fewer acid radicals. That the globulins have a greater affinity for sodium hydroxid than the albumins may be accounted for by the larger size of the molecules of the former class of proteids. The solubility of the substances seems also to be important, as certain insoluble amino acids, such as asparagin, asparagin acid, and leucin failed to reactivate the end-piece.

COMPLEMENT-SPLITTING WITH CARBON DIOXID.

Liefmann splits the complement in the following manner. One half of a cubic centimeter of guinea pig serum (complement) is mixed with two cubic centimeters of distilled water, and carbon dioxid gas is passed through until no more precipitate is formed. The treated serum is then centrifugalized to separate the precipitate from the supernatant fluid. The clear supernatant fluid is carefully decanted and filtered through hardened filter paper into another receptacle and is used as the end-piece after being made isotonic with sodium chlorid. The deposit, which represents the globulin fraction of the serum, is then dissolved in 2.5 cubic centimeters of 0.85 per cent. salt solution. This portion is used as the mid-piece. In a certain number of instances the phenomenon of splitting is ideally shown, the end- and mid-pieces separately having no action upon the sensitized corpuscles (table X). The two components obtained by the carbon dioxid method behave in much the same way as those prepared by the hydrochloric acid method of Sachs. It may be stated, however, that the end-piece portion remains quite active in many instances, as has been observed also by Liefmann and others (tables IX and X).

In considering the processes that lead to the inactivation of the end-piece by carbon dioxid and the subsequent reactivation of this portion by the mid-piece of the globulin fraction, one can conceive that an essential difference exists between the inactivation by carbon dioxid and that by the hydrochloric acid method. In case of the hydrochloric acid-splitting, the carbonates as well as other alkaline salts of the serum are completely converted into chlorids. Small amounts of free carbon dioxid or other weak acids remaining in the fluid cause a partial inactivation of the complement.

On the other hand, the passage of carbon dioxid through a

TABLE VIII.

Inactivation of Complement by Carbon Dioxid.

Dilution 1:10 in water.	0.4 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.04 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.005 c.c.
Native complement in NaCl									C.H.	F.C.H.	No H.
Complement + CO_2, 2 min. } the whole made isotonic						C.H.	Tr.H.	No H.			
Complement + CO_2, 10 min. } the whole made isotonic				C.H.	F.C.H.	No H.	No H.				
Supernatant fluid alone											
Guinea pig complement after passage of CO_2, 2 min.				C.H.	Sl.H.	No H.					
Guinea pig complement after passage of CO_2, 4 min.			C.H.	F.C.H.	F.C.H.	Tr.H.(?)	No H.				
Guinea pig complement after passage of CO_2, 6 min.			C.H.	Tr.H.	No H.						
Guinea pig complement after passage of CO_2, 8 min.		C.H.	Mch.H.	No H.							
Guinea pig complement after passage of CO_2, 10 min.	C.H.	F.C.H.	Mch.H.	No H.							

TABLE IX.

Splitting by Carbon Dioxid.

	Amount of end and mid-piece.							
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.
Native complement 1:10				C.H.	F.C.H.	Mch.H.	Tr.H.	No H.
1:10 complement (CO_2), 10 min. (whole)				C.H.	Mch.H.	Sl.H.	No H.	
Albumin fraction, CO_2, 10 min. (end-piece)	C.H.	F.C.H.	Mch.H.	Tr.H.(?)	No H.			
End-piece[13] (CO_2), 10 min. + mid-piece, 10 min. 1:1			C.H.	F.C.H.	F.C.H.	Tr.H.	No H.	
End-piece (CO_2), 10 min. + mid-piece, 60 min. 1:1,		C.H.	F.C.H.	Mch.H.	No H.			
Albumin fraction (CO_2), 60 min. (end-piece)			C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
End-piece, 60 min., + mid-piece, 10 min., 1:1	F.C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.(?)	No H.		
End-piece, 60 min., + mid-piece, 60 min., 1:1			C.H.	F.C.H.	Mch.H.	No H.		
Mid-piece, 10 min., alone	No H.							
Mid-piece, 60 min., alone	No H.							

[13] 1 : 1 means equal amounts of both end- and mid-pieces; therefore in the column under 0.2 c.c. it means 0.2 c.c. of end-piece + 0.2 c.c. of mid-piece.

TABLE X.

A Case of Successful Splitting by Carbon Dioxid (Very Rare).

	0.7 c.c.	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.
Native complement 1:10 dilution							C.H.	Sl.H.	No H.	
Complement + CO_2, 10 min., made isotonic (whole)							C.H. (delay)	Sl.H.	No H.	
Albumin fractions alone	No H.									
Albumin fraction + globulin fraction 1:5					C.H.	F.C.H.	Sl.H.	Tr.H.	No H.	
Globulin fraction alone	No H.									

A Case of Imperfect Splitting by Carbon Dioxid.

	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.007 c.c.
Native complement 1:10 dilution				C.H.	F.C.H.(?)	F.C.H.	Sl.H.	No H.
Complement + CO_2, 10 min., made isotonic (whole)				C.H.	F.C.H.	Mch.H.	No H.	
Albumin fraction alone				C.H.	Mch.H.	Sl.H.	No H.	
Albumin fraction + globulin fraction 1:5	C.H.	F.C.H.	Sl.H.	Sl.H.	No H.			

A Case of the Complement Twenty-four Hours Old.

	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	0.01 c.c.	0.007 c.c.
Native complement 1:10 dilution	C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	
Complement CO_2, 10 min., made isotonic (whole)		C.H.	F.C.H.	Mch.H.	Tr.H.	No H.	
Albumin fraction alone			C.H.	Mch.H.	Sl.H.	Tr.H.	No H.
Albumin fraction + globulin fraction 1:1	C.H.	F.C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	

TABLE XI.

Inactivation of Complement by Carbon Dioxid in Different Media.

	+ CO_2, 10 min.						
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.
Native complement 1:10 in NaCl							
Complement 1 c.c. + 8.2 c.c. distilled water, isotonized after 30 min.			C.H.	F.C.H.	Sl.H.	Tr.H.	No H.
Complement 1 c.c. + 8.2 c.c. 0.9% NaCl, after 30 min. 0.8 c.c. 0.9% NaCl added					C.H.	Sl.H.	No H.
Complement 1 c.c. + 8.2 c.c. N/250 HCl (in distilled water), neutralized and made isotonic after 30 min.		C.H.	Mch.H.	Mch.H.	Sl.H.	Tr.H.	No H.
Complement 1 c.c. + 8.2 c.c. HCl in 0.9% NaCl, neutralized after 30 min.				C.H.	Mch.H.	Sl.H.	Tr.H.
Complement[14] in isotonic sugar solution 1:10 + 0.3 c.c. NaCl 0.9%	C.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	No H.	

	No CO_2.							
	0.5 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.07 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.
Native complement 1:10 in NaCl						C.H.	F.C.H.	No H.
Complement 1 c.c. + 8.2 c.c. distilled water, isotonized after 30 min.	Tr.H.	No H.						
Complement 1 c.c. + 8.2 c.c. 0.9% NaCl, after 30 min. 0.8 c.c. 0.9% NaCl added					C.H.	C.H.	F.C.H.	No H.
Complement 1 c.c. + 8.2 c.c. N/250 HCl (in distilled water) neutralized and made isotonic after 30 min.		C.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	No H.	
Complement 1 c.c. + 8.2 c.c. HCl in 0.9% NaCl, neutralized after 30 min.		C.H.	C.H.	C.H.	F.C.H.	Sl.H.	Tr.H.	No H.
Complement[14] in isotonic sugar solution 1:10 + 0.3 c.c. NaCl 0.9%						C.H.	Sl.H.	No H.

[14] The addition of sodium chlorid is to prevent complement from complete inactivation.

TABLE XII.

Reactivation of Complement Inactivated by Carbon Dioxid.

Amounts of reactivating substances.		Alanin 3 %.	Mid-piece (CO_2).	Egg-white 1:10.	Sheep serum, 30 min. at 56° C. 1:5.	Protoalbumoses 5 %.	Deuteroalbumoses 2 %.
0.3 c.c. of end-piece from CO_2-splitting in each tube.	1.0 c.c.						
	0.7 c.c.						
	0.5 c.c.						
	0.4 c.c.	C.H.					
	0.3 c.c.	C.H.	Sl.H.				
	0.2 c.c.	C.H.	Mch.H.				
	0.15 c.c.	F.C.H.	Mch.H.			No. H.	
	0.1 c.c.	Mch.H.	C.H.		Sl.H.	Sl.H.	No. H.
	0.05 c.c.		Mch.H.		Sl.H.	Mch.H.	Tr.H.
	0.03 c.c.		Sl.H.	C.H.	F.C.H.	Sl.H.	Mch.H.
	0.02 c.c.			C.H.			Sl.H.
	0.01 c.c.			C.H.			
	0.005 c.c.			C.H.			
	0						

mixture of guinea pig serum and water precipitates the globulin fraction just as in the case of the hydrochloric acid treatment, but the carbonates as well as phosphates remain unaffected by this treatment. The inactivation of the complement in this instance is doubtless due to the overcharging of the serum water with carbon dioxid gas, which by virtue of its acid nature combines with the amphoteric molecules of the serum albumin fraction in which the activity of the complement is residing. In a medium, whether in the undiluted native serum or in a serum diluted with sodium chlorid solution of isotonic concentration, in which the sodium chlorid content reaches a sufficient concentration (table XI), a weak acid like carbon dioxid fails to enter into even a loose combination with the albumin molecules, and hence leaves the complement comparatively uninjured. The reason that carbon dioxid acts upon the complement molecules in serum sufficiently diluted with water is that the strong electrolyte, sodium chlorid, can in that dilution no longer interfere with the action of a weak acid. In diluted serum carbon dioxid causes an acid inactivation of complement and the activity of complement may be restored by certain amphoteric substances (table XII) and also by an alkali (table XV, C), but not by an acid.

The phenomenon of reactivation by means of adequate quantities of sodium hydroxid may raise a question as to why the result-

TABLE XIII.

Substances Subjected to Carbon Dioxid Lose Their Reactivating Power for Complement Inactivated by Carbon Dioxid.

Substances acted upon by carbon dioxid for 15 minutes. The total volume in each tube was 1.5 c.c.

	Alanin 5 %.				Mid-piece (CO_2).				o Control.
	No CO_2.		+ CO_2.		No CO_2.		+ CO_2.		
	0.15 c.c.	0.1 c.c.	0.15 c.c.	0.1 c.c.	0.07 c.c.	0.03 c.c.	0.07 c.c.	0.03 c.c.	
End-piece (CO_2) 0.07 c.c. in all tubes.	C.H.	C.H.	No H.	Tr.H.	C.H.	C.H.	Tr.H.	Sl.H.(?)	No H.
No end-piece (control).............	No H.		No H.		No H.		No H.		
	Egg-white (1:10).				Sheep serum 56° C. (1:5).				o Control.
	No CO_2.		+ CO_2.		No CO_2.		+ CO_2.		
	0.01 c.c.	0.005 c.c.	0.01 c.c.	0.005 c.c.	0.03 c.c.	0.01 c.c.	0.03 c.c.	0.01 c.c.	
End-piece (CO_2) 0.07 c.c. in all tubes.	C.H.	C.H.	Tr.H.	Tr.H.	C.H.	F.C.H.	Tr.H.	Mch.H.	No H.
No end-piece (control).............	No H.		No H.		No H.		No H.		

ant Na_2CO_3 or $NaHCO_3$ no longer exerts the same effect as the free sodium hydroxid and carbon dioxid, since these are readily dissociable in the fluid. But one must not forget that in the stage of reactivation the fluid is now made isotonic with sodium chlorid, and this restrains the dissociation of carbonates or phosphates sufficiently to prevent the free action of the carbon dioxid or sodium ions upon the complement fraction. It is important to notice that an acid has no reactivating property for the end-piece prepared with carbon dioxid, while exactly the opposite is the case with the end-piece made with hydrochloric acid, which is an inactivation by alkali.

In the experiments in table XIII we show that amphoteric substances capable of reactivating complement inactivated by carbon dioxid lose this reactivating property if treated with carbon dioxid before being added to the inactive complement. The way in which amphoteric substances reactivate complement is thus clearly demonstrated.

COMPLEMENT-SPLITTING BY MEANS OF DIALYSIS.

Before Sachs, Liefmann, and others introduced various methods of splitting the complement, Ferrata discovered that by dialyzing complement against water through a fish membrane or a celloidin sac, it could be separated into two inactive components, an albumin fraction corresponding with the end-piece of the later investigators, and a globulin fraction (which precipitates during dialysis) corresponding with the mid-piece. This result, although not always obtained, was nevertheless confirmed by other workers.

On several occasions in the present series of experiments we have also confirmed this finding. Our explanation for this phenomenon is, however, entirely different from that given by previous workers. As will be shown in the following protocols, the supernatant fluid (albumin fraction) when separated from the precipitate (globulin fraction) and made isotonic with sodium chlorid, is quite inactive upon the sensitized corpuscles, but can be reactivated by adding, not only the globulin fraction of the same serum (isotonized with sodium chlorid), but also the globulin fraction prepared by the hydrochloric acid or carbon dioxid methods (table XIV). Indeed,

TABLE XIV.

Reactivation of Complement Inactivated by Distilled Water or Dialysis.[15]

	Alanin 5 %.			Mid-piece (CO_2, 10 min.).			Mid-piece dialysis.		
	0.3 c.c.	0.15 c.c.	0.07 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.	0.3 c.c.	0.2 c.c.	0.1 c.c.
Complement + distilled water (1:10) 2 hours at 37° C., whole made isotonic (NaCl) .... 0.2 c.c.	C.H.	Sl.H.	No H.	Mch.H.	C.H.	F.C.H.	No H.	No H.	No H.
Complement dialyzed at room temperature 6 hours. *End-piece* isotonized (final dilution 1:10) .... 0.2 c.c.	C.H.	F.C.H.	No H.	F.C.H.	C.H.	F.C.H.	No H.	No H.	Sl.H.
End-piece (CO_2) (final dilution 1:10) (control) .... 0.2 c.c.	C.H.	F.C.H.	No H.	Sl.H.	F.C.H.	C.H.	Sl.H.	F.C.H.	No H.
No complement. Control	No H.			No H.			No H.		

	Egg-white (1:10).			Sheep serum (56° C.) 1:5.			Control. No mid-piece.
	0.1 c.c.	0.05 c.c.	0.03 c.c.	0.05 c.c.	0.03 c.c.	0.02 c.c.	
Complement + distilled water (1:10) 2 hours at 37° C., whole made isotonic (NaCl) .... 0.2 c.c.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
Complement dialyzed at room temperature 6 hours. *End-piece* isotonized (final dilution 1:10) .... 0.2 c.c.	No H.	No H.	No H.	No H.	No H.	No H.	No H.
End-piece (CO_2) (final dilution 1:10) (control) .... 0.2 c.c.	Tr.H.	Sl.H.	C.H.	C.H.	C.H.	C.H.	No H.
No complement. Control	No H.			No H.			

[15] Results taken after incubation at 37° C. for 1 hour.

the reactivation may be even better and more constant with mid-pieces prepared by these other methods than with its own mid-piece. Further, it is found that an acid (table XV, B) or certain amphoteric substances which bear no relation to the serum (table XIV)

TABLE XV.

Reactivation of Complement Inactivated by Acid (Carbon Dioxid) Compared to Reactivation of Complement by Alkali (Dialysis or Distilled Water).

		Complement + distilled water (1:10) at room temperature for 1 hr.; the whole made isotonic; 0.5 c.c. used in each tube.					
	Incubation time.	+ Alanin 7.5 %.				No alanin.	
		0 2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
A	HCl N/250 0.1		No H.	No. H.	Tr.H.	No. H.	Sl.H.
	HCl N/250 0.125		No H.	Tr.H.	Sl.H.	Sl.H.	F.C.H.
	HCl N/250 0.15		No H.	Sl.H.	Mch.H.	Sl.H.	F.C.H.
	Control	F.C.H.	F.C.H.	C.H.	C.H.	No H.	No H.(?)
	NaOH N/250 0.1						
	NaOH N/250 0.2						
	NaOH N/250 0.3	No H.	No H.	No H.	No. H.		
	NaOH N/250 0.4	No H.	Tr.H.(?)	No H.	Tr.H.	No H.	No H.
	NaOH N/250 0.5	Tr.H.	Tr.H.	Sl.H.(?)	Sl.H.	No H.	No H.

		End-piece of complement dialyzed for 12 hrs. against running water; made isotonic; final dilution 1:10; 0.3 c.c. in each tube.					
	Incubation time.	+ Alanin 7.5 %.				No alanin.	
		0.2 c.c.		0.3 c.c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
B	HCl N/250 0.1		No H.	No H.	No H.	No H.	No H.
	HCl N/250 0.125		No H.	Sl.H.	Mch.H.	Sl.H.	Mch.H.
	HCl N/250 0.15		No H.	Sl.H.	Mch.H.	Sl.H.	F.C.H.
	Control	F.C.H.	C.H.	C.H.	C.H.	No H.	Tr.H.
	NaOH N/250 0.1						
	NaOH N/250 0.2						
	NaOH N/250 0.3		No H.		No H.		No H.
	NaOH N/250 0.4		No H.		No H.		No H.
	NaOH N/250 0.5		Tr.H.		Tr.H.		No H.

	Incubation time.	End-piece (CO_2) for 10 min.; made isotonic; final dilution 1:10; 0.3 c.c. in each tube.					
		+ Alanin 7.5 %.				No alanin.	
		0.2 c c.		0.3 c c.			
		1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
C	HCl N/250 0.1		No H.		No H.	No H.	Tr.H.
	HCl N/250 0.125		No H.		No H.	No H.	Tr.H.
	HCl N/250 0.15		No H.		No H.	No H.	Tr.H.
	Control	Sl.H.	C.H.	Tr.H.	C.H.	No H.	Sl.H.
	NaOH N/250 0.1	No H.	Tr.H.	No H.	Tr.H.		
	NaOH N/250 0.2	Tr.H.	F.C.H.	Tr.H.	C.H.	C.H.	
	NaOH N/250 0.3	Mch.H.	C.H.	Mch.H.	C.H.	C.H.	
	NaOH N/250 0.4						
	NaOH N/250 0.5						

reactivate this dialyzed end-piece. On the other hand, alkalis do not reactivate it (table XV, B).

From the experiments given above it becomes highly probable that dialyzed serum loses its complement activity chiefly by an alkali inactivation of a reversible character. The cause of this

TABLE XVI.

Rate of Dialysis of Sodium Chlorid and $NaHCO_3$ from the Mixture of Fifty Cubic Centimeters of 1 per cent. Sodium Chlorid + Fifty Cubic Centimeters N/50 $NaHCO_3$.

	NaCl				$NaHCO_3$			
	$AgNO_3$	NH_4SCN	Combined Ag	NaCl	H_2SO_4 N/100	NaOH N/100	Combined acid	$NaHCO_3$ N/50
After[16]								
0	4.0 c.c.	2.0 c.c.	2.0 c.c.	0.02 gm.	6.0 c.c.	1.0 c.c.	5.0 c.c.	2.5 c.c.
20 min.	3.0 c.c.	2.0 c.c.	1.0 c.c.	0.01 gm.	4.0 c.c.	1.0 c.c.	3.0 c.c.	1.5 c.c.
40 min.	2.0 c.c.	1.2 c.c.	0.8 c.c.	0.008 gm.	2.5 c.c.	0.5 c.c.	2.0 c.c.	1.0 c.c.
1 hr.	1.0 c.c.	0.6 c.c.	0.4 c.c.	0.004 gm.	2.5 c.c.	1.3 c.c.	1.2 c.c.	0.6 c.c.
2 hrs.	0.5 c.c.	0.4 c.c.	0.1 c.c.	0.001 gm.	2.0 c.c.	1.3 c.c.	0.7 c.c.	0.35 c.c.
3 hrs.	0.5 c.c.	0.5 c.c.	0	Trace(?)	1.5 c.c.	0.9 c.c.	0.6 c.c.	0.3 c.c.
4 hrs.	0.5 c.c.	0.5 c.c.	0	0	1.5 c.c.	1.0 c.c.	0.5 c.c.	0.25 c.c.
Running water	1.0 c.c.	1.0 c.c.	0	0	1.5 c.c.	1.0 c.c.	0.5 c.c.	0.25 c.c.

[16] At the time indicated, 5 c.c. of the contents of the collodion sac were removed and examined for hydrochloric acid and $NaHCO_3$.

inactivation may be found in the gradual changes taking place in the salt content of the serum within the sac during dialysis. As may be expected, sodium chlorid passes out of the celloidin sac with a greater velocity than the remaining salts, such as carbonates or phosphates, and this together with an increase in volume (by endosmosis of water) results in removing the restraining power of the chlorids upon the dissociation of the carbonates. These salts then inactivate the complement.

A study of the comparative velocity of osmosis of sodium chlorid and Na_2CO_3 through a celloidin membrane is given in table XVI. At the time indicated, five cubic centimeters of the contents of the collodion sac were removed and examined for sodium chlorid and sodium bicarbonate.

SUMMARY.

It is generally accepted that complement may be split into a mid-piece and an end-piece. The mid-piece is thought to be in the globulin fraction, and the end-piece in the albumin fraction. The restoration of complement activity by putting together the albumin and globulin fractions does not prove, however, that each fraction contained a part of the complement, for the albumin fraction can be reactivated in the absence of the globulin fraction.

Complement-splitting as brought about by hydrochloric acid, carbon dioxid, and dialysis, is really an inactivation of the whole complement by certain acids or alkalis, either added in the free state to the serum, or liberated as a result of the dissociation of certain electrolytes.

That the whole complement, and not a part only, is present in the albumin fraction of the serum can be demonstrated by the removal of the inhibitory action of the acid or alkali. This can be effected by the addition, not only of alkali or acid, but also of any amphoteric substance. When hydrochloric acid, carbon dioxid, or dialysis are employed to produce the phenomenon known as complement-splitting, the complement is merely inactivated, not split.

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