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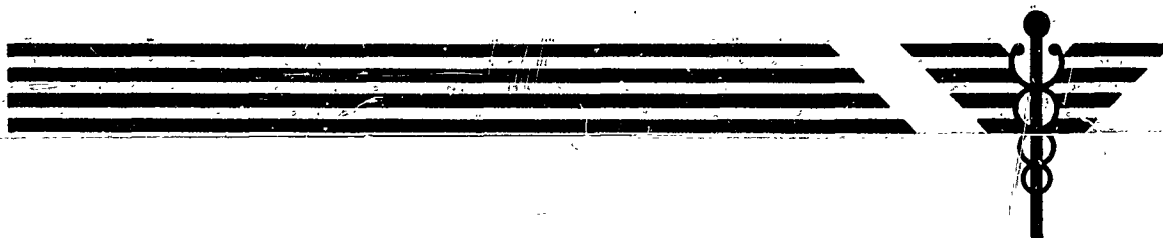
FORT KNOX, KENTUCKY

REPORT NO. 92

1 August 1952

ENDOCRINE INFLUENCE ON THE PLASMIN - PLASMIN
INHIBITOR SYSTEM IN THE BLOOD OF RATS*

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



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REPORT NO. 92

ENDOCRINE INFLUENCE ON THE PLASMIN - PLASMIN
INHIBITOR SYSTEM IN THE BLOOD OF RATS*

by

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from

ARMY MEDICAL RESEARCH LABORATORY
FORT KNOX, KENTUCKY
1 August 1952

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

Report No. 92
Project No. 6-64-12-028
Subtask AMRL S-11
MEDEA

1 August 1952

ABSTRACT

ENDOCRINE INFLUENCE ON THE PLASMIN - PLASMIN INHIBITOR SYSTEM IN THE BLOOD OF RATS

OBJECT

Earlier studies have indicated that the equilibrium of the plasmin - plasmin inhibitor system in blood can be shifted under various experimental conditions (1,2,3,4). The present study is concerned with this system as influenced by cortisone, adrenocorticotropin, thyroxine and thyrotropin and by splenectomy, adrenalectomy, and hypophysectomy.

RESULTS AND CONCLUSIONS

The degree of combination between plasmin and plasmin inhibitor was found to depend on the incubation time of the reactants.

Daily injections of adrenocorticotropin for 3 days produced an increase in antifibrinolytic activity of the blood as measured after 5 or 30 minutes incubation. Thyrotropin, given for 5 days, produced similar though less striking results. Cortisone and thyroxine, administered for 3 and 4 days respectively, produced a slight elevation of the antifibrinolytic activity which was evident only after 30 minutes incubation. Single intraperitoneal injections of adrenocorticotropin, of adrenal cortical extract, and of thyroxine were found to produce no significant change in the plasmin inhibitor titer 15 to 90 minutes after administration.

The antifibrinolytic activity of rat plasma 30 days after removal of the hypophysis or of the adrenals was found to be greatly reduced. Splenectomy did not alter the plasmin inhibitor level of plasma. In splenectomized rats, adrenocorticotropin administration did not produce any change in the antifibrinolytic activity of the blood. Apparently, the influence of the adrenals on the plasmin inhibitor titer is mediated through the spleen. On the other hand, administration of thyroxine to the splenectomized rat was found to produce a slight elevation of the antifibrinolytic titer, suggesting that more than one mechanism is responsible for the control of the plasmin - plasmin inhibitor equilibrium in blood.

The results obtained seem to substantiate the concept that the observed increased antifibrinolytic activity is a result of an actual increase in the plasmin inhibitor in the blood rather than a result of an acceleration of the rate of combination between plasmin and plasmin inhibitor.

RECOMMENDATIONS

The role of the spleen in maintaining the equilibrium between plasmin and plasmin inhibitor in blood should be studied.

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ENDOCRINE INFLUENCE ON THE PLASMIN - PLASMIN INHIBITOR SYSTEM IN THE BLOOD OF RATS

I. INTRODUCTION

Earlier studies have indicated that the equilibrium of the plasmin (fibrinolysin) - plasmin inhibitor (antifibrinolysin) system in blood can be shifted under various experimental conditions (1,2,3,4). The present study is concerned with the plasmin - plasmin inhibitor system as influenced by cortisone, adrenocorticotropin, thyroxine and thyrotropin, and by splenectomy, adrenalectomy, and hypophysectomy. According to Ungar and his associates (4,5) the action of certain endocrine principles on the antifibrinolytic activity of serum appears to result in an acceleration of the rate of combination of plasmin and plasmin inhibitor and not in an actual increase in the plasmin inhibitor. However, the findings presented in this report do not seem to substantiate this concept.

II. EXPERIMENTAL

Male Sprague - Dawley albino rats weighing 220-250 grams were used. The animals were allowed food and water throughout the experiment. Unless noted all injections were given subcutaneously. Normal controls received no injections, while injected controls received a volume of solvent equal to that used in the hormone injections.

Splenectomy was performed on fasting rats anesthetized by ether. Animals regained their normal weight within the second or third post-operative day. The experimental work was carried out 7 days after splenectomy. Hypophysectomized and adrenalectomized rats were obtained from the Hormone Assay Laboratories, Inc., Chicago, Illinois.

The hormones were injected subcutaneously and the following preparations and dosages were used:

Adrenocorticotropin, Armour Acthar, equivalent to 1.0 mg Standard LA-1-A/kg twice daily for 3 days; controls - equivalent volume of 0.9% saline.

Cortisone, Merck Cortone Acetate, 5 mg/kg daily for 3 days; controls - equivalent volume of 0.9% saline.

Thyroxine, Squibb Crystalline Thyroxine, 0.5 mg/kg daily for 4 days; controls - equivalent volume of slightly alkaline 0.9% saline.

Thyrotropin*, Armour Lot 2-D-3, LJW3-16-48 containing 5 Junkman - Schoeller units per mg, 10 mg/kg daily for 5 days; controls - equivalent volume of slightly alkaline 0.9% saline.

Blood samples, 1.8 ml, were taken by heart puncture 2 hours after the last injection, using 0.2 ml of 3.8% sodium citrate as the anticoagulant. Following centrifugation at 2800 rpm for 30 minutes at 5 to 10°C, the plasma was separated and analyzed immediately or stored frozen until tested.

The antifibrinolytic activity of plasma was determined by measuring the residual activity of a standard amount (2 mg**) of bovine plasmin***, after incubation for 5 and 30 minutes with a 4% concentration of the plasma to be tested, as previously described (1). Thrombin, salt-free, (Parke-Davis) and fibrinogen prepared according to Ware, Guest, and Seegers (6) were used. Calculations of antifibrinolytic units were performed as previously reported (7).

The plasmin inhibitor preparation used in these studies was obtained by $(\text{NH}_4)_2\text{SO}_4$ fractionation of a product prepared from bovine plasma according to Loomis, Ryder, and George (8). When tested against standard trypsin, it was found that 60 gamma of this preparation were equivalent to 1 antifibrinolytic unit.

III. RESULTS AND DISCUSSION

In substantiation of earlier observations by Christensen and MacLeod (9) and by Lewis and Ferguson (10), it was found that the inhibition of plasmin required a higher concentration of plasmin inhibitor than is necessary to produce the same percentage of inhibition with trypsin of equal fibrinolytic potency. In agreement with Christensen and MacLeod (9), it also was found that the kinetics of plasmin and trypsin in the presence of plasmin inhibitor were quite distinct. The experimental data of these observations have been omitted since they merely substantiate the findings of the above investigators.

* This preparation was kindly supplied by the Armour Laboratories, Chicago, Illinois.

** This amount of plasmin dissolved the standard fibrin clot in 125 seconds at 37.5°C.

*** Preparations of bovine plasmin were kindly supplied by Dr. E. C. Loomis of the Research Laboratories, Parke-Davis and Company, Detroit, Michigan.

The rate of combination between plasmin and its inhibitor was dependent on the time of incubation of the reactants; a 30 minute incubation produced a greater inhibition than did the 5 minute incubation, both in the case of rat plasma and a purified preparation of bovine plasmin inhibitor (Tables I and II). These findings are in agreement with similar observations reported by Guest, Daly, Ware, and Seegers (11). It was found that the inhibition of trypsin by normal rat plasma after 30 minutes incubation was somewhat greater than after 5 minutes incubation, but the difference was not as pronounced as that observed with plasmin.

TABLE I
INHIBITION OF BOVINE PLASMIN BY NORMAL RAT PLASMA

Plasma Concentration (%)	CDT (Seconds)*	
	5 minute incubation	30 minute incubation
0.0	123	126
0.5	129	136
1.0	135	147
1.5	145	160
2.0	153	175
2.5	166	195
3.0	182	216
3.5	203	249
4.0	234	305
4.5	275	355
5.0	314	420
5.5	382	528
6.0	455	610

TABLE II
INHIBITION OF BOVINE PLASMIN BY PLASMIN INHIBITOR

Plasmin Inhibitor Gamma	CDT (Seconds)*	
	5 minute incubation	30 minute incubation
0	128	129
20	130	138
40	131	145
60	134	154
80	137	163
100	139	166
120	142	177
140	143	193

*Each clot dissolution time (CDT) is the average of duplicate determinations

The antifibrinolytic activity of plasma of normal rats after 5 minutes incubation was 88 ± 8 units and after 30 minutes, 106 ± 6 units (Table III). The plasmas of control rats injected either with saline or alkaline saline had similar antifibrinolytic activities. The injection of adrenocorticotropin (2 mg/kg subcutaneously daily in 2 divided injections (4 hrs. apart) for 3 days) in normal rats produced an increase in the antifibrinolytic activity of the blood, as measured after 5 or 30 minutes incubation; 101 ± 12 and 119 ± 8 units, respectively, (Table III). Thyrotropin, given for 5 days, produced similar though less striking results. Cortisone and thyroxine, administered for 3 and 4 days, respectively, produced a slight elevation of the antifibrinolytic activity which was evident only after 30 minutes incubation.

TABLE III
EFFECT OF REMOVAL OF ENDOCRINE ORGANS AND OF VARIOUS HORMONES
ON THE ANTIFIBRINOLYTIC ACTIVITY OF RAT PLASMA

Treatment	No. of Animals	Incubation Time					
		5 Minutes			30 Minutes		
		CDT (Sec)	AFU/ml plasma Aver. $\pm$ S.D.	p*	CDT (Sec)	AFU/ml plasma Aver. $\pm$ S.D.	p*
Normal Animals							
No Treatment	13	234	88 ± 8		284	106 ± 6	
Saline	10	238	89 ± 9		295	109 ± 8	
Alkaline Saline	10	234	88 ± 8		291	108 ± 9	
ACTH	15	274	101 ± 12	.05	339	119 ± 8	.01
Cortisone	15	240	90 ± 13	>.1	311	113 ± 10	.1
Thyroxine	15	231	85 ± 9	>.1	318	115 ± 6	.02
TSH	15	255	96 ± 8	.1	311	113 ± 8	.1
Hypophysectomy	4				202	60**	
Adrenalectomy	4				230	76**	
Splenectomy***							
Saline Controls	3	239	89 ± 8		284	107 ± 9	
ACTH	9	254	94 ± 13	>.1	298	110 ± 8	>.1
Thyroxine	9	242	91 ± 8	>.1	327	117 ± 7	.02

CDT = Clot dissolution time

AFU = Antifibrinolytic units

* p = .1 considered significant

** Tested against standard trypsin after 30 minutes incubation only. The normal value = 104 AFU per ml of plasma.

*** Previous studies using standard trypsin showed no changes in antifibrinolytic titer after splenectomy.

Single intraperitoneal injections of adrenocorticotropin (1 to 4 mg per rat), of adrenal cortical extract (2 to 4 ml per rat), or of thyroxine (1 mg per rat) were found to produce no significant change in the plasmin inhibitor titer 15 to 90 minutes after administration.

The antifibrinolytic activity of plasma 10 days after removal of the hypophysis or of the adrenals was found to be greatly reduced (Table III). Splenectomy did not alter the plasmin inhibitor level of plasma (Table III). In splenectomized rats, adrenocorticotropin administration (same procedure as in normal animals) did not produce any change in the antifibrinolytic activity of the blood, as measured after 5 or 30 minutes incubation (Table III). Apparently, the influence of the adrenals on the plasmin inhibitor titer is mediated through the spleen. In this connection it may be pointed out that Macfarlane and Biggs (12) have observed that among all the organs, extracts of the spleen had the highest antifibrinolytic activity.

On the other hand, administration of thyroxine (same procedure as in normal animals) to the splenectomized rat was found to produce a slight elevation of the antifibrinolytic titer after 30 minutes incubation (Table III). Apparently, more than one mechanism is responsible for the control of the plasmin inhibitor level in blood.

In general, these findings are in agreement with Ungar's (4,5) observations of an increased antifibrinolytic activity after administration of adrenocorticotropin to normal animals and of no alteration in the plasmin inhibitor titer when administered to splenectomized animals. However, the results presented do not seem to substantiate the concept suggested by Ungar (4,5) that adrenocorticotropin effects the antifibrinolytic activity of plasma by accelerating the rate of combination between plasmin and plasmin inhibitor, rather than by increasing the plasmin inhibitor. Instead, the recorded data tend to show that the observed increased antifibrinolytic activity is a result of an actual rise of the plasmin inhibitor in the blood. However, it should be pointed out that the observed increase in antifibrinolytic activity may also be due to a decrease in fibrinolytic activity. The test employed did not take into account a decrease or increase in fibrinolytic activity, which is always marked by the excess of anti-plasmin over plasmin (or plasminogen) in normal blood.

IV. SUMMARY

The degree of combination between plasmin and plasmin inhibitor was found to depend on the incubation time of the reactants.

Administration of adrenocorticotropin produced a definite increase in antifibrinolytic activity of blood. The injection of cortisone, thyroxine, or thyrotropin caused a slight elevation of the plasmin inhibitor in blood.

The antifibrinolytic activity of plasma of rats, 30 days after removal of the hypophysis or of the adrenals, was found to be greatly reduced. Splenectomy did not alter the plasmin inhibitor level of plasma. In splenectomized rats, adrenocorticotropin administration did not produce any change in the antifibrinolytic activity of the blood. On the other hand, administration of thyroxine to splenectomized rats was found to produce a slight elevation of the antifibrinolytic titer.

V. RECOMMENDATIONS

The role of the spleen in maintaining the equilibrium between plasmin and plasmin inhibitor in blood should be studied.

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Army Medical Research Lab., Fort Knox, Ky. (Report No. 92)

Endocrine Influence on the Plasmin - Plasmin Inhibitor System in the
Blood of Rats - AMRL Project No. 6-64-12-028

Gray, Elizabeth; Volklinger, Evelyn T.; Chamovitz, David L. and Others
1 Aug'52 10pp. tables

Blood - Coagulation
Hormones

Chemistry (52)
Biochemistry (5)

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DIVISION: Chemistry (52)

SECTION: Biochemistry (5)

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