Is Serozyme, a component of the Blood Clotting Mechanism, Identical with Complement, a Factor in Hemolysis?

By

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Introduction.

That the two mechanisms of blood clotting and immunity are closely related has been suggested by many authors as for example, Doyon (1), Nolf (2), Bordet (3), Bergel (5) and Mathews (7). Professor Mathews particularly called my attention to this possibility. It is this possibility which has been investigated. As a result, strong evidence that blood coagulation and hemolysis by specific hemolysin and complement are intermeshing mechanisms has been obtained.

Many important facts are already known which relate blood clotting and hemolysis by amboceptor and complement. In the first place, calcium salts are essential to both systems, as shown by Arthus (8) for blood clotting and Gengou (9) for hemolysis. Excess of calcium salts, as is well known, prevents the action of either system.

Secondly neither blood coagulation nor hemolysis can

29 AG 27

take place in a salt-free medium, as in an isotonic glucose solution. On the other hand, excess of salts prevents blood-clotting or hemolysis.

Then again as serum stands, its ability to clot oxalate plasma or to "complete" hemolysis decreases. Both thrombin and complement disappear or are progressively inactivated.

Complement action for fibrin extracts has been claimed by Ottolenghi (10) and by Bergel (5). However, it is not yet certain whether the complement extracted by NaCl from fibrin is a substance split off fibrin or whether it is a substance from impurities, such as blood platelets, entangled in the fibrin.

It is a very significant fact that serozyme, the precursor of thrombin, coagulates, as Bordet and Delange (4) have shown, at 56 degrees Centigrade, which is just the temperature employed to inactivate the complement in serum. Serozyme is the only protein present in the serum, as far as it is known, that has this coagulation temperature of 56°. This fact alone, makes it probable that serozyme directly or indirectly is concerned in complement activity.

In the phenomenon of hemolysis, attention is naturally focussed on the amount of hemoglobin coming from the cells. This is natural, as in hemoglobin we have a natural indicator which shows the amount of hemolysis and the rate of hemolysis. However, it by no means follows that the most significant aspect of hemolysis is this diffusion of hemoglobin into the medium. What occurs to the stroma in hemolysis?

Two significant observations recently made indicate that in hemolysis by amboceptor and complement, the stroma has been coagulated. If red blood cells are hemolysed by distilled water, the stroma, according to D'Herelle (11), is just as elastic as the intact cell. Placed in a hypertonic medium, the stroma contract, when the medium is made hypotonic by the addition of distilled water, the stroma expand. However, when red blood cells are hemolysed by the addition of a specific hemolysin and complement, the stroma are rigid. They do not expand in a hypotonic medium, nor do they contract in a hypertonic medium. They behave as if they had passed from a sol to a gel state, in other words as if they had been clotted or coagulated.

This observation has been supplemented by Ponder (12) who found that when red blood cells are being hemolysed by a specific hemolysin and complement, they do not change in volume. There is nothing to indicate that the forces of surface tension and osmotic pressure, in so far as they affect the volume of the stroma, have been altered.

Thus we may regard not only blood coagulation but hemolysis by specific hemolysin and complement as phenomena involving gel-formation. This viewpoint is especially significant in view of the fact, discovered by Davide (13), that an anti-fibrinogen serum when injected into the animal that supplied the fibrinogen produces an anemia due to destruction of red

cells that is often fatal. There is then a direct connection between fibrinogen and hemolysis.

With the exception of sheep antifibrinogen serum, all the antifibrinogen sera that Davide examined were able in vitro to hemolyse the red blood cells from the species which had furnished the fibrinogen to prepare the serum in question. Why does guinea-pig fibrinogen, for example, when injected into the rabbit, cause the formation of a substance, that in the presence of complement has the property of hemolysing guinea-pig red blood cells? Davide thinks that a most probable explanation is that supported by Mathews (7) and by Reymann (22): fibrinogen results from the disintegration of red and white, blood cells, and thus there is a close chemical connection between red blood cell globulin and fibrinogen.

It may be then that when red blood cells are hemolysed by a specific hemolysin and complement, the clotting of a fibrinogen-like substance in the stroma of the red blood cells is involved. Trom this point of view also, it is very important to answer correctly the question: Is serozyme identical with complement?

These facts will be more fully discussed later in connection with Bordet's theory of blood clotting.

In the following pages, evidence is presented to show that serozyme, the precursor of thrombin, acts as complement, in effecting the hemolysis of sheep red blood cells by anti-sheep cell amboceptor. Conclusive proof is then advanced that

cephalin, which plays so important a role in blood-clotting, prevents the hemolysis of sheep red blood cells by anti-sheep cell amboceptor and guinea-pig serum (complement). This effect of cephalin, it will be shown, is very probably due to its action on complement. Since cephalin unites in the presence of calcium ions, with serozyme to form thrombin, the anti-complementary action of cephalin reported on here forges another very important link in the chain of evidence connecting immunity and blood-clotting, in general, and serozyme and cephalin, in particular.

The effect of oxidation and of storage on the anti-complementary action of cephalin is then considered, also the action of fibrinogen on hemolysis, and the thrombin activity of "mid-piece", one of the components of complement.

The complementary action of serozyme will now be considered.

EXPERIMENTAL

THE COMPLEMENTARY ACTION OF SEROZYME.

Serozyme was prepared by the method of Mellanby (14). This method assumes that serozyme, or prothrombin, exists in the circulating blood in a salt-like union with fibrinogen. This complex is precipitated when centrifuged plasma is diluted and neutralized by the addition of dilute acetic acid. Part of the precipitates redissolved in dilute saline solution, is clotted by the addition of a calcium salt; the resulting "serum"

which is rich in thrombin is added to the remainder of the solution of precipitate which is then clotted. Thrombin displaces serozyme from combination with fibrinogen. By the addition of thrombin in the right proportions (determined by a preliminary titration) all the fibrinogen is clotted and there remains no excess thrombin; the "serum" contains serozyme and a globulin impurity.

By this method the writer obtained a preparation which showed the following characteristics:

(1) It was not clotted by fresh serum, showing that no fibrinogen was present.

(2) It did not clot fibrinogen solution or plasma, indicating the absence of thrombin.

(3) When cephalin and $CaCl_2$ were added it clotted fibrinogen solution, which was not clotted by $CaCl_2$ or cephalin alone.

This solution of serozyme was dried on a glass plate in an air-current at room temperature; 106 mg. were dissolved in 50 c.c. of distilled water. Solution was incomplete; after standing some days in the ice-box, a flocculent precipitate had appeared. This was removed by centrifuging at high speed for 10 minutes.

The clear supernatant liquid was pipetted off, and it was tested for its ability to replace guinea-pig complement serum in the hemolytic system.

The sheep red blood cells had been washed three times with 0.9% NaCl; they were used in a 5% suspension. The anti-sheep cell rabbit serum had a titre of 1:2200.

The results are given in Table I.

TABLE I.

THE COMPLEMENT ACTION OF SEROZYME

Tube No.	Hemolysin 2 Units	Prothrombin Solution	Distilled Water	NaCl 0.9%	Hemolysis
1	0.9 c.c.	0.1	-	0.5	None
2	" "	0.2	-	0.4	Slight
3	" "	0.3	-	0.3	Partial
4	" "	0.4	-	0.2	Almost Complete
5	" "	-	0.5	-	Faint

37° C. Incubated: 5.15 P.M.
Examined: 8.15 P.M.

This result, as far as it goes, is clear cut evidence that serozyme acts as complement. However, it has not been possible to repeat this very important experiment. One of the reasons is that it is difficult to prepare serozyme, whose properties are not clearly known. Another reason is that as the

below indicates, serozyme seems to be a basic protein, carrying a positive charge. Negatively-charged protein impurities in a serozyme preparation may combine with it to form a salt-like union, thus inactivating it.

The isoelectric point of the serozyme in this one successful preparation was determined as follows: 0.5 c.c. of the clear serozyme solution was pipetted into every one of 12 small tubes, each of which contained 0.5 c.c. of phosphate buffer solution, ranging in ph from ph 6.90 to ph 7.98. The solutions were clear 20 minutes after mixing, but after 14 hours they had become cloudy. Since albumins in aqueous solution are not precipitated out at their isoelectric point, 0.5 c.c. of 95% alcohol was added to each tube to depress the solubility.

The result was very striking. In a few minutes, the solution whose ph was 7.69 became heavily turbid; there was no change in the other solutions. In a few hours, complete precipitation had occurred at ph 7.69.

According to this experiment, the isoelectric point of serozyme is approximately ph 7.7. At the ph of the blood, 7.4, serozyme would be on the acid side of its isoelectric point and would, therefore, function as a base; it would be positively charged. But when blood escapes it becomes more alkaline (unless suitable precautions are taken) due to the loss of CO_2. Serozyme then would be on the alkaline side of its isoelectric point and would therefore function as an acid; it would be positively charged.

Bordet's method of preparing serozyme involves the addition of gelatinous $Ca_3 (PO_4)_2$ to oxalated plasma. The tricalcium phosphates adsorbs the serozyme, which may then be removed from the plasma by centrifuging. If the tricalcium phosphate is negatively charged it will adsorb serozyme from plasma only when the ph is less than 7.7, and as a result the serozyme was positively charged, and therefore not in a proper state for adsorption on the negatively charged tricalcium phosphate.

Another factor that may explain why attempts to demonstrate the complement action of serozyme have, up to the present, failed, is that cephalin acts as anti-complement and thus prevents hemolysis.

THE ANTIHEMOLYTIC ACTION OF CEPHALIN.

The cephalin used in these experiments was prepared by the following method. Hog brains were spread out on a glass plate and dried in an air-current at room temperature. They were then extracted three times at room temperature with acetone. The residue was then extracted three times with ether. These ether extracts were united, and stored in the ice-box until the settling out of the white precipitate that appeared, was complete. The extract was then filtered, and an equal volume of absolute alcohol was added to the filtrate. The precipitate that appeared was filtered, redissolved in ether, and several volumes of absolute alcohol were added. This operation was carried out until the cephalin had been precipitated by

absolute alcohol at least five times.

The cephalin preparations used were free of cholesterol, as entirely negative Liebermann-Burchard and Salkowski tests on chloroform solutions of approximately 0.1 g. samples of cephalin showed. A positive cholesterol test would be very faint but still detectable after the third, but would be absent, after the fourth reprecipitation with alcohol. To entirely remove cholesterol is a necessary precaution, for cholesterol also has the property of preventing hemolysis, as Ransome (23) first showed.

The cephalin was thoroly emulsified in 0.9% NaCl to make a 0.1% solution. Whereas cephalin dissolves in distilled water to give a clear solution, in 0.9% NaCl, cephalin dissolves imperfectly; the solution is opaque and there is generally more or less of a fine residue.

The amboceptor serum used was prepared in the usual manner, by injection a rabbit repeatedly with a well washed 0.9% NaCl suspension of sheep red blood cells. The serum was collected February 3, 1926. It was inactivated by heating to 56° C. for half an hour; an equal volume of glycerine was added for preservation, and it was stored as a stock solution in the ice-box.

Complement serum was obtained by permitting blood drawn from the heart of two or more guinea-pigs to clot and undergo syneresis. The serum was used in a dilution of 1:10, which as controls generally showed, eliminated the effect of

any so-called "natural hemolysin" in the guinea-pig serum.

The sheep red blood cells were always washed three times by centrifuging with 0.9% NaCl. The cells were used in a 10% suspension.

The total volume of the reaction mixtures-amboceptor serum, complement serum, sheep red blood cells, and cephalin solution was thruout kept constant at 2.5 c.c. with 0.9% NaCl solution. In general, the sheep red blood cells were added last; immediately after the addition of the dose of red blood cells to each tube, the tube was inverted several times to insure thorough mixing of the contents. This point of technique is very important, for as Arrhenius (24) has remarked, unless the reaction mixtures are thoroly shaken 30" to 60" after mixing, very large variations in the degree of hemolysis in duplicates often occur. The reason is that the cells and the amboceptor quickly unite, and if there is imperfect mixing, there is an uneven distribution of the amboceptor between the cells.

After the reaction mixtures were pipetted in, the tubes were incubated at 37° and read at the end of an hour.

An example of the method employed - to quantitatively adjust amboceptor and complement that of Kolmer (15) - is given below in Table II.

(Insert Table II)

Since 0.45 c.c. of a 1:500 dilution of rabbit immune serum caused complete hemolysis, its titre is 1:1111.

Table II.

Titration of Amboceptor Serum.

Tube No.	Amount of Amboceptor Serum 1:500 c.c.	Amount of Complement Serum 1:10 c.c.	Amount of 0.9% NaCl c.c.	Amount of 10% Sheep Red Blood Cells c.c.	Hemolysis
1	0.1	1.0	0.9	0.5	Trace
2	0.15	1.0	0.85	0.5	Slight
3	0.20	1.0	0.80	0.5	Slight
4	0.25	1.0	0.75	0.5	Slight
5	0.30	1.0	0.70	0.5	Partial
6	0.35	1.0	0.65	0.5	Partial
7	0.40	1.0	0.60	0.5	Almost complete
8	0.45	1.0	0.55	0.5	Complete
9	0.50	1.0	0.50	0.5	Complete
10	-	1.0	1.0	0.5	None
11	-	1.0*	1.0	0.5	Trace
12	-	-	2.0	0.5	None

37° Incubated one hour.

In titrating the complement serum, the hemolytic dose of amboceptor serum was doubled and kept constant, while the amount of complement serum was progressively increased. The method is illustrated by the data of Table III.

(Insert Table III)

The minimum hemolytic dose of complement (which will be referred to hereafter as the M.H.D. of complement) is thus 0.3 c.c. of a 1:10 dilution, or 0.03 c.c. of the undiluted guinea-pig serum.

In testing the effect of cephalin on hemolysis by amboceptor and complement, a 0.1% solution of cephalin in 0.9% NaCl was added in increasing amounts to tubes containing the amboceptor serum, the complement serum, and the saline diluent. The tubes were shaken well after adding the cephalin solution, then were allowed to stand, generally at room temperature for from 10 to 15 minutes. The dose of sheep red blood cells was added to each tube, they were then incubated at 37° C. and the results were observed after one hour.

Two special controls were always included: the first, in which amboceptor serum and complement serum, in the absence of cephalin, acted on the sheep red blood cells; the second, in which cephalin solution alone acted on the red blood cells. The first control shows that in the absence of cephalin hemolysis occurs, while the second control showed that cephalin does not hemolyze the red blood cells.

An illustration of the method is given below in Table IV.

Table III.

Titration of Complement Serum.

Tube No.	Amboceptor Serum 2 units c.c.	Amount of Complement Serum 1:10 c.c.	Amount of 0.9% NaCl c.c.	Amount of 10% Sheep Red Blood Cells c.c.	Hemolysis
1	0.9	0.1	1.0	0.5	Partial
2	0.9	0.2	0.9	0.5	Almost complete
3	0.9	0.3	0.8		Complete
4	0.9	0.4	0.7		Complete
5	0.9	0.5	0.6		Complete
6	0.9	0.6	0.5		Complete
7	0.9	0.7	0.4		Complete
8	0.9	0.8	0.3		Complete
9	0.9	0.9	0.2		Complete
10	-	-	2.0		None

37° C. Incubated for one hour.

Table IV.

The Effect of Cephalin on Hemolysis by Amboceptor and Complement.

No.	Amboceptor 2 units c.c.	M. H. D. of Complement c.c.	Amount of 0.1% Cephalin c.c.	Amount of 0.9% NaCl	Amount of 10% Sheep Red Blood Cells	Hemolysis
1	0.9	0.3	-	0.8	0.5	Complete
2	0.9	0.3	0.1	0.7	0.5	Partial
3	0.9	0.3	0.2	0.6	0.5	Slight
4	0.9	0.3	0.3	0.5	0.5	None
5	0.9	0.3	0.4	0.4	0.5	None
6	0.9	0.3	0.5	0.3	0.5	None
7	0.9	0.3	0.6	0.2	0.5	None
8	0.9	0.3	0.7	0.1	0.5	None
9	0.9	0.3	0.8	-	0.5	None
10	-	-	-	2.0	0.5	None
11	-	-	-	1.0	0.5	None
12	-	1.0	1.0	.10	0.5	None

All the components of the reactions mixtures except the red blood cells were added to the tubes. They were then incubated at room temperature for 10 minutes, after which the red blood cells were added. The results were observed after one hour incubation at 37°.

As Table IV shows when the amboceptor and complement were quantitatively adjusted to each other, the addition of 0.3 c.c. of a 0.1% solution of cephalin entirely prevented hemolysis, after an hour's incubation. This represents,when diluted by the reaction mixture, an activity of cephalin in a final dilution of approximately 1:26,000.

The preparation of cephalin used above had the following chemical characteristics:

N = 1.83%

P = 3.99%

N:P ratio = 1:2.18

Iodine No. = 45

Tests for cholesterol : negative.

This preparation of cephalin will be in the future referred to as "Preparation A".

Different cephalin preparations, amboceptor sera, and complement sera were tested to determine the minimum amount of 0.1% cephalin solution, in 0.9% NaCl, that would entirely prevent hemolysis after an hour's incubation at 37° C. in systems in which amboceptor and complement were adjusted by previous titrations to each other. The data is given below in Table V.

Table V.

The Minimum Amount of Cephalin Required to Prevent Hemolysis.

Titre of Amboceptor Serum	M. H. D. of Complement Serum c.c.	Incubation Time	Minimum amount of 0.1% Cephalin c.c.	Remarks
1: 1428	0.02	10'	0.12	
1: 1428	0.05	15'	0.30	
1: 1428	0.04	18'	0.20	The cephalin reduced oxyhemoglobin
1: 3300	0.02	16' (38° C.)	0.15	
1: 6666	0.03	15' (38° C.)	0.05	The cephalin reduced oxyhemoglobin
1: 6666	0.03	15'	0.40	
1: 1111		10'	0.30	Prep. A of cephalin. Oxyhemoglobin not reduced
-		10'	0.15	
-		12'	0.10	
1: 1111		11' (36.5°C.)	0.20	Preparation A of cephalin

The average of the minimum amounts of 0.1% cephalin solution required to completely prevent hemolysis is 0.2 0.02 c.c. This represents a final dilution of 1:100,000 g. of cephalin, which completely prevented hemolysis under the conditions described above.

It is certain that cephalin prevents hemolysis in this system. The question as to how cephalin acts, whether on amboceptor or on complement, will now be taken up.

The Nature of the Anti-hemolytic Action of Cephalin.

Obviously, the ideal way to determine how cephalin acts in preventing hemolysis would be to test it in a "pure" system of three components, red blood cells, amboceptor, and complement. If cephalin acts on amboceptor, then by increasing the amount of amboceptor (meanwhile keeping the doses of cephalin, red blood cells, and complement constant), amboceptor in excess of the amount bound by cephalin, should combine with the red blood cells and complement, and cause hemolysis. The same argument applies if cephalin combines with complement.

Due to the lability of complement it has not yet been isolated, so instead of a pure solution of complement, complement must be used in the form of a mixture, serum. Tho it is possible to obtain amboceptor in a relatively high degree of purity, it was also used in the form of the mixture, serum. Applying the above method of experimentation, the results obtained will not be conclusive, but will be at most an inference

of a high degree of probability, since the serum contains at least one other antihemolytic substance, namely cholesterol.

Amboceptor and complement sera were quantitatively adjusted to each other. The titre of the immune serum was 1:1111; the M. H. D. of complement was 0.03 c.c. Then the dose of cephalin just sufficient to prevent entirely hemolysis in such a system, after one hour's incubation at 36° C., was found to be 0.2 c.c. of a 0.1% saline solution.

A new rack of tubes was now set up in which the amounts of red blood cells, of complement, and of cephalin were kept constant, but in which the amount of amboceptor was progressively increased. The results are given in Table VI.

(Insert Table VI)

The amboceptor, NaCl solution, complement serum, and cephalin were added to each tube, then they were incubated 10 minutes at 36.5° C. The sheep red blood cells were then added, and the tubes were reincubated for one hour.

Hemolysis was completed in Tube No. 9 in 15 minutes, but in Tube No. 8 there was no hemolysis at the end of one hour, and only slight hemolysis at the end of 14 hours, No hemolysis had occurred at the end of 14 hours in Tube No. 10.

The fact that there is no hemolysis when the amboceptor is increased is evidence for the view that cephalin acts either on the red blood cells or on complement. It must be remembered that cephalin was present in the above mixtures, not in excess,

Table VI.

The Effect of Increasing Amboceptor in the Presence of Cephalin.

No.	Amboceptor	Complement	Cephalin 0.1 %	NaCl 0.9%	Sheep Cells S 10 %	Hemolysis
1.	0.9 c.c.	0.3 c.c.	------	0.8 c.c.	0.5 c.c.	Complete
2.	"	"	0.2 c.c.	0.6	"	None
3.	1.0.	"	"	0.5	"	"
4.	1.1	"	"	0.4	"	"
5.	1.2	"	"	0.3	"	"
6.	1.3	"	"	0.2	"	"
7.	1.4	"	"	0.1	"	"
8.	1.5	"	"	---	"	"
9.	1.5	"	-----	0.2	"	Complete
10.	----	"	-----	1.7	"	None
11.	----	"	1.0	1.0	"	None
12.	----	"	--------	2.0	"	

36.5° C Incubated for one hour.

but in an amount just sufficient (in Tube No. 2) to completely prevent hemolysis.

The effect of increasing complement, while amboceptor, cephalin and red blood cells were kept constant was next noted. The reagents in the previous experiment were used in this experiment. The technique also was similar: all the components of the reaction mixtures except the red blood cells, were mixed and incubated 10 minutes at 36.5°. Then the sheep red blood cells were added, and the tubes were incubated at 32° C. for one hour. The results are given in Table VII.

(Insert Table VII)

These results show that as the complement increases, the effect of the cephalin is neutralized; the excess complement is then free to combine with amboceptor and so "complete" hemolysis.

The thromboplastic action of cephalin is not affected by hydrogenation, as Kuwashima (25) showed. It is of importance to learn whether breaking the double bonds of the unsaturated fatty acids of cephalin affect its property of acting as anti-complement.

THE EFFECT OF OXIDATION ON THE ANTICOMPLEMENTARY ACTION OF CEPHALIN.

There are two lines of evidence which indicate that oxidation of its unsaturated fatty acids does not destroy the anticomplementary action of cephalin. The first is that

iodized cephalin prevents hemolysis by amboceptor and complement. The second line of evidence is that cephalin which has an iodine number of 45, and which shows little or no ability to transform oxy- to met-hemoglobin, prevents hemolysis just as well as cephalin which vigorously changed oxyhemoglobin, as seen by reference to Table IV.

As stated before the iodine number of Preparation A was found to be 45. In this determination, the chloroform layer containing the iodized cephalin was allowed to stand for 24 hours, then it was separated from the aqueous layer, dissolved in ether, and precipitated with absolute alcohol. The weight of this precipitate was 0.0434 g., which is 7.06% of the total amount - 0.6141 g.- of iodized cephalin removed

~~(Insert Table VII)~~

from the chloroform layer. The precipitate was emulsified in 50 c.c. of 0.9% NaCl, giving an 0.086% solution.

Tested for its anticomplementary action, using the usual technique it was found that 1.0 c.c. of the cephalin solution prevented hemolysis.

After filtering off the precipitate produced by the addition of alcohol to the chloroform layer, a portion of the filtrate was copiously diluted with distilled water. No precipitate was obtained tho the solution became opalescent. The solution was evaporated to dryness on the steam bath. The residue was taken up with ether, evaporated in vacuo and

Table VII.

The Effect of Increasing Complement in the Presence of Cephalin

No.	Vol. of 1:500 Amboceptor	Vol. of 1:10 Complement	Vol. of 0.1% Cephalin	Vol. of 0.9% NaCl	Vol. of 10% Red Cells	Hemolysis
1	0.9 c.c.	0.3 c.c.	-	0.8 c.c.	0.5 c.c.	Complete
2	0.9 "	0.3 "	0.2 c.c.	0.6 "	0.5 "	None
3	0.9 "	0.4 "	0.2 "	0.5 "	0.5 "	"
4	0.9 "	0.5 "	0.2 "	0.4 "	0.5 "	"
5	0.9 "	0.6 "	0.2 "	0.3 "	0.5 "	Slight
6	0.9 "	0.7 "	0.2 "	0.2 "	0.5 "	Almost complete
7	0.9 "	0.8 "	0.2 "	0.1 "	0.5 "	Complete
8	0.9 "	0.9 "	0.2 "	-	0.5 "	"
9	-	0.9 "	-	0.2 "	0.5 "	"
10	-	0.9 "	-	1.1 "	0.5 "	None
11	-	-.	-	2.0 "	0.5 "	None

weighed. The residue, weighing 7.1 mg. was emulsified in 7 c.c. of 0.9% NaCl making a 0.1% solution.

At the end of an hour, 0.15 c.c. of this solution had completely prevented hemolysis in a system in which the amboceptor and complement had been previously adjusted to each other.

Regarding the second line of evidence showing that the degree of unsaturation of its fatty acids is not related to the anticomplementary action of cephalin, it must be said that the evidence so far obtained is inferential. Some cephalin preparations change the color of oxyhemoglobin, whether it is in solution or whether it is present in the red blood cells from red to brown; other preparations do not. The correlation between the iddine number of a cephalin preparation and its ability to transform oxyhemoglobin has not yet been worked out. But it has been found, as stated before that cephalin preparations even tho they do not affect oxyhemoglobin, are very active as anticomplement.

That the color change from red to brown, produced by adding cephalin to oxyhemoglobin, consists in the transformation of oxyhemoglobin to methemoglobin was proved by the spectroscopic examination of the brown solution. The instrument used was a spectrophotometer (Adam Hilger, London); the thickness of the cell, containing the solution, was 1 cm.

Four bands were found: their positions were as follows:

	Positions of Bands			
Determination No.	1	2	3	4
1	632	579	539	500
2	630	575	542	490
3			540	510
Average	631	577	540	500

The control to which no cephalin has been added was a bright red color. Two bands were found whose positions were:

	Position of Band	
Determination No.	1	2
1	578	546
2	578	542
3	578	541
Average	578	543

The rapidity with which the oxyhemoglobin is changed by active cephalin is shown by the following experiment. An excess of amboceptor and complement were used. The amboceptor serum, whose titre was 1:1200, was used in a dilution of 1:400. The guinea-pig serum used was 24 hours old. The effect of the cephalin on this system is shown in Table VIII.

Table VIII.

The Effect of Cephalin on Hemolysis by Amboceptor and Complement.

No.	Amboceptor 1:400	Complement 1:10	Cephalin 0.1%	NaCl 0.9%	Distilled H_2O	Sheep Red Cells 5%	Hemolysis
	c.c.		c.c.	c.c.		c.c.	
1	1.0	1.0	0.5	-	-	0.5	Partial
2	1.0	1.0	-	0.5	-	0.5	Complete
3	-	1.0	0.5	1.0	-	0.5	None
4	1.0	-	0.5	1.0	-	0.5	None
5	-	-	0.5	-	2.0	0.5	Complete

The tubes were read after one hour's incubation at 38° C. Within seven minutes after adding the red blood cells to the distilled water, a marked fading from red to brown had occurred. Within four minutes from the time of adding the red blood cells to Tubes 3 and 4 there was a marked contrast of color between them: No. 3 was red and No. 4 was a muddy brown, while in Tubes No. 3 and 4, the cells had completely settled to the bottom; in No. 3 the sediment was red, while in No. 4 the sediment was brown.

One may infer that the absence of color change in Tube 3 was due to the combination of cephalin with one or more of the proteins of the guinea-pig serum. This use of oxyhemoglobin as an indicator may prove of service in a study of the nature and extent of the combination of cephalin with serum proteins.

According to Howell (16), Waksman (18), and McLean (19), cephalin in aqueous solution gradually loses it thromboplastic activity on standing. It lacks then its characteristic property of accelerating the clotting time of recalcified plasma, or the clotting time of plasma and fresh serum.

It is of interest to inquire whether the anticomplementary property of cephalin is lost when cephalin is stored in solution, and whether when the thromboplastic action is lost, the anti-complementary property is still present.

THE EFFECT OF STORAGE ON THE ANTICOMPLEMENTARY PROPERTY OF CEPHALIN.

The effect of storage of a saline solution of cephalin was first investigated. A 0.1% solution of cephalin in 0.9% NaCl was prepared February 3, 1927. The cephalin was from preparation A. The solution was stored in a stoppered bottle in the ice-box and was tested at intervals. The technique in all cases was that described on page . The data obtained is summarized in Table IX.

(Insert Table IX)

As the above data shows, the property of preventing hemolysis is fully retained after standing in the form of a saline solution, for two months and a half. The variations, from 0.1 to 0.3 c.c. are within the limit of experimental error.

The next question is, does this cephalin still show any thromboplastic activity? May the anticomplementary property of cephalin be present, unchanged, after the thromboplastic activity of cephalin has disappeared?

The cephalin was tested first, for its property of accelerating clotting time of recalcified plasma and second, for its property of accelerating the clotting time of plasma by serum.

The citrated horse plasma had been kept stored in the ice-box for a week. The serum was obtained by clotting 50 c.c. of the above plasma with 10 c.c. of 1% $CaCl_2$; the clotted plasma was stored in the ice-box; 24 hours later the serum was expressed

Table IX.

The Effect of Storage on the Anticomplementary Activity of Cephalin, Dissolved in 0.9% NaCl.

Date of Titration	Time of Incubation	Amount of Cephalin required to prevent hemolysis
2/3/27	10'	0.15 c.c.
2/13/27	12'	0.10 c.c.
4/7/27	10'	0.30 c.c.
4/19/27	11'	0.20 c.c.

and used. The activity of the cephalin is shown in Table X.

(Insert Table X)

Table X shows that the cephalin, in this emulsion, two and a half months old, is not only very active as anticomplement, but as an accelerator of clotting. It is interesting to note that in the case of Tube 5, the cephalin was added to the plasma, then about a minute later the serum was added. Under these conditions the cephalin failed to accelerate clotting. But in Tubes No. 7 and 8, the cephalin was added to the samples of serum, incubated about a minute, then the cephalinized serum was added to the plasmas.

A 0.1% solution of cephalin (from preparation A) was made using distilled water as the solvent. In strong contrast to the turbidity of a saline solution of cephalin, cephalin in distilled water gives a clear, transparent solution. Its thromboplastic activity was tested at once, using citrated horse plasma (this plasma had been standing in the ice-box 3 days) and fresh horse serum, derived from the above plasma by recalcification. The results obtained are given below.

(Insert Table XI)

After standing twenty-three days in the ice-box, the solution of cephalin in distilled water was again tested, using citrated horse plasma and serum derived by recalcifying that plasma. The results are given in Table XII.

Table X.

The Thromboplastic Activity of Cephalin.

Tube No.	Plasma c.c.	Serum c.c.	0.1% cephalin in 0.9% NaCl	0.9% NaCl	1% $CaCl_2$	Clotting Time
1	1.0	-	-	0.8	0.2	2' 0"
2	1.0	-	0.1 c.c.	0.7	0.2	1' 30"
3	1.0	-	0.2 "	0.6	0.2	1' 15"
4	1.0	0.8	-	0.2	-	2' 30"
5	1.0	0.8	0.2 "	-	-	2' 30"
6	1.0	0.8	-	0.2	-	2' 30"
7	1.0	0.8	0.2 "	-	-	0' 30"
8	1.0	0.8	0.2 "	-	-	0' 20"

Table XI.

The Thromboplastic Activity of a Freshly prepared Cephalin Solution, dissolved in distilled water.

Tube No.	Citrated Horse Plasma	Horse Serum	$CaCl_2$ 1%	NaCl 0.9%	Cephalin 0.1%	Clotting Time
	c.c.	c.c.	c.c.	c.c.	c.c.	
1	1.0	-	0.2	0.8	-	3' 30"
2	1.0	-	0.2	0.6	0.2	2' 0"
3	1.0	0.8	-	0.2	-	1' 30"
4	1.0	0.8	-	-	0.2	30"
5	1.0	0.8	-	0.2	-	1' 45"
6	1.0	0.8	-	-	0.2	37"

Table XII.

The Thromboplastic Activity of an Aqueous Solution of Cephalin, After 23 Days Storage.

Tube	Citrated Horse Plasma	Horse Serum	1% $CaCl_2$	0.1% Cephalin in dist. H_2O	0.9% NaCl	Clotting Time	Remarks
	c.c.	c.c.	c.c.		c.c.		
1	1.0	-	0.2	-	0.8	3' 18"	Three Determinations
2	1.0	-	0.2	0.2	0.6	3' 15"	Two Determinations
3	1.0	0.8	0.2	0.2	-	22"	
4	1.0	-	-	-	0.2	27"	

Storage for 23 days then resulted in the complete loss of thromboplastic activity of the cephalin dissolved in distilled water. The cephalin solution was not sterile, for on microscopic examination, bacteria were noticed in the solution. Nevertheless this solution still retained the property of preventing hemolysis, as seen in Table XIII (the cephalin solution was mixed with an equal volume of 1.8% NaCl to make it isotonic).

(Insert Table XIII)

As usual, the cephalin, immune serum, complement serum and 0.9% NaCl were incubated at room temperature for 10 minutes, then the red blood cells were added, 0.8 c.c. of 0.05%, or 0.4 c.c. of 0.1% cephalin were sufficient to prevent all but a trace of hemolysis at the end of an hour's incubation.

It remains to be worked out whether cephalin will lose its thromboplastic activity when stored in aqueous solution under sterile conditions. It will be interesting also to determine how long the anticomplementary action of cephalin persists when cephalin is dissolved in sterile distilled water. Why does the anticomplementary action of cephalin remain after the thromboplastic action had disappeared? That result may mean that the anticomplementary action of cephalin is due to one part of the cephalin molecule, while the thromboplastic action is due to another part of the molecule, which is altered by standing in distilled water. On the other hand, the anticomplementary

Table XIII.

The Anticomplementary Action of Cephalin, stored 23 days in aqueous Solution.

No.	Amboceptor 1:500	Complement	NaCl 0.9 %	Cephalin 0.05 %	Sheep Cells 10 %	Hemolysis
1.	1.0 c.c.	0.1 c.c.	0.9 c.c.	----	0.5 c.c.	Complete
2.	"	"	0.5	0.4 c.c.	"	"
3.	"	"	0.4	0.5	"	Almost Complete
4.	"	"	0.3	0.6	"	Partial
5.	"	"	0.2	0.7	"	Slight
6.	"	"	0.1	0.8	"	Trace
7.	--"--	--- ------	2.0	-----	"	None

36° C. Incubated for one hour.

action of cephalin may be due not to cephalin, but to an impurity which is unaffected by storage in distilled water.

The sera of horses, mules and asses not only do not act to complete the hemolytic system investigated here, but they prevent hemolysis when guinea-pig serum is used as complement. Globulins as ordinarily prepared are not pure proteins, but are combined with cholesterol and phospholipins, such as cephalin. The antilytic action of horse globulin then may not be due to the protein fraction but to the splitting off of cholesterol or cephalin which then acts upon complement. Since this antilytic action is exerted also by horse plasma as well as horse serum, and since fibrinogen is a globulin, it is of interest to know whether horse fibrinogen can prevent hemolysis.

There is a further reason why it is important to learn the effect of horse fibrinogen on hemolysis. If there is any component of thrombin which is identical with one of the components of complement, then it should follow that when guinea-pig serum is clotted by fibrinogen, the serum should show an absence or at least a marked decrease of complement.

In the next section, the effect of fibrinogen on hemolysis is discussed.

THE EFFECT OF FIBRINOGEN ON HEMOLYSIS BY AMBOCEPTOR AND COMPLEMENT.

Amboceptor and complement were by previous titrations adjusted to each other. The titre of the immune serum was 1:1250; the M. H. D. of complement was 0.03 c.c. The complement serum was from three male guinea-pigs and was two days old. The fibrinogen solution was also two days old: it had been prepared by adding NaCl to the point of half-saturation to 100 c.c. of citrated mule plasma that had previously been centrifuged an hour and a half. The fibrinogen precipitated out was washed with 250 c.c. of saturated NaCl solution, then was dissolved in 100 c.c. of 0.9% NaCl. Its activity at the time of this experiment (2 days later) is shown below:

1.0 c.c. of fibrinogen solution + 0.05 c.c. of 1% $CaCl_2$ + 0.05 c.c. of 0.9% NaCl - No clot.

1.0 c.c. of fibrinogen solution + 0.05 c.c. of 0.1% cephalin solution (Preparation A) + 0.05 c.c. of 1% $CaCl_2$ - Clot in 2' 30".

2.0 c.c. of fibrinogen solution + 2.0 c.c. undiluted complement serum-sliding clot in 2 hours.

2.0 c.c. of fibrinogen solution and 20 c.c. of the undiluted complement serum were mixed, and increasing amounts of the mixture were added to the two units of hemolysin and 0.5 c.c. of 5% sheep red blood cells.

The tubes were incubated 1 hour at 37° C. In the first 5 tubes, the complement is in a dilution of 1:2; in the next 5 tubes the complement was diluted 1:10; that is 1:0 c.c.

of the mixture of complement and fibrinogen solution was diluted with 4.0 c.c. of 0.9% NaCl. The results are given in Table XIV.

(Insert Table XIV)

These results show that (1) the fibrinogen was capable of being clotted; (2) that the guinea-pig serum, two days old, and in a dilution of 1:5 had thrombin activity as well as complement activity and (3) that the fibrinogen under these experimental conditions did not interfere with hemolysis. These results were confirmed in other experiments in which preparations of horse fibrinogen were used.

That the fibrinogen of horse or mule does not affect hemolysis is further shown by the following experiments. Two preparations of fibrinogen were made from centrifuged oxalated horse plasma by salting out with NaCl. The one preparation was redissolved and used after the first precipitation, the second preparation was further purified by reprecipitation with NaCl. Three c.c. of each fibrinogen solution were treated with 3 c.c. of guinea-pig serum (24 hours old); as a control, 3 c.c. of 0.9% NaCl were also treated with 3 c.c. of complement serum. The three tubes were then stored in the ice-box.

Examined the next day, fibrin networks were seen in the tubes containing fibrinogen. These were carefully lifted out with a stirring rod in such a way, that as little as possible of the sera were removed with them.

Table XIV

The Effect of Fibrinogen on Complement Activity

No.	Vol of 1:500 Amboceptor	Complement Fibrinogen	Vol. of 0.9% NaCl	Vol. of 5% Sheep Cells	Hemolysis	
1	0.7cc	0.1cc	0.7cc	0.5cc	Complete;	Fluid
2	"	0.2	0.6	"	"	"
3	"	0.3	0.5	"	"	"
4	"	0.4	0.4	"	"	"
5	"	0.5	0.3	"	"	Gelled
6	"	0.3	0.5	"	"	"
7	"	0.4	0.4	"	"	Fluid
8	"	0.5	0.3	"	"	"
9	"	0.6	0.2	"	"	"
10	"	0.7	0.1	"	"	"
11	-	0.7	0.8	"	±	"
12	-	-	2.0	"	-	

N. B. In. Tube Nos. 4 and 5, hemolysis had occured before clotting.

Each lot, including the control, was then titrated to determine the M. H. D. of complement. Using 0.5 c.c. of 5% sheep red blood cells and 2 units of amboceptor, the M. H. D. of complement in the control and in the complement sera treated with fibrinogen was 0.03 c.c. of undiluted complement serum. Thus clotting which removed thrombin from the guinea-pig serum, did not seem under these experimental conditions to affect its complement activity.

It is well known that complement can by methods which precipitate the globulin of sera, such as dialysis or dilution and acidification, be separated into two parts. The part carried down by the globulin precipitate is called "mid piece" at the suggestion of Brand (21), while the part that stays in solution with the albumin is called "end piece". Neither portion when added to amboceptor and red blood cells will complete hemolysis, but a mixture of "mid-piece" and "end-piece" is actively complementary for amboceptor and red blood cells. The terms "mid-piece" and "end-piece" refer to the fact that sensitized red blood cells (cells that is, in combination with amboceptor) will combine with the globulin, but not the albumin fraction of the serum: the latter fraction, combines with the sensitized cells only after the globulin fraction has been bound.

In view of Howell's statement (17) that the isoelectric of thrombin is on the acid side of neutrality and in view of the possible identity of complement and thrombin it is interesting to inquire, when guinea-pig serum has been split into globulin

and albumin fractions, whether thrombin, like complement, is split into two parts, or whether it is contained in one fraction only.

The Thrombin Activity of "Mid-piece", a Component of Complement.

Complement serum, 8 hours old, from the heart blood of 2 male guinea-pigs, was used. It was fractionated according to the method of Fränkel (26); 1.9 c.c. of serum was diluted to 19 c.c. with distilled water and CO_2 was passed thru the diluted serum for 10 minutes. It was allowed to stand for 27 minutes, during which time a flocculent precipitate settled out, then was centrifuged for 10 minutes. The volume of the packed precipitate was 0.1 c.c. The supernatant liquid was decanted off, and the sediment was stirred up in 2 c.c. of 0.9% NaCl. This solution and the albumin fraction of the original serum were tested for thrombin activity with uncentrifuged citrated horse plasma.

(Insert Table XV)

As Table XV shows, thrombin activity, was present only in the "mid-piece fraction; in other words, thrombin unlike complement is not split into two fractions, but is concentrated in the globulin precipitate.

This result however cannot be regarded as invariable, since only one experiment of this kind was performed. If it is established that the globulin fraction of serum always

Table XV.

No.	Volume of Plasma	Soln. of Precipitate	Albumin Fraction	Untreated Comp. Serum 1:10	0.9% NaCl	Result
	c.c.		c.c.		c.c.	
1	0.5	-	0.5	-	1.5	No clot over night
2	0.5	-	1.0	-	1.0	" " " "
3	0.5	-	1.5	-	0.5	" " " "
4	0.5	-	2.0	-	-	" " " "
5	0.5	-	-	-	2.0	" " " "
6	1.0	1.0	1.5	-	-	No clot
7	0.5	0.5	-	-	-	Clot over night
8	0.5	1.0	-	-	-	" " "

contains thrombin, the result is of great interest in connection with the experiments of Bordet (4) who found that "mid piece" - that is, the globulin fraction, of guinea-pig serum - when injected intravenously into a guinea-pig, usually killed it within five minutes. The toxicity of this faction was not destroyed by heating at 60° C. for half an hour, but is destroyed by heating to 70° C. for half an hour. Intraperitoneal or subcutaneous injections were harmless. In some non-fatal cases the coagulation time of the blood was greatly prolonged, insome cases as long as 24 hours. McKinley (20) who had previously worked with Bordet on this phenomenon, later found that death could be always secured following injection if a small amount of lipoid mixture (obtained from Wasserman antigen and undoubtedly containing some cephalin) were added to the globulin fraction.

That the globulin fraction causes death by intravascular clotting is an explanation of the phenomenon that has not hitherto been offered. In view of the fact that thrombin is thrown down with the globulin precipitates, and that death always occurs when lipoid is added (evidently an increase of thrombin concentration due to the action of cephalin) this explanation seems probable. If this explanation is correct, it will provide further evidence for the contention of Mills and Mathews (6) that thrombin is capable of intravascular clotting and is hence a true physiological mechanism.

Discussion.

Bordet (3) in a recent paper has given a very stimulating discussion of the theory of blood-coagulation. Mills and Mathews (6), adopting his theory, regard thrombin clotting as a physiological mechanism paralleling the clotting of blood by tissue juice, which Wooldridge (27) claimed was the only physiological process. Blood-clotting according to them is a resultant due to the effect of substances in the tissues and in the blood stream on fibrinogen. They have presented this synthesis of the ideas of Bordet and Wooldridge in a diagram which is reproduced on the following page.

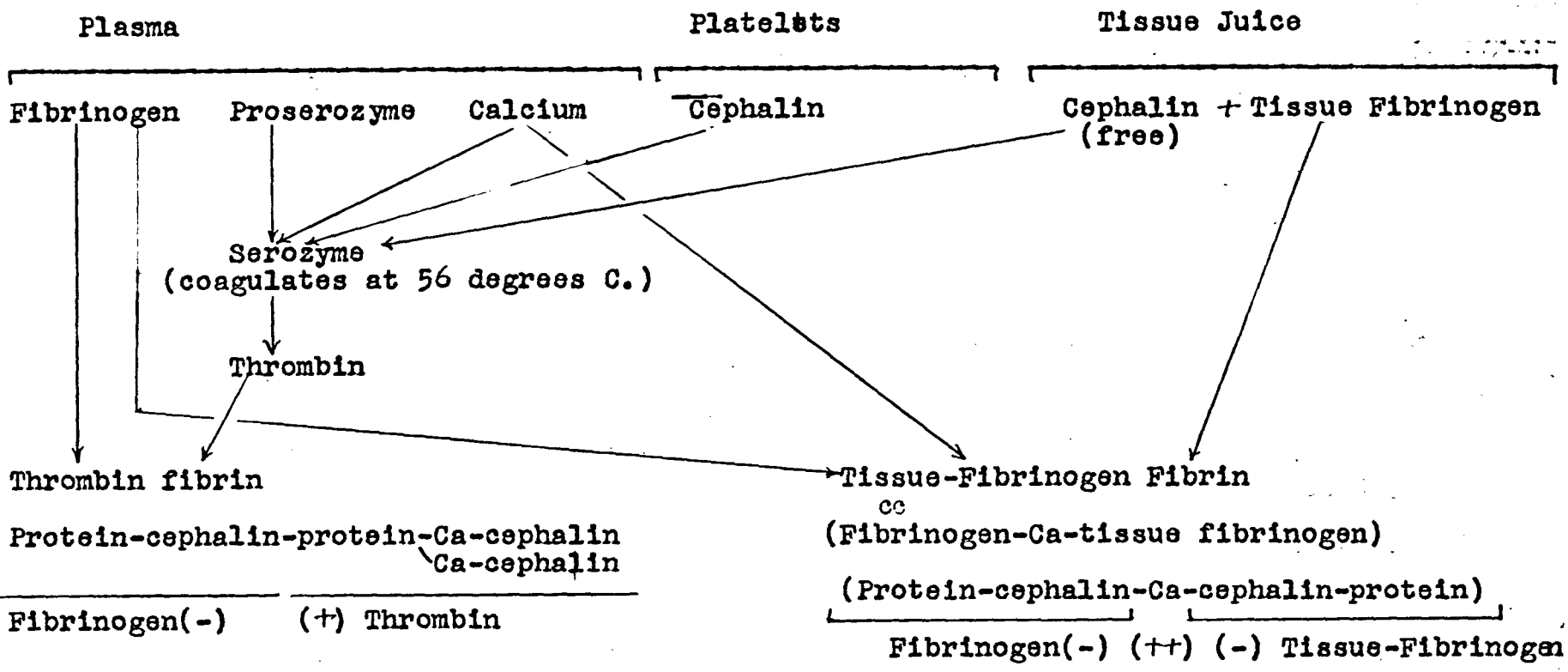
According to this diagram, there are two kinds of blood clotting, thrombin clotting and tissue fibrinogen clotting. The former is due to the action of substances in the blood on fibrinogen; the latter is due to the action of substances in the tissues on fibrinogen. In thrombin clotting, thrombin unites with fibrinogen; as a result, gelatinous fibrin is formed.

However, in tissue fibrinogen clotting, a lipo-protein, tissue fibrinogen, coming from the tissues, unites with blood fibrinogen in the presence of ionic calcium, to form tissue fibrinogen fibrin.

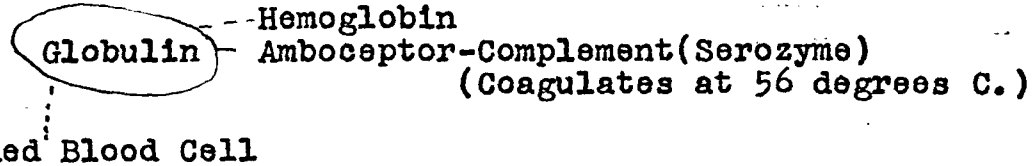
At the same time, free cephalin in the tissue juice, and cephalin derived from the disintegration of blood platelets, unite with ionic calcium and a protein, serozyme, to form thrombin, which then acts on fibrinogen.

The Two Physiological Mechanisms of Blood Clotting.

(After Mills and Mathews)



Hemolysis of the Sheep Red Blood Cell by Amboceptor and Complement.



Below this diagram of blood clotting, is a crude representation of hemolysis. According to the diagram, complement unites with the sheep red blood cell thru amboceptor (Ehrlich and Morgenroth). As far as the experimental evidence goes, complement could be pictured as uniting directly with the cell after the cell had been acted on by the specific hemolysin (Bordet).

It is known that red blood cell stroma in part consists of a globulin. It is probable that this globulin is chemically similar to blood fibrinogen. Since red blood cells contain cephalin, this protein may be regarded, like blood fibrinogen, as a protein-cephalin compound.

Since there is a constant ratio between the amount of hemoglobin in red blood cells and their surface area, it is probable that hemoglobin is united in an easily broken chemical union with the stroma at the surface, - perhaps with the eu-globulin constituent. When the cell is hemolysed, one may, for lack of a better hypothesis, picture hemoglobin as displaced from combination with the euglobulin, by amboceptor-complement. The hemoglobin diffuses into the surrounding medium, and the euglobulin is clotted.

How is this euglobulin, fibrinogen-like substance clotted? Remembering that calcium ions are as essential in hemolysis as in blood-clotting and that serozyme like complement is inactivated at 56°, and that cephalin is concerned in

hemolysis, as well as in blood-clotting, it might be argued that the clotting in hemolysis was due to thrombin acting in the presence of amboceptor, on the fibrinogen-like protein of the stroma.

But Burnett (28) found that thrombin does not act as complement. His experimental evidence however is open to serious criticism. The one "thrombin" preparation he tried was an 8% extract of fibrin, whose source was not given. The thrombin activity of this extract was not given. This crude extract very probably contained impurities which might more or less neutralize any complement activity of the thrombin present.

On the other hand, Bergel found that saline extracts of guinea-pig fibrin were hemolytic for rabbit red cells, and that extracts of rabbit fibrin were hemolytic for guinea-pig red cells. Thrombin, as a constituent of fibrin, might be a factor. However, the active principle of the extracts might be some constituent of cellular elements, such as the blood platelets, entangled as impurities in the fibrin.

Referring to the diagram, cephalin is a constituent of thrombin. Its additionto serum increases the concentration of thrombin, an inference supported experimentally by the fact that serum treated with cephalin, shows a sudden great increase in clotting, that is, thrombin, activity. Now if thrombin acts as complement, there should be a similar great increase in complement activity when serum is treated with cephalin.

As the data of this thesis show, when guinea-pig serum is treated with cephalin, the complement activity decreases

or disappears. That is, such serum when added to amboceptor serum and sheep red cells cause no diffusion of hemoglobin into the surrounding medium. But hemolysis also involves a clotting of the stroma. It may be that the addition of cephalin results in a clotting of the stroma, but under such conditions that the diffusion of hemoglobin is prevented.

Moreover, if thrombin were identical with complement, the addition of fibrinogen to complement serum should "fix" or deviate complement. But our experiments show that this is not the case.

Since complement is separated into two fractions by dilution and acidification of the serum thrombin should be similarly fractionated, if it is identical with complement. The evidence, as far as it goes, is against this idea. In guinea-pig serum, thrombin is concentrated in the globulin fraction. However not enough work has been done on this point to permit a definite conclusion.

The facts known at present are best explained by the following temporary working hypothesis. Serozyme and complement are identical, being a basic protein coagulating at 56°. When this protein combines with calcium ions and cephalin, thrombin is formed. In the absence of cephalin and in the presence of calcium ions, this basic protein is capable of acting thru the specific hemolysin on the red blood cells used to produce the hemolysin in such a way that the stroma of the cells clot and their hemoglobin diffuses into the surrounding medium.

This hypothesis is worthy of consideration for at least one reason: it is capable of direct experimental proof or disproof. If it is true the protein in red blood cells can be clotted in the presence of purified amboceptor and calcium ions by serozyme. With the right technique this fundamental point is capable of direct experimental attack.

As the hypothesis indicates, the basic protein may be inactivated in two ways: first by combination with cephalin, and second, by combination with a negatively charged protein. The presence of cephalin and negatively charged proteins in serozyme preparations, account in large part for past failures to demonstrate that complement and serozyme are identical.

Another reason, we believe, lies in the fact that the wrong plasma was used for the preparation of serozyme. The serozymes in various sera very probably are different proteins, just as are the fibrinogens of different plasmas. The chemical nature of horse serozyme may be unsuited for the demonstration of its complement activity in the hemolytic system used in these experiments. Horse serum when fresh possesses only a slight complement activity (which quickly disappears) as far as this hemolytic system is concerned. A serum such as guinea-pig , or hog serum, which has a powerful complement action in this system would prove perhaps much better material.

Cholesterol, as well as cephalin, prevents hemolysis. For this reason, the proof that cephalin acts as anticomplement

is conclusive, for it may be objected that cephalin displaces cholesterol from combination with serum proteins and that the cholesterol thus displaced, acts on complement. But that same objection is valid when cholesterol is added to complement serum: cholesterol may merely liberate cephalin which then combines with complement.

Whatever the mechanism of the action of cephalin, it is certain that it does prevent hemolysis and that in this, as in other ways, blood coagulation and hemolysis are related.

Summary.

Evidence has been presented to show that serozyme, a component of the blood clotting mechanism, is identical with complement. This evidence is admittedly incomplete and more work must be done to demonstrate their identity. The factors that at present make this domonstration difficult are discussed. Conclusive proof is given that cephalin (cholesterol-free), another factor in blood-clotting, prevents hemolysis by specific hemolysin and complement. Evidence is offered that cephalin acts as anticomplement. This constitutes an important link between the mechanism of blood clotting and the mechanism of hemolysis.

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