STUDY OF FIBRINOGEN AND PLASMINOGEN CONCENTRATIONS IN RABBITS DURING ANAPHYLACTIC SHOCK

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Study of fibrinogen and plasminogen concentrations in rabbits during anaphylactic shock

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In order to evaluate the effect of anaphylaxis on the last stages of coagulation, the different clotting parameters of these stages were determined in blood obtained from rabbits before and after induction of anaphylactic shock.

Two cubic centimeters of dog serum, injected into an ear vein on 2 consecutive days, served to sensitize the animals. Sixteen days after the last injection anaphylaxis was provoked with 0.4 to 1.2 cm.³ of dog serum.

Blood obtained from an aortic catheter was processed for its fibrinogen and plasminogen concentration and the plasmin and plasminogen activator activity. Fibrinogen concentrations were also studied in animals pretreated with epsilon aminocaproic acid before injection of the anaphylaxis-provoking dose of dog serum.

It was found that fibrinogen concentrations invariably declined after induction of anaphylaxis. This was not found in the group of animals pretreated with epsilon aminocaproic acid.

Plasminogen concentrations also declined invariably after induction of anaphylaxis. Concomitant with the decline in plasminogen concentration there occurred a substantial increase in plasminogen activator activity. These changes are consistent with the hypothesis that activation of the plasminogen-plasmin system occurs in anaphylaxis in rabbits.

In anaphylactic shock several abnormalities of the coagulation system have been described. Jaques and Waters[11] have shown that the clotting time is prolonged in anaphylaxis in the dog. They have indicated that the marked prolonged clotting times are a result of increase in circulating heparin.

Pesci,[17] and more recently, Quick and associates,[19] have shown that agglutination of platelets and severe thrombocytopenia occur during anaphylaxis. Associated with disintegration of platelets there may be intravascular coagulation.

Plasminogen activation in vitro by antigen-antibody reaction has been incriminated by Ungar and Mist.[23] This activation has not been reproduced by others.[12]

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Fibrinogen and plasminogen-plasmin system were studied during anaphylaxis in rabbits. The results indicated that the fibrinogen concentrations do fall after induction of anaphylactic shock. Considerable activation of the plasminogen-plasmin system was demonstrated after induction of anaphylactic shock.

Materials and methods

Albino rabbits weighing 2 kilograms were used for all the experiments referred to in this paper. Each of these animals were given, as a sensitizing dose, 2 intravenous injections of 2 ml. of whole dog serum on 2 consecutive days.[20]

Twelve to 16 days following the administration of the sensitizing dose, the animal was subjected to intubation of the aorta under Nembutal anesthesia. An anaphylaxis-provoking dose of 0.4 to 1.2 ml. of dog serum was then given through the cannula in the aorta.

One group of animals was pretreated with epsilon aminocaproic acid (E.A.C.A.)* in a dose of 6 to 8 ml. (0.15 M) per rabbit.

The severity of the ensuing anaphylactic reaction was judged by the extent of the blanching of the ear veins, dyspnea and cyanosis, the increase in small bowel motility, and the distention of the abdominal veins.[4, 20] Whole blood (5 ml.) was withdrawn before and after injection of dog serum. These samples were collected in tubes containing 0.25 ml. of 10 per cent Sequestrene.

The blood was centrifuged for 5 minutes at 2,000 r.p.m. and the plasma separated. Plasma samples were placed on the different fibrin plates (see below), after which the rest of the plasma was quick frozen. Thawing and rewarming of the samples was done at 37° C. in a constant temperature bath. All samples of one rabbit were processed simultaneously on the day of collection for their fibrinogen and plasminogen contents.

Fibrinogen concentrations. These were determined by the method of Kjell-Jacobsson.[10] The plasma is diluted with buffer and clotted with a standard thrombin preparation. The clot is washed and dissolved in a 40 per cent urea solution. The solution is read in a Beckman spectrophotometer at 279, 320, and 360 mμ against a urea blank.

Plasminogen concentrations. These were determined by the casein digestion method. The plasmin and plasminogen of serum or plasma are freed from their inhibitors by being precipitated in the euglobulin fraction at pH 5.2. The euglobulin solution is incubated with standard amounts of streptokinase, dilution of human serum and casein for 30 minutes, and the amount of casein digestion determined by measuring the tyrosine content of the reaction mixture in the Beckman spectrophotometer at 280 mμ. Plasminogen determination by this method yields the same values for both plasma and serum. Therefore, the euglobulin plasminogen values are not dependent on fibrinogen concentration.

FIBRIN PLATES. The plates were prepared according to directions given by Astrup and Mullertz.[3] Fibrinogen is precipitated from bovine oxalated plasma with ammonium sulfate. The fibrinogen fraction is dissolved in barbiturate buffer (pH 7.8) to a final concentration of 200 mg. per cent. Ten cubic centimeters of this solution is pipetted into standard Petri dishes and clotted with 50 u. thrombin.†

HEATED PLATE. The above Astrup plate is heated at 80° C. for 30 minutes.[13]

Plasminogen was obtained from plasma Fraction III‡ by a fractionation method described by Kline.[12A] Ten grams of Fraction III is acidified by addition of 200 cm.³ of 0.05 N sulfuric acid. After stirring and centrifugation, the top layer of lipids is skimmed off. The remainder is brought to pH 11 with 1 N NaOH. After exactly 1 minute the pH of the solution is brought to 5.3 with 1 N HCl. After refrigeration for 3 hours, the pH is again brought to 2. Solution is centrifuged in the cold for 1 hour at 2,500 r.p.m. The gelatinous residue is discarded and the supernatant is brought to pH 8.6 and dialyzed against sodium

*Ortho Research Laboratory, Ortho Pharmaceutical Corporation Raritan, N. J.
†Topical Thrombin, Parke, Davis & Company, Detroit, Mich.
‡Plasma Fraction III obtained through the courtesy of Dr. Gerlough of E. R. Squibb & Sons, New York, N. Y., and Dr. J. N. Answorth of the American Red Cross.

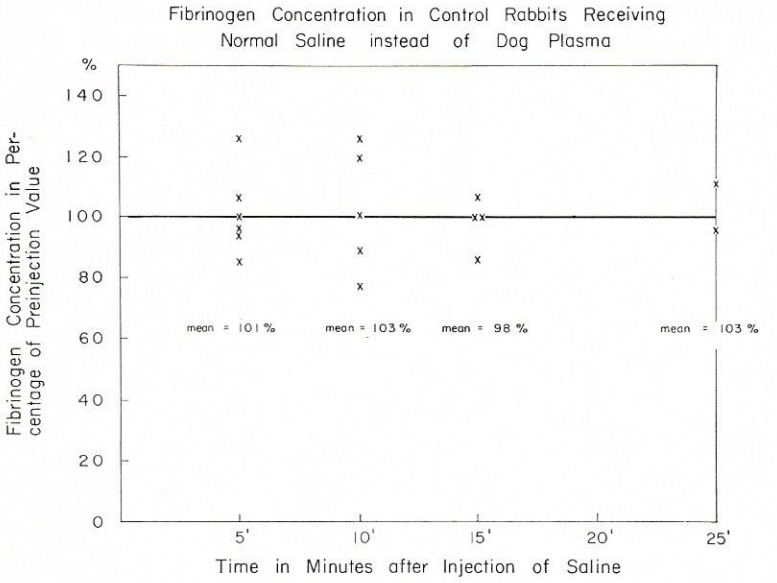

Fig. 1. Fibrinogen concentrations expressed in percentage of preinjection value, in 6 control rabbits receiving saline instead of dog serum.

phosphate buffer (0.025 M, pH 6.0). Dialysate is refrigerated 1 hour and resulting precipitate is separated by centrifugation at 2,500 r.p.m. for 1 hour. Precipitate is dissolved in 25 cm.^{3} of distilled water to which is added one drop of 0.1 N HCl. This plasminogen solution is stored in refrigeration.

PLASMINOGEN REINFORCED PLATE. Of the above standard plasminogen solution, 0.1 cm.^{3} was added to the usual constituents of the Astrup plate.

Epsilon aminocaproic acid was used in a 0.15 M solution. The E.A.C.A. (1 cm.^{3}) was added to the constituents of the plasminogen reinforced plate. These plates are described as epsilon aminocaproic acid–enriched plates (E.A.C.A. plates).

The amount of lysis of the fibrin plates was determined as described by Astrup[3] by measuring the greatest diameter of the lysed area after incubation at 37° C. for 20 hours. Duplicate applications of 30λ were placed on surface of each plate.

Results

Fibrinogen concentrations. Since the initial fibrinogen concentration varies normally from one animal to the other, the concentrations found after injecting the animals were expressed in a percentage of preinjection values.

Six control rabbits received saline 10 to 15 days after the primary sensitizing injections of dog serum. No marked changes were observed after saline injection (Fig. 1).

Upon challenging 12 animals with dog serum, however, and then provoking anaphylactic shock, a decline in the fibrinogen concentration invariably occurred (Fig. 2). The average decrease in fibrinogen value for these 12 animals was found to be 44 per cent.

The group of 12 rabbits pretreated with E.A.C.A. prior to injection of the provoking dose of dog serum showed no significant changes in the fibrinogen concentrations (Fig. 3).

Plasminogen plasmin system. The effect of in vitro combination of dog serum and rabbit blood obtained from previous sensitized animals was studied specifically. This addition did not result in the activation of the plasminogen-plasmin system. The lack of activity was demonstrated by incubating samples of this combined plasma and its euglobulin fractions on all the types of fibrin plates.

Plasminogen concentration. No changes of any significance of the plasminogen values were found in the group of control animals (Fig. 4).

However, plasminogen concentrations as determined by Norman's[16] method invariably declined after the induction of anaphylaxis (Fig. 5). The maximum

Table I. Fibrinogen concentrations in mg. % before and after induction of anaphylatic shock in rabbits

No. rabbit	*Fibrinogen value (mg. %) Time (in minutes) after induction of anaphylaxis*						Remarks
	Control	*5*	*15*	*30*	*45*	*60*	
129	363	215					Dead within 8 minutes
132	398	148					Dead within 6 minutes
149	360	205					Dead within 6 minutes
158	335	295	230	260	180	228	No shock
160	200	72	74 c. p.*				Dead within 15 minutes
167	354	212	177	155 c. p. at 20			Dead within 30 minutes
169	322	116	103 c. p.				Dead within 15 minutes
170	497	244	495	475			No shock
2,288	386	320	230	210			No shock, marked anaphylaxis
2,289	442	306	226 c. p.				Dead within 15 minutes
2,296	230	197	141				Dead in 18 minutes
2,297	310	272	252				No shock

*c. p. = Cardiac puncture.

Table II. Fibrinogen concentrations in mg. % before and after induction of anaphylaxis in rabbits pretreated with epilson aminocaproic acid

No. rabbit	*Fibrinogen value (mg. %) Time (in minutes) after induction of anaphylaxis*						Remarks
	Control	*5*	*15*	*30*	*45*	*60*	
142	255	275	268 c. p.*				Dead within 15 minutes
143	396	373	366 c. p.				Dead within 15 minutes
145	328	312	392 c. p.				Dead within 15 minutes
146	481	561	425	412			Animal killed with Nembutal
170	315	295	372	420	410		No shock
171	483	398	412 c. p.				Dead within 15 minutes
173	355	335	322				Dead within 20 minutes
175	311	335	335				Dead within 18 minutes
177	300	288 c. p.					Dead within 5 minutes
180	413	398 c. p.					Dead within 5 minutes
181	304	357 c. p.					Dead within 5 minutes
182	290	290	265 c. p.				Dead within 15 minutes

*c. p. = Cardiac puncture.

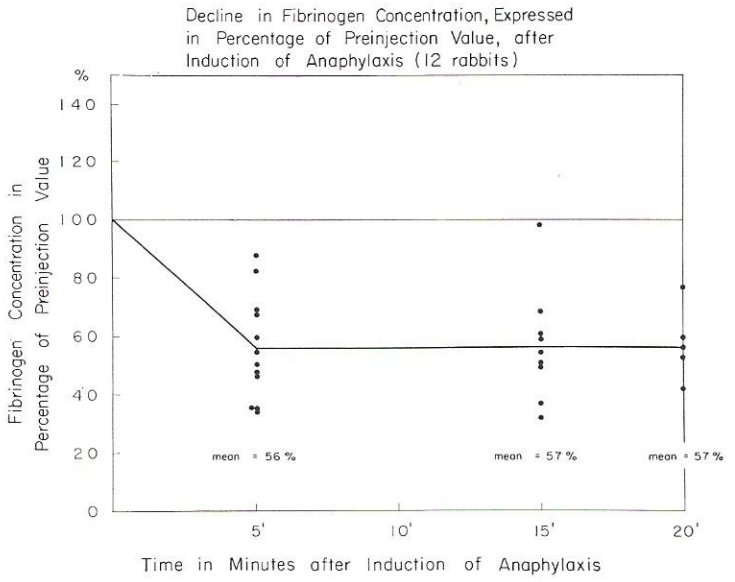

Fig. 2. Decline in fibrinogen concentration, expressed in percentage of preinjection value, after induction of anaphylaxis in 12 rabbits.

decline occurred in the first 5 minutes and decreased to 58 per cent of the original concentration. No activity was noted in the heated (Lassen[13]) plates. Therefore, no free plasmin was found.

Plasminogen activator activity. Concomitant with the plasminogen decrease found after induction of anaphylaxis, a substantial increase in plasminogen activator activity was noted. This increased plasminogen activator activity was determined by the extent of lysis of the plasminogen reinforced fibrin plates

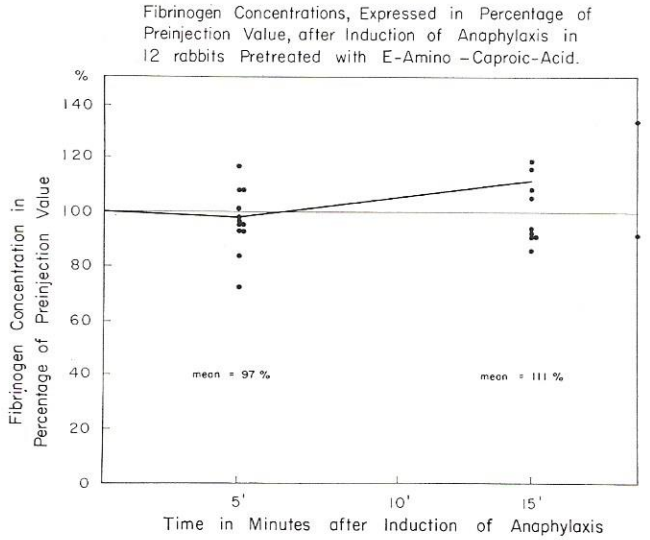

Fig. 3. Fibrinogen concentrations, expressed in percentage of preinjection value, after induction of anaphylaxis in 12 rabbits pretreated with epsilon aminocaproic acid.

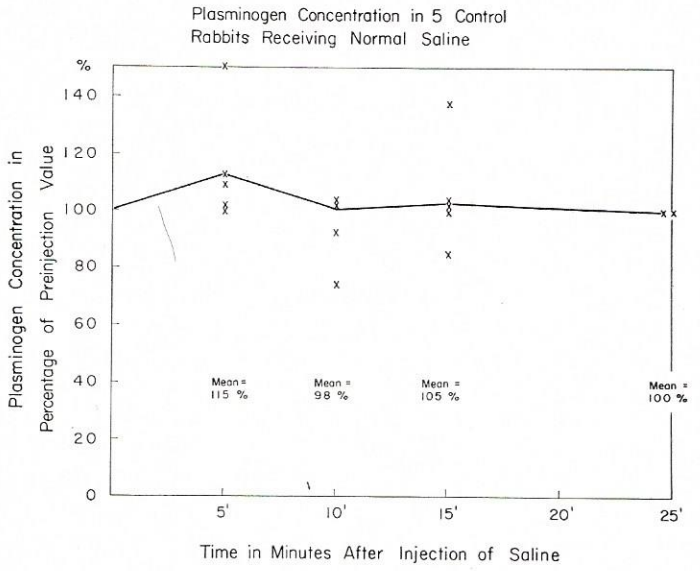

Fig. 4. Plasminogen concentrations, expressed in percentage of preinjection value in 5 control rabbits receiving normal saline.

produced by euglobulin samples. The increase in plasminogen activator activity was found in the 5 minute sample and reached a maximum in the 15 minute sample (Fig. 6). Lysis on the plates increased from an average control level of 40 mm. to a maximum of 160 mm.[2]

Course of anaphylactic reaction. No difference in the symptoms of anaphylactic reaction could be detected between the E.A.C.A.–pretreated rabbits and the unpretreated animals (Tables I and II).

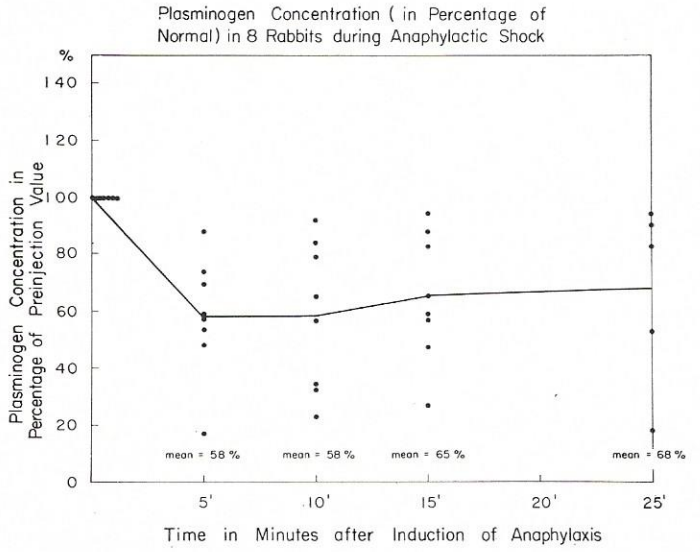

Fig. 5. Plasminogen concentrations, expressed in percentage of preinjection value, in 8 rabbits after induction of anaphylaxis.

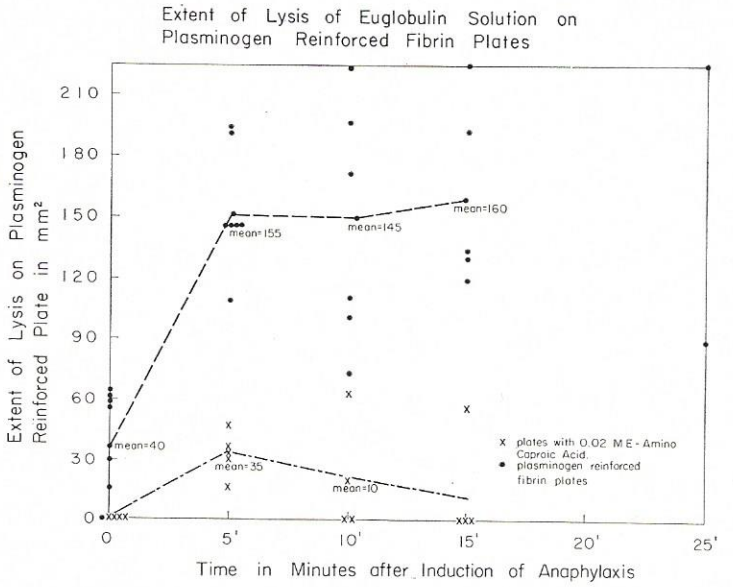

Fig. 6. Increase in plasminogen activator activity, as is evident from the extent of lysis of the plasminogen reinforced fibrin plates, after the incubation of euglobulin samples prepared from the plasma of 8 rabbits. Increase in plasminogen activator activity, as evident from the extent of lysis of plasminogen reinforced fibrin plates, after the incubation of euglobulin samples prepared from the plasma of 8 rabbits. Note here that E.A.C.A. fibrin plates have minimal lysis. This inhibition by E.A.C.A. indicates that plasminogen activator activity was responsible for the observed lysis.

Discussion

In reviews of Doerr[4] and Richet[20] the methodology for producing anaphylactic shock was outlined. The symptoms of anaphylactic shock of the rabbits in the present experiment were those described by these authors. The effect on the rabbits reported here were impressive in that there was a dramatic occurrence of dyspnea, cyanosis, hyperperistalsis with defecation, and vasoconstriction and death occurred in the animals at the time of induction of anaphylaxis.

These animals undergoing anaphylactic shock were studied in order to evaluate the effect of the condition on the fibrinogen and plasminogen-plasmin system.

The first change noted was the decline in fibrinogen concentration. This partial change occurred immediately after induction of the anaphylaxis. The average decline was found to be 44 per cent of the original fibrinogen concentration. This fall in fibrinogen was consistent in the experimental animals and absent in the control animals.

The apparent decrease of the fibrinogen concentration can be explained by several different postulates. These postulates, however, have to take into account the fact that the noted fibrinogen changes were prevented by pretreating the animals with E.A.C.A. which acts as a powerful inhibitor of plasminogen activation. The effect of several activators of plasminogen, like streptokinase,[1] urokinase,[15] and human plasminogen activator[9] is completely inhibited by the

compound E.A.C.A. This, then, supplied correlative indirect evidence suggesting that the fibrinogen decline was the result of the activation of the plasminogen-plasmin system, since the decline in fibrinogen level was prevented by pretreating the animals with E.A.C.A.

Direct evidence for activation of the plasminogen-plasmin system was obtained by observing a decrease in plasminogen concentration and a rise in plasminogen activator activity. The plasminogen decline of 58 per cent from the original value was not present in the control animals. The plasminogen activator activity rose in inverse proportion to the decline in plasminogen.

The interpretation of the above noted findings must include a conclusion that anaphylaxis activates the plasminogen-plasmin system. E.A.C.A. did not protect the animals against signs and symptoms of anaphylaxis.

The lack of lysis on the heated fibrin plates indicates no demonstrable plasmin activity. Plasmin is known to be readily inactivated. Therefore absence of detectable free plasmin activity remains compatible with an activation of the plasminogen plasmin system. Indeed, hyperplasminemia is probably only rarely noted in conditions of active fibrinolysis.[22]

In the past, several investigators have tried to demonstrate plasminogen activation during anaphylaxis. Rocha e Silva,[21] in experiments on anaphylaxis, added protamine to neutralize the heparin released in dogs. Subsequently, however, Downie and Cliffton,[5] showed that protamine itself has marked plasminogen activating properties. Because of this, the mechanism of plasminogen activator which Rocha e Silva observed remains unsolved.

Subsequently, Ungar and Mist[23] demonstrated in an in vitro system that antigen addition to guinea pig blood containing sufficient quantities of antibodies resulted in the activation of plasminogen. Several investigators,[6, 12] however, were unable to confirm these findings. Similarly, the experiments presented here are unable to demonstrate plasminogen activation in vitro.

Rocha e Silva[21] has postulated that the proteolytic mechanism releases histamine, serotonin, and bradykinin. In the present experiments, plasminogen activator activity is inhibited by pretreatment with E.A.C.A., yet anaphylaxis still occurs. Suggestively, proteolysis as represented by the plasminogen-plasmin system cannot be implicated in the causative mechanism of signs and symptoms of anaphylaxis.

The activation of the plasminogen-plasmin system observed after anaphylaxis is in marked contrast to the lack of such activation in the rabbit following the induction of shock from other causes such as adrenalin[24] or endotoxin.[14] This laboratory has been able to confirm the observation that endotoxin shock in rabbits does not activate the plasminogen-plasmin system.

The ability of the rabbit to activate the plasminogen-plasmin system in response to anaphylaxis but not to other types of insult remains a paradox. Each type of shock produced may have a particular end-organ response, i.e., lungs in anaphylaxis, kidneys in endotoxin, and intestines in hemorrhagic shock. Perhaps the plasminogen activator activity of these organs may be sufficiently different to explain the above apparent discrepancies. Further investigation along these lines is in progress.

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