Antifibrinolytic Activity in Plasma after Exposure to Cold and after Chymotrypsin Administration

By H. Jensen, Ph.D., David L. Chamovitz, M.D., Evelyn Volkringer, Elizabeth Gray, B.A., James E. Gosney, B.S. and Walter Kocholaty, Ph.D.

CONTINUING PREVIOUS INVESTIGATIONS on the equilibrium between fibrinolysis and antifibrinolysis in blood during various conditions of stress,[1, 2] the antifibrinolytic activity in the blood of rats and of human subjects was measured after exposure to cold.

Since intravascular clotting has been shown to occur in frostbitten tissue,[3] it was felt that the induction of an increase in fibrinolytic activity (decreased antifibrinolytic activity) might be a method of treating this injury. Therefore, the effect of the administration of chymotrypsin on the antifibrinolytic activity of the blood was studied; the findings are also reported in this paper.

Methods

Cold Exposure and Antifibrinolytic Activity

Sprague-Dawley male albino rats averaging 200 Gm. were housed in a constant temperature room at 4 C. for eighteen days. A control group was kept at 22 C. Purina Laboratory Chow was fed to both groups.

Blood (1.8 ml.) was withdrawn by cardiac puncture into 0.2 ml. of 3.8 per cent sodium citrate. The sample was centrifuged at 2800 r.p.m. for 30 minutes at 5 C. The supernatant plasma was removed, frozen immediately at −30 C. and stored at this temperature. Antifibrinolytic activity determinations were done within two days after withdrawal of the blood.

An opportunity to study the effect of cold exposure on the antifibrinolytic titer in man was afforded during Army maneuvers at "Exercise Snowfall," Camp Drum, N. Y., during January and February, 1952. All of the men in the exercise had been intermittently exposed to wintry conditions at temperatures ranging from 5 C. to − 29 C. for one to three months. During the final phase of the maneuvers the exposure (as low as −25 C.) was continuous for three to eight days.

All patients with cold injury were studied by a research team assigned to Camp Drum. Plasma samples from patients and controls were obtained and flown in the frozen state to Fort Knox.

The men were classified into four groups: (1) controls, including men prior to cold exposure (43 total); (2) normal men three days after continuous cold exposure (6 total); (3) minor cold injury cases including those with either first degree frostbite or ill-classified cold condition of the feet (13 total); (4) patients with frostbite (7 had fourth degree injury,* 2 had third degree injury and 65 had second degree injury).

Chymotrypsin and Antifibrinolytic Activity

Crystalline salt free chymotrypsin (Armour Laboratories) was dissolved in Krebs' buffer (pH 7.4) and immediately administered intracardially to Sprague-Dawley male albino

From the Department of Biochemistry, Army Medical Research Laboratory, Fort Knox, Ky.

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* These patients with fourth degree injury were Korean veterans receiving treatment at the Percy Jones General Hospital, Battle Creek, Mich.

rats (average weight, 250 Gm.) and to New Zealand male albino rabbits (average weight, 2400 Gm.). Doses (0.5 to 1.0 ml.) ranging from 8 to 40 mg. per Kg. body weight were administered to rats and doses of 3 to 15 mg. per Kg. body weight were administered to rabbits.

Blood samples were obtained by cardiac puncture at intervals of 10 minutes to 48 hours following chymotrypsin administration.

TABLE 1.—*The Changes in the Antifibrinolytic Activity in Plasma of Rats Exposed to Cold*

No. of Rats	Exposure		Antifibrinolytic Activity, Units per ml. of Plasma (Avg.)
	Temperature	Duration	
30	+4 C	18 days	127 ± 10.2
30	+22 C	18 days	105 ± 8.4*

* p = <0.001.

TABLE 2.—*Effect of Cold on the Antifibrinolytic Activity in the Blood of Man*

	Group	No. of Determinations	Antifibrinolytic Activity Units per ml. of Plasma	
			Average	Range
I	Controls			
	1. Normal (prior to cold exposure)	17	122	99–149
	2. Army Laboratory Technicians	26	122	96–152
	3. Combined	43	122 ± 14.4	
II	Normal after cold exposure	6	143	108–180
III	Minor cold injury*	13	151 ± 20.1	119–176
IV	Frostbite, total*	74	151 ± 18.7	
	1. 24 hrs. after injury	15	158	135–201
	2. 48 hrs. after injury	9	154	125–191
	3. 72 hrs. after injury	10	155	140–164
	4. 96 hrs. after injury	8	146	131–167
	5. 120 hrs. after injury	2	140	
	6. 144 hrs. after injury	3	139	
	7. 18–22 days after injury	10	147	129–185
	8. 34–58 days after injury	10	146	126–161
	9. 3 or more months after 4th degree injury	7	158	122–209

* See text for description.

Determination of Antifibrinolytic Activity

The antifibrinolytic activity was obtained by determining the residual fibrinolytic activity of a standard amount of crystalline trypsin after incubation with appropriately diluted unknown plasma samples, as previously described.[2] Since the plasmin preparations, presently available, are rather crude and give inconsistent results, trypsin was used instead of plasmin for reasons of purity and reproducibility. The interaction of antiplasmin with trypsin differs quantitatively but not qualitatively from that with plasmin.[4]

One antifibrinolytic unit is defined as that amount of plasmin inhibitor which will neutralize one fibrinolytic (trypsin) unit after 30 minutes incubation at 25 C. and pH 7.25.

Results

"Antifibrinolytic activity" as measured here is the resultant of the reaction between the fibrinolytic enzyme and the inhibitor present. The average antifibrinolytic titer of 30 rats exposed to 4 C. for eighteen days was 126.7 ± 10.2

Table 3.—*Antifibrinolytic Activity of Plasma of Rats after Intracardiac Administration of Chymotrypsin*

Chymotrypsin (mg./Kg. Body Weight)	Time After Chymotrypsin Injection	No. of Animals	Antifibrinolytic Activity, Units per m . of Plasma (Avg.)
None (Control)	—	65	104
8	10 min.	3	78
12	10 min.	6	71
16	10 min.	3	60
24	10 min.	3	64
40	10 min.	3	51
40	30 min.	3	40
40	2 hrs.	3	45
40	5 hrs.	3	70
40	24 hrs.	3	121
40	48 hrs.	2	132

Table 4.—*Antifibrinolytic Activity of Plasma of Rabbits after Intracardiac Administration of Chymotrypsin*

Chymotrypsin (mg./Kg. Body Weight)	No. of Animals	Time After Chymotrypsin Injection	No. of Observations	Antifibrinolytic Activity, Units per ml. of Plasma (Avg.)
Normal	25	0	25	43
Control (1.0 ml. Krebs' Buffer)	3	15 min.	3	48
		24 hrs.	3	49
		48 hrs.	3	50
3	6	15 min.	2	41
		30 min.	2	47
		3 hrs.	2	43
15	9	15 min.	3	20
		1 hr.	2	24
		4 hrs.	2	26
		6 hrs.	2	26
		8 hrs.	2	48
		24 hrs.	3	58
		48 hrs.	7	57

units (table 1). This was 22.0 ± 2.4 units higher than the normal of 30 rats, 104 ± 8.4 units, exposed to room temperature, 22 C. The average antifibrinolytic titer in 43 normal human subjects was 122.0 ± 14.4 units per ml. of plasma (table 2). Of 93 determinations in men either exposed to or injured (frostbite) by cold, the average antifibrinolytic level was 150.8 ± 19.4 units. This repre-

sents an increase of 28.8 ± 2.9 units. No difference was noted between the subjects with frostbite and those with minor cold injury. Although the antifibrinolytic activity of the blood of the subjects uninjured by cold was not as high as those injured by cold, there were not sufficient determinations of the former to establish any statistical significance.

Administration of chymotrypsin to rats (table 3) and to rabbits (table 4) produced a lowering of the antifibrinolytic titer. This was achieved with 8 to 40 mg. per Kg. in the rat and less than 15 mg. per Kg. in the rabbit. These single doses maintained a lowered antifibrinolytic activity longer than 5 to 6 hours. At intervals of 24 and 48 hours after administration of the enzyme the antifibrinolytic titer was found to be above normal. The 40 mg. per Kg. dose in rats caused a mortality of 13 per cent. Animals which survived this dose exhibited pallor and mild flaccidity.

Discussion

The observation of an elevated antifibrinolytic titer in blood after exposure to cold possibly may be correlated with the development of intravascular occlusion observed in frostbite.[3] Guest, Daly, Ware and Seegers have reported an increased antifibrinolytic activity in certain clinical cases in some of which the possibility of thrombosis exists.[5]

The fibrinolysis-antifibrinolysis system is generally considered not to be strictly a part of the blood clotting process. However, one may consider blood clotting (fibrin formation) as a dynamic process which is continuously taking place at a restricted but definite rate. The comparatively rapid turnover of prothrombin, fibrinogen and platelets substantiates such an assumption. Fibrinolysis may be the natural physiologic means whereby this continuous fibrin formation is limited. In other words, there may exist a dynamic equilibrium between fibrin formation and fibrin lysis which maintains the fluidity of the blood.

It has been suggested that the normal integrity of the vascular wall may be dependent upon a normal process of fibrin formation.[6, 7] It may be possible that whenever this process is accelerated (decreased fibrinolytic activity, increased antifibrinolytic activity), intravascular clotting may occur, while in conditions when clot formation is retarded (increased fibrinolytic activity, decreased antifibrinolytic activity), abnormal bleeding may occur. The present observations that increased antifibrinolytic activity may be associated with intravascular clotting and the previous findings[1, 2] that decreased antifibrinolytic activity may be associated with hemorrhage seem to agree with the above concept.

The finding of a lowering of the antifibrinolytic activity after chymotrypsin administration indicates its possible efficacy in the dissolution of an intravascular clot.

Summary

Exposure of rats and of human subjects to cold was associated with an elevation of the antifibrinolytic activity of blood. Administration of chymotrypsin to rats and rabbits caused a decrease in the antifibrinolytic activity of blood The possible significance of these and earlier observations on the fibrinolysis antifibrinolysis system in blood is discussed.

REFERENCES

[1] Westphal, U., Kocholaty, W., McDonald, B., King, E. and Jensen, H.: Studies on proteolytic, fibrinolytic, antitryptic, and antifibrinolytic activities in the blood of rats. Proc. Soc. Exper. Biol. & Med. *75:* 862, 1950.

[2] Kocholaty, W., Ellis, W. W. and Jensen, H.: Effect of total body x-irradiation on plasmin inhibitor titer in blood of rats. Proc. Soc. Exper. Biol. & Med. *80:* 36, 1952.

[3] Lange, K., Weiner, D. and Boyd, L.: Frostbite: physiology, pathology and therapy. New England J. Med. *237:* 383, 1947.

[4] Gray, E. J., Volkringer, E. T., Chamovitz, D. L., Kocholaty, W. F. and Jensen, H.: Endocrine influence on the plasmin-plasmin inhibitor system in the blood of rats. In press.

[5] Guest, M. M., Daly, B. M., Ware, A. G. and Seegers, W. H.: A study of the antifibrinolysin activity in human plasmas during pathological states. J. Clin. Investigation *27:* 793, 1948.

[6] Nolf, P.: Contribution à l'étude de la coagulation du sang (5e memoire). La fibrinolyse. Arch. internat. de physiol. *6:* 306, 1908.

[7] Allen, J. G., Moulder, P. V., Enerson, D. M. and Glotzer, D.: The dynamics of coagulation. J. Lab. & Clin. Med. *34:* 1579, 1949.