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Mechanism of Anaphylaxis in the Rabbit. Further Evidence Against Plasma Protease Mechanism.* (19989)

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The theory that the development of anaphylaxis depends upon the activation of plasma protease (plasmin, or fibrinolysin) has numerous adherents. Although this theory lacks the support of direct and unequivocal evidence, and in spite of direct evidence in opposition(1), it enjoys sufficiently wide acceptance to be indicated as the probable mechanism of anaphylaxis in a recent review(3). The literature pertinent to the activation of plasma protease by immune reactions was recently reviewed briefly by Geiger(4), who, as a result of his own careful experiments, considered it "unlikely that anaphylactic symptoms in rabbits can be attributed solely to activation of plasma protease." In our studies of the mechanism of the anaphylactic reaction in the rabbit, we have utilized as a criterion the release of histamine from blood cells, which follows the addition of antigen to the blood from a sensitized rabbit *in vitro*. In an earlier publication(1), we indicated that the participation of plasma protease in the *in vitro* release of histamine in rabbit blood was doubtful because: 1. Excessive quantities of soybean trypsin inhibitor failed to inhibit the histamine release, and 2. Large amounts of bovine fibrinolysin failed to release significant amounts of histamine.

To obtain an even more direct type of evidence, we have now activated plasma protease of rabbit blood by means of streptokinase both *in vitro* and *in vivo*, and have compared the effects of antigen and streptokinase with respect to the activation of protease, the release of histamine *in vitro*, and the development of anaphylactic symptoms *in vivo*. The results of the *in vitro* experiments, Table I, indicate that there is a complete lack of correlation between histamine release and protease activation. Homologous antigen released a large amount of histamine and failed to activate a detectable amount of plasma protease; conversely, streptokinase markedly activated plasma protease and in so doing did not release significant amounts of histamine. From the data in Table II, it is apparent that there is no correlation between protease activation *in vivo* and the development of anaphylaxis. There is no significant difference in protease level between the blood of normal rabbits and blood withdrawn from challenged rabbits at the time of anaphylactic death, prior to death, or during severe anaphylactic symptoms. Conversely, rabbits treated with streptokinase showed no anaphylactic symptoms even though there was a several-fold elevation of the plasma protease level in all cases but one. The evidence presented here not only casts extreme doubt upon the plasma protease theory of the mechanism of anaphylaxis in the rabbit, but also calls for a more critical attitude toward this theory in relation to other species.

Experimental. The streptokinase (SK) preparation, kindly supplied by Mr. Frank Ablondi, of Lederle Laboratories, was dissolved in physiological sodium chloride solution immediately before use. The SK units indicated are Lederle units.

The details of rabbit sensitization, and the *in vitro* histamine release technic were presented previously(1,2). The *in vitro* histamine release was carried out both at 37.5°C,

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TABLE I. Histamine Release and Protease Activation in Sensitized Rabbit Blood *In Vitro*.

	Histamine release,* μg/ml blood				Clot lysis time,† min.			
	1	2	3	4	1	2	3	4
SK 1750 U‡	0	0	0.06	0.29	5, 7	6, 7	5	5.5
875 U	0.19	0	0	0	7, 8	6, 12	6	5
350 U	0	0.13	0	0.27	35	25	7	5.5
Antigen	4.4	1.7	2.3	2.1	No lysis in 3 hr			

* At 37.5°C.
† Time required for complete lysis of the plasma clot at 29.5°C.
‡ Lederle units of streptokinase per ml of blood.

TABLE II. Relation of Plasma Protease Level to Anaphylactic Symptoms.

Non-sensitized animals Untreated	Sensitized animals Egg white i.v.†		Non-sensitized animals Streptokinase i.v.‡	
Protease units*	Protease units	Symptoms	Protease units	Symptoms
4.2	5	D§ 6 min.	27	0
5.8	4	D 30 "	6	0
8.8	13	D 4 hr	24	0
6.6	3	D 1 "	19	0
4.2	8	D 6 min.	43	0
8.6	4.9	Severe, recovered	41	0

* Per 100 ml of plasma. † 1 ml whole egg white per rabbit. ‡ 105,000 Lederle S.K. units per 2.5 kg. § D indicates anaphylactic death.

and at 29.5°C; the results at the two temperatures were essentially the same. The following procedure was used for the protease activation and clot lysis determination *in vitro*: In a 10 x 75 mm test tube, at 29.5°C, 0.3 ml of plasma (from the sensitized rabbit blood) was mixed with 0.2 ml of saline, a saline solution of SK, or a saline solution of egg white diluted 1:100; 0.1 ml of thrombin in 50 percent glycerol was added immediately, and the contents of the test tube were mixed thoroughly. The lysis time was taken from the time the thrombin was added until the clot appeared completely fluid. Lysis time determinations were run in duplicate, and when the duplicates did not agree both results are indicated. The clot lysis was demonstrated more readily at 29.5° than at 37.5°C. At the higher temperature, the SK-treated samples showed a rapid partial lysis which stopped short of complete lysis, perhaps because of a rapid inactivation of the protease at the higher temperature. In the *in vivo* experiments, blood samples were withdrawn by cardiac puncture into syringes containing 1 ml of M/10 sodium oxalate for every 7 ml of blood. Blood was withdrawn 10 minutes after the i. v. administration of SK or egg white, except in cases where the animals died in less than 10 minutes. In such cases, the blood was taken just as the animal appeared to be dying. Blood cells were removed by centrifugation, and protease was isolated from the plasma by the method of Ungar(5). Quantitative protease determinations were done by the method of Geiger(4), and the results are expressed in terms of the unit defined by Geiger.

Summary. The plasma protease theory of the mechanism of anaphylaxis in the rabbit appears untenable because: (1) Homologous antigen will release histamine *in vitro* or will produce fatal anaphylaxis *in vivo* without significant activation of plasma protease. (2) Streptokinase will activate plasma protease both *in vitro* and *in vivo* without producing any symptoms of anaphylaxis *in vivo* or releasing any histamine from the blood cells *in vitro*.

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