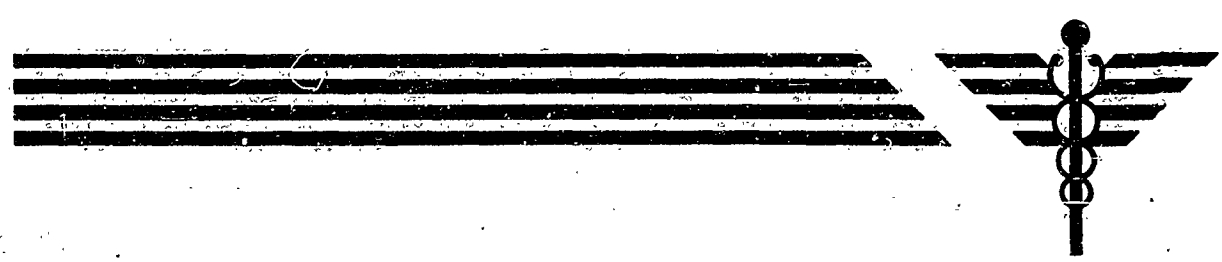
# ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 88
14 July 1952

## EFFECT OF COLD AND OF CHYMOTRYPSIN ADMINISTRATION ON THE ANTIFIBRINOLYTIC ACTIVITY OF BLOOD*

* Subtask under Environmental Physiology, AMRL Project No. 6-64-12 -028, Subtask, Enzyme, Endocrine and Metabolism Studies in Shock.



MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

REPORT NO. 88

# EFFECT OF COLD AND OF CHYMOTRYPSIN ADMINISTRATION ON THE ANTIFIBRINOLYTIC ACTIVITY OF BLOOD*

by

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from

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Report No. 88 14 July 1952
Project No. 6-64-12-028
Subtask AMRL S-11
MEDEA

ABSTRACT

# EFFECT OF COLD AND OF CHYMOTRYPSIN ADMINISTRATION ON THE ANTIFIBRINOLYTIC ACTIVITY OF BLOOD


## OBJECT

To continue previous investigations (1, 2) on the equilibrium between fibrinolysis and antifibrinolysis in blood during conditions of stress, the antifibrinolytic activity in the blood of rats and of human subjects was measured after exposure to cold. The effect of chymotrypsin administration on the antifibrinolytic activity in the blood of rats and of rabbits was also studied.

## RESULTS AND CONCLUSIONS

In rats, exposed to a temperature of +4°C for 18 days, it was found that the antifibrinolytic activity was increased by 20 per cent. It was observed that exposure of rats to +4°C for 18 days caused a fall in the plasma fibrinogen concentration of about 23 per cent.

In man, the normal antifibrinolytic titer was found to be 121 units per ml of plasma. In 93 determinations in men either exposed to or injured (frostbite) by cold the average antifibrinolytic titer was found to be 151 units. This was 25 per cent higher than the average control.

Administration of chymotrypsin to either rats or to rabbits produced a lowering of the antifibrinolytic titer, the degree and length of effect being dependent on the quantity of chymotrypsin administered.

The possible significance of these and earlier observations (1, 2) on the fibrinolysis-antifibrinolysis system in blood is discussed.

## RECOMMENDATIONS

Correlation between the fibrinolysis-antifibrinolysis level in blood and the tendency for thrombosis or hemorrhage should be established.

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# EFFECT OF COLD AND OF CHYMOTRYPSIN ADMINISTRATION ON THE ANTIFIBRINOLYTIC ACTIVITY OF BLOOD

## I. INTRODUCTION

Continuing previous investigations on the equilibrium between fibrinolysis and antifibrinolysis in blood during certain conditions of stress (1, 2), the antifibrinolytic activity in the blood of rats and of human subjects was measured after exposure to cold. "Antifibrinolytic activity" as used here is the resultant of the amounts of "fibrinolytic" enzyme and inhibitor present.

Because of the possible value in dissolving an intravascular clot, the effect of chymotrypsin administration on the antifibrinolytic activity of blood was studied in rats and rabbits.

## II. EXPERIMENTAL

### A. Cold exposure and antifibrinolytic activity.

Sprague-Dawley male albino rats averaging 200 grams were housed in a constant temperature room at +4°C for 18 days. A control group was kept at +22°C. Purina Laboratory Chow was fed to both groups. The "cold" animals gained an average of 20 grams and the control group, an average of 75 grams.

Blood (1.8 ml) was withdrawn by cardiac puncture into 0.2 ml of 3.8% sodium citrate. The sample was centrifuged at 2800 rpm 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 the 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, New York, during January and February, 1952. All of the men in the exercise had been intermittantly 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 4 groups: 1) controls, including men prior to cold exposure (43 total); 2) normal men 3 days after continuous cold exposure (6 total); 3) minor cold injury cases including those with either 1st degree frostbite or ill-classified cold condition of the feet (13 total); 4) patients with frostbite (7 had 4th degree injury, 2 had 3rd degree injury, and 65, 2nd degree injury).

B. Chymotrypsin and antifibrinolytic activity.

Crystalline salt-free chymotrypsin (Armour) was dissolved in Krebs' buffer (pH 7.4) and immediately administered intracardially to Sprague-Dawley male albino rats (average weight, 250 grams) and to New Zealand male albino rabbits (average weight, 2400 grams). Doses (0.5 to 1.0 ml) ranging from 8 to 40 mgm/kgm body weight were administered to rats and doses of 3 to 15 mgm/kgm body weight were administered to rabbits.

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

C. Determination of antifibrinolytic activity.

The antifibrinolytic activity was obtained by determining the residual fibrinolytic activity of a standard amount of trypsin after incubation with appropriately diluted unknown plasma samples, as previously described (2). Crystalline trypsin was used instead of plasmin for reasons of purity and reproducibility.

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

D. Fibrinogen determination.

The fibrinogen concentration in plasma was determined according to the method of Ratnoff and Menzie (3).

## III. RESULTS

Exposure of rats to +4°C for 18 days produced an increase of about 20% in the antifibrinolytic activity of the blood (Table I). The finding that exposure to cold causes a fall in the plasma fibrinogen concentration (Table I) agrees with similar observations of Henriques, Henriques and Selye (4).

The average antifibrinolytic titer in normal human subjects was 122 units per ml of plasma (Table II). Of 93 determinations in men either exposed to or injured (frostbite) by cold, the average antifibrinolytic level was 151 units. This represents an increase of approximately 25% above normal. No difference of statistical significance was noted between the injured and uninjured group after cold exposure.

Administration of chymotrypsin to rats (Table III) and to rabbits (Table IV) produced a lowering of the antifibrinolytic titer. This was achieved with 8 to 40 mgm/kgm in the rat and 15 mgm/kgm in the the rabbit. At intervals of 24 and 48 hours after administration of the enzyme the antifibrinolytic titer was found to be above normal. The 40 mgm/kgm dose in rats caused a mortality of 13%. Those which survived this dose exhibited pallor and mild flaccidity.

## IV. 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 (5). Guest, Daly, Ware and Seegers have reported an increased antifibrinolytic activity in certain clinical cases in some of which the possibility of thrombosis existed (6).

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 observed comparatively rapid turnover of prothrombin, fibrinogen, and platelets substantiates such an assumption. Fibrinolysis may be the natural physiological means whereby this continuous fibrin formation is limited. In other words, there may exist a dynamic equilibrium between fibrin formation and fibrinolysis 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 (7, 8). 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 that chymotrypsin produces a lowering of the antifibrinolytic activity indicates its possible efficacy in the dissolution of an intravascular clot.

## V. SUMMARY

Exposure of rats and of human subjects to cold produced an elevation of the antifibrinolytic titer in the 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.

## VI. RECOMMENDATIONS

Correlation between the fibrinolysis - antifibrinolysis level in blood and the tendency for thrombosis or hemorrhage should be established.

## VII. BIBLIOGRAPHY

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TABLE I

THE CHANGES IN THE ANTIFIBRINOLYTIC ACTIVITY AND FIBRINOGEN CONCENTRATION IN PLASMA OF RATS EXPOSED TO COLD

| No. of Rats | Exposure | | Antifibrinolytic Activity, Units per ml of Plasma (Aver.) | Fibrinogen mgm/100 ml of Plasma |
|---|---|---|---|---|
| | Temperature | Duration | | |
| 30 | + 4°C | 18 days | 127 ± 10.2 | 211* |
| 30 | +22°C | 18 days | 105 ± 8.4 | 274* |

* Average value obtained for 10 rats.

TABLE II

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 Lab. 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.

TABLE III

ANTIFIBRINOLYTIC ACTIVITY OF RAT PLASMA AFTER INTRACARDIAC ADMINISTRATION OF CHYMOTRYPSIN

| Chymotrypsin (mgm/kgm body weight) | Time After Chymotrypsin Injection | No. of Animals | Antifibrinolytic Activity, Units / ml of Plasma (aver.) |
|---|---|---|---|
| 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 IV

ANTIFIBRINOLYTIC ACTIVITY OF RABBIT PLASMA AFTER

INTRACARDIAC ADMINISTRATION OF CHYMOTRYPSIN

| Chymotrypsin (mgm./kgm. body weight) | No. of Animals | Time After Chymotrypsin Infection | No. of Determinations | Antifibrinolytic Activity, Units / ml of Plasma (aver.) |
|---|---|---|---|---|
| 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 |

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Army Medical Research Lab., Fort Knox, Ky. (Report No. 88)

Effect of Cold and of Chymotrypsin Administration on the Antifibrinolytic Activity of Blood - AMRL Project No. 6-64-12-028

Volkringer, Evelyn T.; Chamovitz, David L.; Gray, Elizabeth and Others
14 July'52 12pp. tables

Blood - Coagulation
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Chemistry (52)
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*Blood Coagulation
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(over)

DIVISION: Chemistry (52)
SECTION: Biochemistry (5)
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