ACTIVATION OF PROFIBRINOLYSIN BY ANTIGEN-ANTIBODY REACTION AND BY ANAPHYLACTOID AGENTS; ITS RELATION TO COMPLEMENT*

By GEORGES UNGAR, M.D., EVELYN DAMGAARD, AND FRED P. HUMMEL

(From the Rheumatic Fever Research Institute, Northwestern University Medical School, Chicago)

(Received for publication, June 1, 1953)

It was shown previously that an antigen-antibody reaction induced in guinea pig tissues or serum causes the activation of the enzyme precursor profibrinolysin (1, 2). These observations were confirmed by Geiger (3) while von Euler and Heller (4) and Jemski, Flick, and Stinebring (5) were unable to duplicate them. The present paper reports further observations on protease activation both by antigen-antibody reaction and by "anaphylactoid" agents and attempts to explain the discrepancies between results published by different workers. Observations are also reported suggesting that profibrinolysin activation requires the intervention of a kinase system strikingly similar to serum complement.

Methods

Profibrinolysin Activation.—As described in detail previously (2), the enzyme precursor present in guinea pig serum is activated by mixing a certain amount of serum (usually 0.1 or 0.2 ml.) with the activating agent. After 3 minutes' contact, with constant shaking, the mixture is diluted twentyfold with distilled water and the pH adjusted to 5.2 with an 0.2 per cent solution of acetic acid. The precipitate formed is separated by centrifugation and redissolved in buffered saline (0.15 M phosphate in 0.15 M NaCl, pH 7.4). In the control samples, saline is added to serum and the mixture treated as described above.

Estimation of Fibrinolytic Activity.—The presence of active protease in the euglobulin fraction is detected by estimating fibrinolytic activity. The method used in previous studies (1, 2) was based on the digestion of fibrinogen and estimation of the remaining fibrinogen by determining its clotting time after addition of thrombin. The technique used in the present investigation is also based on the digestion of fibrinogen, but this is determined with greater accuracy by measuring the ultraviolet absorption (at $\lambda = 280$ mμ) of the fraction of fibrinogen rendered incoagulable by the action of fibrinolysin.

The technique is an adaptation of the procedure described by Kunitz (6) for determination of tryptic activity. The details have been given elsewhere (7) and only the main points will be mentioned here. The solution obtained after activation of 0.1 or 0.2 ml. of serum is made up to 3.7 ml. with buffered saline (pH 7.4), mixed with 2 ml. of a 0.2 per cent fibrinogen[1]

* This investigation was supported by the Medical Research and Development Board, Office of The Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-404.

[1] Bovine fibrinogen, The Armour Laboratories, Chicago.

solution and placed for 20 minutes in a water-bath at 37.5°C. After this interval, 0.3 ml. of a solution of thrombin[2] (containing 325 units per ml.) is added to clot the remaining fibrinogen. After further incubation of 30 minutes the solution is filtered and the filtrate read on the Beckman spectrophotometer. Readings are made against appropriate blanks to correct for the ultraviolet absorption of all the reagents.

The optical density readings can be converted into milligrams of fibrinogen hydrolyzed and these are, as seen in Table I, directly proportional to enzyme concentration. As routine, however, it seemed more appropriate to express the results in units defined according to Kunitz (6): one unit of fibrinolysin is the amount of enzyme which, under the conditions

TABLE I

Comparison between the Action of Bovine and Guinea Pig Fibrinolysins on Fibrinogen and Fibrin

		Amount digested	
		Fibrinogen	Fibrin
		mg.	*mg.*
Guinea pig euglobulin*	0.05 ml.	0.95	—
	0.1	1.8	—
	0.15	2.75	—
	0.2	3.25	0.35
	0.3	—	0.55
	0.5	—	0.85
Bovine fibrinolysin	0.1 mg.	0.75	—
	0.2	1.6	—
	0.3	2.45	—
	0.4	3.2	0.3
	0.5	—	0.4
	0.75	—	0.6
	1.0	—	0.75

Fibrinogen digestion was done according to the procedure described in the text. For fibrin digestion, fibrinogen was clotted with thrombin and incubated for 30 minutes before addition of the enzyme. In all samples the initial fibrinogen concentration was 4 mg.

* Serum from guinea pigs sensitized against rabbit serum was activated by the specific antigen.

just described, gives rise to an increase of one optical density unit per minute. The fibrinolysin content of the samples is more conveniently expressed in 10^{-3} units. These will be referred to in the text as "units."

The control samples (serum + saline) nearly always showed a certain amount of fibrinolytic activity (see Table II). This "spontaneous" activation seemed to occur when the blood sample had been contaminated with fluid from traumatized tissues or in sera which had been allowed to stand for several days before the experiment. Sera showing a spontaneous activity over 40 units per ml. were discarded for reasons which will be discussed below. In all tables the number of units listed as resulting from the activation of profibrinolysin represent the excess over the spontaneous activity of the corresponding controls.

The protease activity appearing in the sera submitted to various treatments is attributed

[2] Kindly supplied by Dr. E. C. Loomis, Research Laboratories, Parke, Davis & Co., Detroit.

in this study to the enzyme termed fibrinolysin or plasmin. These terms, used interchangeably, probably designate a mixture of enzymes. For example, Mullertz (8) described recently under the name of plasmin a protease which attacks fibrin but not fibrinogen. Table I shows that the protease described in the present report has a higher affinity for fibrinogen than for fibrin. It is also seen that its respective affinity for these substrates closely resembles that of a bovine fibrinolysin obtained by chloroform treatment of plasma (9).[2]

Immunological Techniques.—Guinea pigs were sensitized to egg albumin by a single subcutaneous injection of the antigen[3] (0.5 ml. of a 1 per cent solution). Sensitization against rabbit serum was induced by intracardial injection of 0.5 ml. The animals were bled after a stated interval by heart puncture. The guinea pig serum was used either immediately after separation or after a few days' storage at −20°C.

Antibody nitrogen was determined according to the technique described by Germuth *et al.* (10) using the ultraviolet absorption of the redissolved antigen-antibody complex. Fractionation of complement and complement titration were done according to the techniques described by Kabat and Mayer (11).

RESULTS

Profibrinolysin Activation by Antigen-Antibody Reaction.—In view of the contradictory results, mentioned above, published by certain workers (3–5) experiments were performed to determine the precise conditions required for profibrinolysin activation. Experiments similar to those previously reported (2) were repeated using a more accurate technique.

Each serum was divided into three samples, usually of 0.2 ml. each. Samples A and B were mixed with 0.2 ml. of saline and sample C with 0.2 ml. of a 0.2 or 0.5 per cent solution of egg albumin. After 3 minutes' contact, the mixtures were diluted to 8 ml. with distilled water and the pH adjusted to 5.2 as described above. The resulting euglobulin precipitate was centrifuged off and redissolved in 3.7 ml. buffered saline. 2 ml. of a 0.2 per cent solution of fibrinogen were added to samples B and C and the same volume of buffered saline was added to sample A. After 20 minutes' incubation at 37.5°C., 0.3 ml. of thrombin was added to all samples and after a further incubation of 30 minutes the solutions were filtered. The ultraviolet absorption of the filtrates was read at 280 mμ.

The reading of sample B against A, corrected by subtracting the absorption of a fibrinogen-thrombin blank, gives the spontaneous fibrinolytic activity. The reading of sample C against B, corrected by subtracting the absorption of an egg albumin blank (which had been treated by dilution and acidification like the serum samples), gives the fibrinolytic activity produced by the antigen-antibody reaction. Optical densities were converted into units according to the definition given in the preceding section.

Since under the conditions of the method, activities above 25 units cannot be measured in a given sample, most of the sera were also tested at 0.1 ml. In this case the amount of antigen added was reduced by half.

The results of these experiments are expressed in Table II in terms of 10^{-3} units of fibrinolytic activity per ml. of guinea pig serum. It is seen that spontaneous activation occurs in most sera (15.0 ± 11.0 units per ml. of serum) but under the influence of the antigen-antibody reaction fibrinolytic activity is significantly increased. Addition of 2 mg. of egg albumin to the homologous

[3] Soluble egg albumin, Merck & Co., Inc., Rahway, New Jersey.

TABLE II

Activation of Profibrinolysin in Guinea Pig Antisera by Addition of Homologous Antigen

Serum	Antigen	Fibrinolysin 10^{-3} U/ml. after addition of	
		Saline	Antigen
Anti-egg albumin	Egg albumin (2 mg./ml. serum)	21.00	132.50
" "	" " "	16.25	127.50
" "	" " "	20.00	137.50
" "	" " "	12.50	95.00
" "	" " "	17.00	129.75
" "	" " "	20.00	25.00
" "	" " "	13.00	125.00
" "	" " "	0	87.50*
" "	" " "	38.50	149.50‡
		17.6±10.1§	112.1±38.2§
Anti-egg albumin	Egg albumin (5 mg./ml. serum)	26.00	80.00
" "	" " "	20.50	92.50
" "	" " "	0	55.00*
" "	" " "	29.00	112.50‡
		18.9±12.8§	85.0±24.1§
Anti-rabbit serum	Rabbit serum (0.04 ml./ml. serum)‖	12.00	82.50
" "	" " "	0	95.00
" "	" " "	6.50	115.00
" "	" " "	22.50	102.50
" "	" " "	0	67.25
		8.2±9.4§	92.5±18.4§
Normal	Egg albumin (2 mg./ml. serum)	18.00	2.00
"	" " "	0	7.50
"	" " "	27.50	0
"	" " "	7.25	2.75
"	" " "	18.00	5.50
		14.15±10.7§	3.5±3.0§

* Pooled serum A (see text).

‡ Pooled serum B (see text).

§ Mean and standard deviation.

‖ Sensitized guinea pigs were bled 6 weeks after sensitization.

antisera produced 112.1 ± 38.2 units, while 5 mg. of the same antigen added to the same serum produced only 85.0 ± 24.1 units per ml. When another antigen, rabbit serum, was used, similar results were obtained (92.5 ± 18.4 units per ml.). When however, normal guinea pig sera were treated with egg albumin only an insignificant increase in proteolytic activity (3.5 ± 3.0 units per ml.) was observed.

As stated above, results obtained with sera showing high spontaneous activity (over 40 units per ml.) were discarded and are not listed in Table II. Such sera do not undergo further activation when treated with the antigen. Six samples with an average spontaneous activity of 85.5 ± 27.0 units showed an additional activity of 11.5 ± 10.5 units per ml. when mixed with 2 mg. per ml. of egg albumin. The reason for this may be either that all the available profibrinolysin of these sera has already been converted into fibrinolysin or, more probably, that free fibrinolysin destroys a kinase, as will be discussed below (12).

To show whether profibrinolysin activation was caused by an antigen-antibody reaction the following experiment was carried out.

Antibody concentration was determined in two pooled samples of guinea pig serum. Serum A was collected 4 weeks after sensitization and its antibody N was 0.03 mg. per ml. Serum B was collected from the same guinea pigs 1 week later; its antibody N was 0.083 mg. per ml. The antibody content of these sera was also tested by passive anaphylaxis. Four guinea pigs were injected with each of the serum samples (0.5 ml. given intracardially to guinea pigs with a mean weight of 300 gm.). 48 hours later, shock was elicited by intracardial injection of 0.5 ml. of a 1 per cent solution of egg albumin. With serum A, two guinea pigs showed signs of shock but none died. With serum B, three guinea pigs died and autopsy showed typical anaphylactic lung changes. It is seen in Table II that serum B produced higher proteolytic activity than serum A.

The results shown in Table II suggest that increased concentrations of antigen do not necessarily produce higher fibrinolytic activity. This was tested in a series of experiments in which a pooled serum sample from guinea pigs sensitized to egg albumin was mixed with varying amounts of the antigen. The results summarized in Table III show that profibrinolysin activation first increases with antigen concentration but, after having reached an optimum, declines, and with high enough concentrations falls to zero.

Profibrinolysin Activation by Anaphylactoid Agents.—It was shown previously that proteolytic activity can be induced in normal guinea pig sera by addition of peptone and certain high molecular weight carbohydrates (2). These substances have one common property: when injected into animals, they can reproduce some of the symptoms and tissue changes observed in anaphylaxis. The manifestations thus produced are often called "anaphylactoid" and the substances which cause them may be designated as anaphylactoid agents.

Peptone activation was repeated using the spectrophotometric technique for the estimation of fibrinolytic activity. Peptone[4] (20 mg. per ml. of serum) was added to fourteen individual guinea pig serum samples. No activation occurred in three sera but the remaining eleven showed high proteolytic activity. The mean value was 110.1 ± 66.9 units per ml. of serum.

A number of substances have been mentioned in recent literature as being able to produce anaphylactoid phenomena. Some of these are polymers, such as polyoxyethylene sorbitan monolaurate (tween 20) (13) and a mixture of the di-, tri- and tetramers of *p*-methoxyphenethyl-methylamine known as substance 48/80 (14). The same effect is exerted by certain alkaloids of opium (15) and curare (16). Even simple amines, like octylamine, decylamine (17), and octadecylamine (18) were shown to produce anaphylactoid manifestations.

TABLE III

Influence of Antigen Concentration on Profibrinolysin Activation

Egg albumin/ml. guinea pig serum	Fibrinolysin 10^{-3} U/ml. serum
mg./ml.	
0.1	32.5
0.5	87.5
1.0	145.00
2.5	152.50
5.0	147.50
10.0	107.50
25.0	57.50
50.0	0

Guinea pigs were bled 6 weeks after sensitizing injection.

Some of these agents were tested for profibrinolysin activation and the results of these tests are shown in Table IV. It is seen that tween 20,[5] morphine, octylamine,[6] octadecylamine[7] and 48/80,[8] all activate profibrinolysin to a varying extent. It should be noted that some of these agents, as in the case of antigen, also cause less activation at concentrations above the optimum level.

Serofibrinokinase and Complement.—In a previous report attention was called to the fact that activation of profibrinolysin by antigen-antibody reaction or anaphylactoid agents does not seem to result from the direct action of these activators on the enzyme precursor (2). It was observed that addition of peptone to partially purified profibrinolysin fails to bring about activation. It was also reported that serum previously heated to 56°C. for

[4] Neopeptone, Difco Laboratories, Detroit.

[5] Batch 225 B, by courtesy of Atlas Powder Co.

[6] 2-Amino-n-octane, Eastman Kodak Co., Rochester, New York.

[7] Kindly supplied by Dr. F. C. McIntire, Abbott Laboratories, North Chicago.

[8] Kindly supplied by Dr. F. C. MacIntosh, McGill University, Montreal.

TABLE IV

Profibrinolysin Activation in Guinea Pig Serum by Anaphylactoid Agents

Agent/ml. serum		Fibrinolysin 10^{-3} U/ml. serum
Tween 20	0.2 μl.	8.30
	0.6	76.50
	0.8	120.00
	"	100.25
	"	85.00
	"	55.00
	"	90.00
	"	112.50
	"	50.25
	"	125.50
	"	92.50
Morphine sulfate	0.75 mg.	87.50
	1.1	93.50
	1.3	92.75
	1.5	97.50
	1.75	51.25
	2.2	35.00
	3.0	35.00
Octylamine	0.25 mg.	105.50
	"	97.25
	"	85.25
	"	115.50
Octadecylamine	0.07 mg.	49.00
	0.14	58.00
	"	63.00
	"	77.00
	0.28	26.00
	"	36.00
	"	37.50
48/80	0.2 mg.	60.00
	1	125.00
	1.5	55.00
	2.0	45.00
	5.0	27.50

30 minutes cannot be activated by peptone. Under both these conditions streptokinase (an activator produced by certain strains of streptococci) is still capable of activating profibrinolysin. It was therefore assumed that the anaphylactic or anaphylactoid type of activation, as opposed to the streptokinase type, requires the intervention of a kinase system present in whole,

fresh guinea pig serum. This kinase system was given the name of serofibrinokinase.

Experiments were carried out first to determine whether the anaphylactoid agents listed above as profibrinolysin activators also acted through serofibrinokinase. Table V shows that addition of these agents to serum previously heated to 56°C. for 30 minutes or to the euglobulin fraction of serum did not result in any significant activation of profibrinolysin, as compared with the effect of these agents on whole, fresh serum. The fact that streptokinase[9] is active under all conditions investigated is evidence that profibrinolysin was not destroyed by heating or precipitation. All anaphylactoid agents investigated seem therefore to act through serofibrinokinase.

TABLE V

Comparison of the Effects of Profibrinolysin Activators Added to Fresh Serum, Heated Serum, or to Globulin Fraction

Activator/ml. serum	Fibrinolysin 10^{-3} U/ml. serum Activator added to		
	Fresh serum	Heated serum	Globulin
Egg albumin 2 mg.*	137.50	40.00	15.00
Rabbit serum 0.04 ml.*	95.00	25.00	2.50
Peptone 20 mg.	180.00	27.50	25.00
Tween 20 0.8 μl.	120.00	12.50	0
Morphine sulfate 1.5 mg.	97.50	0	5.00
Octylamine 0.25 mg.	105.50	35.00	12.50
48/80 1 mg.	125.00	17.50	7.25
Streptokinase 2.5 mg.	125.00	95.00	140.00

* Added to sensitized guinea pig serum.

Since serofibrinokinase was not present in the euglobulin fraction of serum, it was first assumed that it must have remained in the supernatant. No significant activation was observed, however, when the supernatant of an activated serum preparation was added to normal euglobulin or to whole fresh serum. Negative results were also obtained when the euglobulin was first precipitated, the activating agent added to the supernatant, and the two fractions mixed again.

From these observations the conclusion was reached that under the conditions of the test serofibrinokinase occurred only in whole, fresh serum. Results shown in Table VI supply evidence that the addition of fresh normal serum to heated antibody-containing serum increases profibrinolysin activation, presumably by increasing serofibrinokinase concentration. There is, however, an objection to this interpretation since the fresh serum, besides serofibrino-

[9] Kindly supplied by Dr. B. W. Carey, Lederle Laboratories, Pearl River, New York.

kinase, also contains profibrinolysin and this contributes to the final amount of fibrinolysin detected. Completely satisfactory results could be obtained only by separating the kinase from the proenzyme.

Since fresh guinea pig serum is known to contain a system which can be brought into action by the antigen-antibody reaction, an attempt was made to determine whether this system, complement, was in any way related to

TABLE VI

Action of Fresh Non-Sensitized Guinea Pig Serum on Profibrinolysin Activation by Antigen-Antibody Reaction

Fresh immune serum	Heated immune serum	Fresh normal serum	Fibrinolysin 10^{-3} U/ml. serum
0.1	—	—	102.5
—	0.1	—	20.0
—	0.1	0.01	62.5
—	0.1	0.05	65.0
—	0.1	0.1	92.5
—	0.1	0.2	130.0
—	—	0.2	12.5

The antigen was egg albumin 2 mg./ml. of serum.

TABLE VII

Fractionation of Complement and Profibrinolysin Activation

Activator	Whole serum	Fibrinolysin 10^{-3} U/ml. serum		
		Yeast-treated	NH_3-treated	Reconstituted*
None	12.5	1.5	6.5	—
Egg albumin,‡ 2 mg./ml.	95.0	12.5	25.0	85.0
Tween 20, 0.8 μl./ml.	85.0	17.5	16.5	82.5
Streptokinase, 2.5 mg./ml.	100.0	93.5	87.5	—
Complement titer§	80–120	0–20	0	80–100

* Reconstituted by mixing equal parts of yeast-treated and NH_3-treated samples.

‡ Added to guinea pig serum sensitized to egg albumin.

§ 50 per cent hemolysis method.

serofibrinokinase. It is known that complement consists of at least four components and that the loss of any one of these components results in inactivation. In a first series of experiments profibrinolysin activation was studied in sera in which the third and fourth components of complement were destroyed. It is seen in Table VII that treatment of serum with yeast (which adsorbs the third component) or with ammonia (which inactivates the fourth component) results in inhibition of profibrinolysin activation by the antigen or an anaphylactoid agent. The table shows that when the two deficient serum fractions

are mixed together, fibrinokinase activity, as well as complement function, again became manifest. It is also seen that activation by streptokinase is not affected significantly by the inactivation of complement.

In other experiments, complement was fixed with an antigen-antibody system and fibrinokinase activity tested in the supernatant.

TABLE VIII

Complement Fixation and Profibrinolysin Activation

Activator	Fibrinolysin 10^{-4} U/ml. serum	
	Non-sensitized red cells	Sensitized red cells
None	27.5	15.0
Egg albumin,* 2 mg./ml.	102.5	35.5
Tween 20, 0.8 μl./ml.	105.0	15.5
Streptokinase, 2.5 mg./ml.	105.0	125.0
Complement titer‡	90	10–15

* Added to guinea pig serum sensitized to egg albumin.

‡ 50% hemolysis method.

Since comparatively large amounts of serum were required, an excess of both antigen (sheep blood cells) and antibody (rabbit anti-sheep hemolysin) had to be used. To 1 ml. of a 20 per cent sheep cell suspension 1 ml. of a 1/100 dilution of hemolysin[10] and 0.2 ml. of guinea pig serum were added and the mixture kept at 2°C. for 45 minutes. After this interval, the samples were centrifuged and the supernatant submitted to the activating agents according to the technique described above. Control samples were set up at the same time, containing all the reagents except hemolysin so that the sheep cells were not sensitized. Table VIII shows that, when complement was fixed by the sheep cell-hemolysin system, profibrinolysin activation occurred only with streptokinase and not with the specific antigen or tween 20. In the control samples in which complement was not fixed, activation occurred with all three agents.

From the results of these experiments it can be concluded that serofibrinokinase activity disappears from sera in which complement is split, fixed or, as seen in Table V above, inactivated by heating. It was also observed that, when serum is treated with an anticomplementary substance such as Congo red (19), profibrinolysin activation is inhibited. Another compound, polylysine (a 31-mer of L-lysine),[11] was first found to inhibit profibrinolysin conversion. When tested on a hemolytic system, polylysine also proved to be an inhibitor of complement.

DISCUSSION

The observations just described can probably explain some of the contradictory results published on protease activation by the antigen-antibody reaction.

[10] Bacto antisheep hemolysin, Difco Laboratories, Detroit.

[11] Kindly supplied by Dr. E. Katchalski, Weizmann Institute of Science, Jerusalem, Israel.

It is seen that this activation takes place only when whole serum is used. This explains the negative results obtained by workers using the euglobulin fraction of serum or any other partially purified preparation (4, 5). The present study shows that maximum activation occurs at an optimal ratio between antibody and antigen; addition of too little or too much antigen to serum produces degrees of activation too small to be detected by most methods. It is also shown above that sera with high spontaneous activity cannot be further activated by the antigen. This invalidates the negative results observed in series in which high spontaneous activity was a regular occurrence (5). Development of spontaneous fibrinolytic activity seems to depend on certain details of handling the blood samples; technique of bleeding, storage, and probably other factors. A last cause of error is the absence of antibody from the sera used (5); negative results obtained with such sera confirm rather than contradict the assumption that profibrinolysin activation is caused by the antigen-antibody reaction.

The results indicate that profibrinolysin is activated in guinea pig serum by a number of comparatively simple chemical compounds. These compounds, like the antigen-antibody complex, have the ability to produce anaphylactic manifestations when injected into animals of the appropriate species. These substances are also called "histamine liberators" (15) because they have the ability to release histamine from tissues *in vivo* as well as *in vitro*. There is reason to believe that there is a causal link between the properties of these substances: they produce anaphylactoid phenomena because they release histamine and they release histamine because they activate profibrinolysin. In a series of experiments conducted simultaneously with those reported in this paper a high correlation was observed between histamine release and proteolysis when guinea pig lung slices were subjected to an antigen-antibody reaction or to anaphylactoid stimuli (20). All these results tend to confirm the view that activation of a proteolytic enzyme system occupies a key position in the chain of events leading to local and systemic allergic phenomena (21, 22).

The exact identity of this proteolytic enzyme system can only be determined by its isolation. Fibrinolysin (or plasmin) is a term which has come to designate several different enzymes, as shown by the example mentioned in the section on Methods. Whether the fibrinolysin which results from the activation process dealt with in this paper is a unique protease or a mixture of several enzymes is as yet undecided.

Activation of profibrinolysin under the conditions described above seems to require the intervention of a kinase system. In all instances investigated, this kinase system behaves in a way indistinguishable from complement. It would be meaningless to say that serofibrinokinase is identical with complement since the identity of complement itself is but vaguely defined. The presence of complement in serum can be detected because of its involvement in the antigen-antibody reaction. The present findings suggest, however, that

complement may also play a role in non-specific reactions taking place in the absence of an antibody. They also indicate that complement is required for the production of anaphylactoid phenomena as well as for true anaphylaxis.

There are other indications that the fibrinolytic system is somehow connected with complement. Geiger believes that activation by streptokinase also requires a "complement-like" factor (23). Pillemer *et al.* have shown that fibrinolysin inactivates complement (12). The latter observation explains perhaps the fact mentioned above that sera with high spontaneous activity do not undergo further activation by the antigen-antibody reaction.

Profibrinolysin and serofibrinokinase exist in normal serum side by side without any apparent interaction. The available evidence suggests that, under the influence of the activating agents, serofibrinokinase undergoes some change which renders it capable of reacting with profibrinolysin and converting it into fibrinolysin. The nature of this change is unknown.

SUMMARY

The activation of profibrinolysin in sensitized guinea pig serum when mixed *in vitro* with the homologous antigen was confirmed with a more accurate and more reliable method than the one previously used. A study was made of some of the conditions required for obtaining maximum activation.

Profibrinolysin activation was also induced in normal guinea pig serum by addition of certain "anaphylactoid" agents such as peptone, tween 20, morphine, octylamine, octadecylamine, and 48/80.

The specific antigen and the anaphylactoid agents produce activation only when added to whole, fresh, unheated serum. Profibrinolysin activation by these agents, as opposed to activation by streptokinase, seems to require the intervention of a kinase system (serofibrinokinase) inactivated by fractionation of serum and by heating to 56°C.

Whenever serum was submitted to treatments which caused fractionation, fixation or inhibition of complement, serofibrinokinase was also inactivated. Under the conditions investigated the behavior of this kinase was indistinguishable from that of complement.

BIBLIOGRAPHY

1. Ungar, G., *Lancet*, 1947, **1**, 798.
2. Ungar, G., and Mist, S. H., *J. Exp. Med.*, 1949, **90**, 39.
3. Geiger, W. B., *J. Immunol.*, 1952, **68**, 11.
4. von Euler, H., and Heller, L., *Ark. Kemi*, 1950, **2**, 313.
5. Jemski, J. V., Flick, J. A., and Stinebring, W. R., *J. Exp. Med.*, 1953, **97**, 439.
6. Kunitz, M., *in* Northrop, J. H., Kunitz, M., and Herriott, R. M., Crystalline Enzymes, New York, Columbia University Press, 2nd edition, 1948.
7. Ungar, G., Damgaard, E., and Hummel, F. P., *Endocrinology*, 1951, **49**, 805
8. Mullertz, S., *Bull. Soc. chim. biol.*, 1953, **35**, 190.

9. Loomis, E. C., Ryder, A., and George, C., Jr., *Arch. Biochem.*, 1947, **12**, 1.
10. Germuth, F. G. Jr., Oyama, J., and Ottinger, B., *J. Exp. Med.*, 1951, **94**, 193.
11. Kabat, E. A. and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, C. C. Thomas, 1948.
12. Pillemer, L., Ratnoff, O. D., Blum, L. and Lepow, I. H., *J. Exp. Med.*, 1953, **97**, 573.
13. Krantz, J. C. Jr., Carr, C. J., Bird, J. G., and Cook, S., *J. Pharmacol.*, 1948, **93**, 188.
14. Paton, W. D. M., *Brit. J. Pharmacol.*, 1951, **6**, 499.
15. Feldberg, W., and Paton, W. D. M., *J. Physiol.*, 1951, **114**, 490.
16. Rocha e Silva, M., and Schild, H. O., *J. Physiol.*, 1949, **109**, 448.
17. Mongar, J. L., and Schild, H. O., *Brit. J. Pharmacol.*, 1953, **8**, 103.
18. McIntire, F. C., Roth, L. W., and Sproull, M., *Am. J. Physiol.*, 1951, **167**, 233.
19. Gordon, J. and Watkin, N., *J. Path. and Bact.*, 1945, **57**, 451.
20. Ungar, G., Damgaard, E., and Hummel, F. P., Communication to International Physiological Congress, Montreal, 1953, **844.**
21. Ungar, G., *Lancet*, 1952, **2**, 742.
22. Ungar, G., *Internat. Arch. Allergy and Appl. Immunol*, 1953, **4**, 258.
23. Geiger, W. B., *J. Immunol.* 1952, **69**, 597.