REGULATION OF THE LIQUID STATE OF THE BLOOD IN THYMECTOMIZED RATS AFTER INTRAVENOUS INJECTION OF THROMBIN

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According to recently published reports immunogenesis is linked with the blood clotting system and fibrinolysis [4, 12, 14, 15]. However, information on the role of factors of cellular and humoral immunity in the regulation of the liquid state of the blood is not to be found in either the Soviet or the non-Soviet literature. Yet it was shown comparatively recently that removal of the thymus leads to the development of hypercoagulation [5]. It can accordingly be postulated that in thymectomized animals the regulation of blood clotting and of fibrinolysis is disturbed. This investigation was devoted to a study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 30 noninbred albino rats aged 5-6 months. The thymus was removed from 15 rats at the age of 2-3 months, but no operation of any kind was performed on the rest. Preliminary experiments conducted by members of the staff of the department on 100 rats undergoing a mock operation (thoracotomy without removal of the thymus) showed no abnormality in the blood clotting system or in fibrinolysis in these animals 2 weeks after the operation.

The program of the experiments was as follows. A thymectomized and a control rat were used in the experiment on the same day. The animals were tied to a frame and 2.5 ml blood was removed from the jugular vein. The same volume of blood was again taken 30 min after slow intravenous injection of thrombin in a dose of 100 units/kg body weight. The blood clotting time [10], plasma recalcification time [9], prothrombin time [14], thrombin time [8], fibrinogen concentration [7], and fibrinolysis [11] were determined. Because of the small size of the experimental animals, some of the methods used (determination of the fibrinogen concentration, the euglobulin lysis time) were modified: The volume of blood for plasma used and also the reagents added were taken in half the quantities.

The numerical results were subjected to statistical analysis for independent observations.

EXPERIMENTAL RESULTS

The rats developed hypercoagulation 3-4 months after thymectomy and their fibrinogen level rose. The rest of the indices studied showed no significant changes. The blood clotting time and plasma recalcification time were lengthened in both intact and thymectomized rats 30 min after the injection of thrombin (100 units/kg body weight), the fibrinogen concentration was lowered, the prothrombin and thrombin times were increased, and fibrinolysis was activated. However, the intensity of the reactions differed in the thymectomized and intact animals. In intact rats the blood clotting time and thrombin time were lengthened after injection of thrombin a little more, and fibrinolysis was activated considerably more than in the thymectomized rats, in which the protective reactions in response to injection of thrombin were much weaker. Other differences also were found. Often after injection of thrombin the thymectomized rats developed apnea, to be followed after a short time by dyspnea. The control animals responded to the injection by vigorous vocal and motor reactions, but

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TABLE 1. Changes in Blood Clotting in Intact and Thymectomized Rats after Intravenous Injection of Thrombin (100 units/kg body weight)

Index studied	Statistical index	Control		Expt.	
		1	2	1	2
Blood clotting time, sec	M	194,4	245,6	125,4	212,6
	$\pm m$	16,0	26,4	12,2	37,8
	P_1	—	<0,2	—	<0,2
	P_2	—	—	<0,03	0,4
Recalcification time, sec	M	81,2	99,6	73,6	92,9
	$\pm m$	5,4	9,1	5,1	12,4
	P_1	—	<0,2	—	<0,2
	P_2	—	—	0,3	0,5
Prothrombin time, sec	M	22,0	28,4	20,9	24,8
	$\pm m$	1,8	3,1	1,6	2,7
	P_1	—	<0,1	—	0,3
	P_2	—	—	0,5	0,4
Fibrinogen concentration, mg %	M	307,6	216	370	263
	$\pm m$	18,3	21,6	21,3	34
	P_1	—	<0,01	—	<0,03
	P_2	—	—	<0,5	0,3
Thrombin time, sec	M	30,4	44,0	31,0	37,0
	$\pm m$	0.2	6,3	0,7	2,9
	P_1	—	<0,05	—	<0,1
	P_2	—	—	0,5	0,3
Euglobulin lysis time, min	M	166,0	71,3	162,0	98,0
	$\pm m$	18,0	9,0	15,9	13,0
	P_1	—	<0,001	—	<0,05
	P_2	—	—	0,5	<0,1

Legend. 1) Before injection of thrombin, 2) 30 min after injection; P_1) significance of differences between indices in control and thymectomized rats before and after injection of thrombin, P_2) significance of differences between indices before and after injection of thrombin in control and in experiment.

this type of response was not always given by the thymectomized rats. In the control group only one animal died during the first hour after injection of thrombin (the rest tolerated the experimental procedures relatively well), whereas six rats of the thymectomized group died ($\chi^2 = 4.28$; $P < 0.05$).

An attempt was made to study changes in the circulation in the intact and thymectomized rats after injection of thrombin. For this purpose the animals were anesthetized with phenobarbital, laparotomy was performed, and the mesentery was exposed and examined in transmitted light under a microscope (ocular 15, objective 40). In animals with an intact thymus, aggregates of blood cells formed after injection of thrombin (sludging syndrome) and fibrin clots appeared, dissolving fairly rapidly. In the thymectomized rats lysis of the clots was not observed, for after injection of thrombin the animals with an exposed mesentery died rapidly. Like other workers [1-3, 6], we observed the development of hypocoagulation and stimulation of fibrinolysis, due to utilization of blood clotting factors and an increase in the level of natural anticoagulants, after the injection of thrombin. At the same time the protective reaction after injection of thrombin was much weaker in the thymectomized rats, and this led to their more frequent death. For instance, the thrombin time was lengthened by a lesser degree in the thymectomized rats. This result cannot be explained by the greater fibrinogen consumption, for its concentration remained higher in the thymectomized animals. Consequently, the less marked lengthening of the prothrombin time in the experimental rats must be explained by a lower level of antithrombin.

Enzymic fibrinolysis was activated much less strongly in the thymectomized animals in response to injection of thrombin (54 ± 4% in the control, 36 ± 8% in the experimental animals; $P < 0.05$). The less marked stimulation of fibrinolysis in the thymectomized rats may perhaps lead to a very small increase in the level of fibrinogen and fibrin degradation products, which have an antithrombin action.

After injection of thrombin into the animals complex compounds of heparin with biologically active substances and hormones (thyroxine, adrenalin, noradrenalin, serotonin, etc.) are formed in the blood stream and have an anticoagulant and nonenzymic fibrinolytic action [3]. This protective reaction helps to save the animal's life. The possibility cannot be ruled out that thymus hormones can also form similar complexes. However, this problem was not studied.

We know that the thymus regulates the state of cellular immunity. After thymectomy the killer, helper, and suppressor functions of the T lymphocytes are disturbed. Meanwhile, when activated, T lymphocytes liberate a mediator which stimulates the release of plasminogen activator by monocytes and macrophages [13]. It can accordingly be postulated that this mechanism may play a definite role in the regulation of the liquid state of the blood in response to injection of thrombin; however, the possibility cannot be ruled out that the thymus participates in the regulation of blood clotting and fibrinolysis in other ways than through its action on T lymphocytes.

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