THE FUNCTION OF THE PHYSIOLOGICAL ANTICOAGULATION SYSTEM IN RETICULOENDOTHELIAL BLOCK

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Experimental data have been introduced in previous reports [2, 3, 4, 6], dealing with the existence and significance of a neuro-humoral anticoagulation system in the animal's organism; on the basis of this, a new schema has been worked out for the process of blood coagulation [5].

According to the data obtained, when thrombin appears in the circulating blood, it sets off a reflex arc via the chemoreceptors of the circulatory bed, leading to secretion of humoral agents into the circulating blood which block blood coagulation. Under these conditions, the disruption of blood coagulation in vivo is caused by the secretion of a relative excess of heparin or heparin-like substances into the blood, the release of a plasminogen activator, and a marked reduction in the concentration of fibrinogen. This defense reaction to the appearance of thrombin in the circulatory bed is doubtlessly dependent upon functioning of some cell or tissue structures located near the blood vessels, which secure the formation of the anticoagulatory humoral agents and their secretion into the blood stream.

In searching for the source of these effects, we undertook a study of the role of the reticuloendothelial system in the functioning of the physiological anticoagulation system.

EXPERIMENTAL METHOD

The experiments were carried out on white rats, weighing 150-160 grams. The animals were maintained on the usual natural laboratory ration. The functional state of the anticoagulation system in the experimental and control rats was ascertained by the reaction of the animal organism to the injection of an appropriate dose of thromboplastin solution into the jugular vein; the thromboplastin was obtained from rat brain tissue.

Blockade of the reticuloendothelial system was accomplished by the intravenous injection of a 0.1 or 0.5% solution of trypane blue in physiological saline. Using a Sahli hemometer, we determined the relative quantitative concentration of the stain in the oxalated blood plasma of the experimental rats, which was expressed in indices [1]. This permitted us an objective means of judging the degree of saturation of the reticuloendothelial system with the stain injected into the blood stream. Blood was drawn from the jugular vein, using a syringe washed with physiological saline, and quickly transferred to a test tube, which was placed in a water bath at 37° for determination of the time required for spontaneous coagulation.

The time required for fibrinolysis of the clot, in the blood taken from the jugular vein, was determined in a test tube at 37°, from the moment following its coagulation to the moment of complete resorption of the clot.

Determination of the thrombin time was carried out, using coagulation of the oxalated blood (0.2 ml) following the addition of 0.2 ml of thrombin solution.

EXPERIMENTAL RESULTS

A 0.1% solution of trypane blue was injected intravenously, using 2 ml at each injection. Three injections were given, at intervals of 48 hours. Determination of the stain concentration in the plasma was performed 4 and 24 hours after each injection. Table 1 shows that the maximum reduction in the index of the active stain saturation for the cells of the reticuloendothelial system took place after the 2nd injection. However, after the 3rd injection, a certain elevation of the indices appeared, signaling the onset of suppression of the RES (reticuloendothelial system).

Using a 0.5% stain solution in the experiments, and a dose of 2 ml, suppression of the reticuloendothelial system was observed 72 hours after a single injection.

TABLE 1. Degree of Saturation of the Circulating Blood with a 1% Solution of Trypane Blue

Interval of the blood analysis following each injection	Index of stain saturation following		
	1st injection	2nd injection	3rd injection
After 4 hours	1.7	1.23	1.25
After 24 hours	6.0	4.07	4.30

On the basis of the data obtained, the animals were used in the experiment at the appropriate intervals following the injections of trypane blue, when the reticuloendothelial system was in the blocked state, i.e., in the first case, 48 hours after the 2nd stain injection, and in the second case, 72 hours after the single injection of stain.

Into the jugular vein of these animals, we injected a solution of thromboplastin, obtained from rat brain and capable of clotting oxalated rat blood plasma, with recalcification and at 37°, within 19-20 seconds. Table 2 shows that with blockade of the reticuloendothelium system the mortality of the animals following intravenous injection of thromboplastin was 6 times higher than for the animals that received the same dose of thromboplastin without preliminary blockade of the reticuloendothelial system. The negative activity of thromboplastin on the animals with the reticuloendothelial system block has a specific character, and is connected with its thrombogenic properties.

TABLE 2. The Effect of Trypane Blue Induced Reticuloendothelial System Block on the Functional State of the Physiological Anticoagulation System

Nature of the trial	Number of animals	Thromboplastin dose (in ml)	Mortality of the animals (in %)
Reticuloendothelial system blockade + + intravenous injection of thromboplastin	87	1.0-1.3	37
Reticuloendothelial system blockade + + injection of inactivated thromboplastin	15	1.0-1.3	0
Injection of thromboplastin without reticuloendothelial system blockade	79	1.0-1.3	6

It can also be seen from Table 2 that inactivation of the thromboplastin, by heating at 65° for 15 minutes, led to complete elimination of its action on the animals with the reticuloendothelial system blockade.

Coagulation studies on the blood taken from the remaining living experimental animals showed (Table 3) that, despite the reticuloendothelial system blockade, the defense reaction in them to the injection of thromboplastin into the blood stream was still carried out; this indicates that the functional state of the physiological anticoagulation system was sustained in their organism. However, if we judge by the degree to which blood coagulation was restored 1-1½ hours after the thromboplastin injection, the level of this defense reaction was lower than in the control animals, receiving the same dose of thromboplastin.

Therefore, in the animals with the reticuloendothelial system blockade the anticoagulation reaction was manifested to a lesser degree.

Table 4 shows that in the experimental animals, clot resorption occurred somewhat later than in the control animals (without reticuloendothelial system blockade), which is evidence that the fibrinolytic activity in the experimental animals, following injection of the thromboplastin, increased to a lesser degree than in the control.

As an example of one of the experiments, Table 5 shows the data from determination of the level of heparin, using the thrombin time following incubation with protaminsulfate, in animals with reticuloendothelial system blockade who have been injected with thromboplastin. It is apparent from Table 5 that the thrombin time after incubation with protaminsulfate was lower in the animals with reticuloendothelial system blockade. These data obviously indicate that in animals with reticuloendothelial block the injection of thromboplastin is followed by less secretion of heparin or heparin-like substances into the circulatory bed than in animals without the block.

TABLE 3. Coagulation Time of Whole Blood, Taken 1½ Hours after the Injection of Thromboplastin, in Rats with Reticuloendothelial System Blockade (Mean Data)

Group of animals	Number of animals	Dose of thromboplastin (in ml)	Coagulation time of the whole blood (in seconds)	
			before injection of the thromboplastin	1½ hours after injection of the thromboplastin
Experimental (with reticuloendothelial system blockade)	16	1-1.3	65	93
Control	20	1-1.3	68	113

TABLE 4. Time Required for Complete Fibrinolysis of Blood Taken 1-1½ Hours after Injection of Thromboplastin in Rats with RES Blockade

Character of the experiment	Number of animals	Time required for complete fibrinolysis (in seconds)
Reticuloendothelial system blockade + injection of thromboplastin	27	342
Injection of thromboplastin without reticuloendothelial system blockade	33	218

TABLE 5. Thrombin Time of Whole Blood before and after Incubation with Protaminsulfate (0.2 ml of Oxalated Blood + 0.2 ml of a Thrombin Solution + 0.03 ml of a 0.2% Solution of Protaminsulfate) in Those Animals with Reticuloendothelial System Blockade that Received a Thromboplastin Injection

Character of the experiment	Number of animals	Thrombin time (in seconds)	
		before incubation with protaminsulfate	after incubation with protaminsulfate
Reticuloendothelial system blockade + thromboplastin injection	5	> 1800	68
Injection of thromboplastin without reticuloendothelial system blockade	5	> 1800	141

The data obtained serve as evidence that with blockade of the animals' reticuloendothelial system the reaction of the physiological anticoagulation system is somewhat reduced. It is known, from the available literature, that one cannot cause complete blockade of the reticuloendothelial system, even by the use of various means. In this case, it has been shown that a varying degree of reticuloendothelial block leads to a varying change in its function, depression or activation [7]. In line with this, in experiments with blockade one must take into consideration the tremendous variability in the functional state of the reticuloendothelial system within the individual experimental animals, which can influence the final results of the trial. The histological control showed that with blockade induced by the 0.1% stain, solution the trypane blue was deposited in the Kupfer cells of the liver. With blockade by the 0.5% stain solution deposition of trypane blue was noted in the Kupfer cells of the liver and in a large number of connective tissue histiocytes, and minimal deposition was seen in the spleen. In the renal tubule cells we observed the processes of excretion of the injected stain.

SUMMARY

Trypane blue block of reticuloendothelium in rats provoked some reduction in the protective function of the physiological anticoagulation system. Mortality rate after intravenous administration of thromboplastin (in moderate doses), obtained from the brain tissue of rats, is considerably great in animals with reticuloendothelial block than that in the controls receiving the same thromboplastin doses. The protective reaction of the organism to the thromboplastin administered in the jugular vein is less pronounced during reticuloendothelial block, the rise of the blood fibrinolytic activity in response to the administered thromboplastin being less marked in the experimental animals than in the controls. As compared to controls thrombin time of blood subjected to preliminary incubation with protaminsulfate is reduced in the experimental animals, pointing to a lesser discharge or of heparin-like substances in their blood. The restoration of the normal blood coagulation time in the surviving experimental animals was relatively more rapid than in controls.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
