THE FUNCTIONAL INHIBITION OF THE PHYSIOLOGICAL ANTICOAGULANT SYSTEM CAUSED BY THE FEEDING OF AN ATHEROGENIC DIET

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(Presented by Active Member, Academy of Medical Sciences, USSR, S. E. Severin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 10, pp. 26-30, October, 1961
Original article submitted November 20, 1960.

It has been established in previous communications [2-9] that the preservation of the blood circulating in the mal body in a liquid state depends on the function of the neurohumoral anticoagulant system. The function of this system is based on the following principle: whenever thrombin appears in the blood stream in a concentration threatening the animal's life, the chemoreceptor apparatus of the blood vessels sends an impulse along the reflex arc; this impulse evokes an effector act, consisting in the excretion into the circulation of substances which prevent the coagulation of the blood. These substances include, in particular, heparin and heparin-like agents as well as activators of the fibrinolytic process. In case of an experimentally produced dysfunction of the physiological

TABLE 1. The Functional State of the Physiological Anticoagulant System in Rats, Kept for 45 Days on an Atherogenic Diet. (Average Values.)

Conditions of experiment	Average weight of the animals (in g)	Dose of the intravenous thrombin injection (in ml)	Number of animals		
			Total No. of experimental animals	Number of surviving animals	Number of animals perished due to thrombosis
Experimental group : animals kept for 45 days on a fat-cholesterol diet.	228	0.5 - 1.0	16	7	9
Control group: animals kept on the natural laboratory diet.	221	0.5 - 1.0	17	14	3

anticoagulant system [5, 7] the appearance of thrombin in the circulating blood fails to elicit a defence reaction and culminates in generalized thrombosis. In view of these findings we took an interest in the investigations of Wilgram [16, 17] and also of Thomas and Hartroft [15] which authors observed spontaneous myocardial infarction in rats fed on a diet, rich in fat and cholesterol.

We assumed that the atherogenic diet used in the experiments mentioned above, exerts an inhibitory effect upon the function of the protective physiological anticoagulant system; this creates a prethrombotic state, finally complicated by myocardial infarction.

In the present paper we set ourselves the task to verify the above working hypothesis in animal experiments.

EXPERIMENTAL METHOD

The experiments were carried out on sexually mature male white rats. Healthy animals, weighing between 280 and 350 g were kept, instead of the natural laboratory ration, on a diet representing a slight modification of

the mixture used by Wilgram [16, 17]; actually two variants of this diet were used by us.

The first variant – egg powder: 35 parts, 30% wheaten meal: 10 parts, soybean (meal): 10 parts, (beet-) sugar: 10 parts, bran: 6 parts, dripping: 25 parts, salt mixture*: 2 parts, vitamin-sugar mixture: 1 part, fat-soluble vitamins: 1 part; to this mixture we further added cholesterol: 1%, methyl-thiouracil: 0.3%, a preparation called "chologon" (dehydrocholic-acid): 0.2%, vitamin D_2 (commercial preparation): 1400 units per kg. diet, vitamin E (commercial preparation): 60 mg per kg diet, vitamin B_{12}**: 30μg per kg diet.

The second variant – the ingrediants of the diet were the same as in the first variant with the exception of the vitamin-sugar mixture, which was replaced by dried brewer's yeast, added daily to the ration in a dose of 60 g yeast per kg diet.

TABLE 2. Depression of the Function of the Physiological Anticoagulant System in Rats, Kept for a Prolonged Period on an Atherogenic Diet -(Average Values).

Conditions of experiment	Average weight of the animals in different groups (in g)	Dose of the intravenous thrombin[1] injection (in ml)	Number of Animals		
			Total No. of experimental animals	Number of surviving animals	Number of animals perished due to thrombosis
Experimental group: animals kept between 5 and 7½ months on a fat-cholesterol diet.	221 - 276	1.0 - 1.2	25	3	22
Control group 1: animals kept on a natural laboratory diet.	223 - 276	1.0 - 1.2	25	22	3
Control group 2: animals kept for 5 months on a fat-cholesterol diet.					
Thrombin inactivated by heating.	251	1.7(inactivated)	12	12	0

[1] The thrombin solution coagulated an equal volume of blood at 37° C in 5 sec.

TABLE 3. Changes in the Fibrinogen Concentration and in the Fibrinolytic Activity of the Blood in Rats Kept on an Atherogenic Diet (Average Values)

Conditions of experiment	Number of animals	Fibrinogen concentration		Fibrinolytic activity (in %)
		in mg %	in relative %	
Experimental group: animals kept for 5-8 months on a fat cholesterol diet. . . .	40	433.4	142.4	7.5
Control group: animals kept on a natural laboratory diet.	40	312.7	100.0	48.0

TABLE 4. Changes in the Heparin Tolerance of the Plasma in Rats Kept on an Atherogenic Diet (Average Values).

Conditions of experiment	No. of animals		Average time required for the formation of a solid clot in recalcified plasma in the presence of heparin	
	Experimental	Control	experimental group	control group
The experimental animals were kept between 5 & 7½ months on a fat-cholesterol diet; the control animals were kept on the natural laboratory diet	5	5	3 min 49 sec	22 min 48 sec
	5	5	3 min 25 sec	16 min 25 sec
	6	6	2 min 25 sec	10 min 08 sec
	7	7	3 min 08 sec	7 min 31 sec
	7	7	4 min 46 sec	12 min 35 sec
	8	7	6 min 28 sec	16 min 56 sec
	8	7	5 min 44 sec	8 min 24 sec

* According to Best [10] – the vitamin sugar mixture was daily added to the ration.

** Vitamin B_{12} was daily added to the diet before the feeding time.

Male white rats of the corresponding weight and kept permanently on the natural laboratory diet served as control animals to the experimental group. The blood samples were taken by means of a syringe from the jugular vein. Intravenous injections of various substances used for the experiments were administered by the same route. The total blood coagulation time in the experimental and in the control animals respectively was established in glass test tubes (of 8 mm diameter), without addition of any reagents, in a water bath at 37° C. The activity of the thrombin used for the experiments (a commercial preparation made from horse blood and a pure laboratory preparation) was arbitrarily assessed on the basis of the coagulation time (in sec) at 37° C of oxalated rat blood mixed with an equal volume of thrombin; the activity of the thromboplastin (prepared from the brain of rats) was assessed on the basis of the coagulation time (in sec) at 37°C of oxalated rat blood (0.1 ml) in the presence of a 0.025 M $CaCl_2$ solution (0.1 ml) after addition of 0.1 ml thromboplastin extract. The fibrinolytic activity of the plasma and fibrinogen concentration were estimated by the method of Bidwell [11]; the total blood cholesterol level by the method of Grigant [13]. The heparin tolerance of the plasma was estimated by the method of Gormsen [12]. The trypsin inhibitor was isolated from soy beans by the method of Kunitz [14].

TABLE 5. The Protective Effect of Trypsin Inhibitor Against Thrombus Formation Caused by Intravenous Injection of Lethal Thromboplastin Doses in Rats Kept for 5 Months on an Atherogenic Diet.

Conditions of experiment	Dose of a 5% trypsin inhibitor solution (intravenously, in ml)	Dose of thromboplastin (in ml)	Average weight of the animals (in g)	Number of animals		
				Total No. of experimental animals	Number of surviving animals	Number of animals perished due to thrombosis
Experimental group: (fat-cholesterol diet). . .	1.4	2.1	254	10	10	0
Control group: (fat-cholesterol diet). . .	0.	2.1	253	10	0	10

EXPERIMENTAL RESULTS

Throughout the experiment (up to 8 months), no appreciable difference in the development of pathological symptoms could be found between the rats kept on the first variant, and those kept on the second variant of the experimental diet respectively; for this reason the results obtained with both variants of the diet were analysed and discussed together. All animals kept for a prolonged period on the experimental diet gradually lost weight. Autopsy revealed in the experimental rats the accumulation of large quantities of fat under the peritoneum.

As reported in our earlier publications [2-9], rapid intravenous injection of moderate doses of thrombin solution fails to cause thrombosis in the majority of healthy animals and leads to a temporary ceasing in the function of the blood coagulating system. This defence reaction, however, does not take place, if the function of the physiological anticoagulant system is impaired [2-9]. On the basis of these findings we used intravenous injections of thrombin solutions to assess the functional state of the physiological anticoagulant system in the experimental animals, depending on the length of time for which they had been kept on the atherogenic diet. The first experiment, consisting in the intravenous injection of thrombin, was carried out after the animals had been kept for 45 days on the experimental diet. The data set forth in Table 1 show that by that time a certain degree of inhibition in the function of the physiological anticoagulant system can already be observed in the rats kept on the atherogenic diet.

After the experiment had been continued for 5 months (and more) the fat-cholesterol diet inhibited function of the physiological anticoagulant system to a considerable degree, and this created a prethrombotic state in the animal body. The data set forth in Table 2 show that, in these animals, intravenous injection of moderate thrombin doses causes instantaneous death due to thrombosis, whereas the control animals survive intravenous injections of similar thrombin doses.

In the animals in which a prethrombotic state had been created, we found an increased fibrinogen concentration and a marked fall in the fibrinolytic activity of the plasma (table 3). At the same time the experimental animals were characterized by an increased herapin tolerance of the plasma (Table 4). The total blood cholesterol level showed a 10-fold increase (experimental group – 943 mg%, control group – 95 mg%).

The above findings enable us to state that the described syndrome- increased fibrinogen concentrations, a fall in the fibrinolytic activity and an increase in the herapin tolerance of the plasma – can be regarded as diagnostic for

the prethrombotic state.

The results of the experiments described above show that the atherogenic diet creates a prethrombotic state in the animal body. Experimental studies concerning the function of the physiological anticoagulant system have shown, however, that the prethrombotic state turns into an actual state of thrombosis only if adequate quantities of thrombin are present in the circulating blood [2-9].

As (under normal conditions) the formation of thrombin in the blood stream is prevented by the lack of thrombokinase, it is obvious that, to achieve a transition from the prethrombotic state into an actual state of thrombosis, thromboplastin must be present in the circulating blood. In consequence it is possible, in principle, to prevent the formation of a thrombus in an animal, in which a prethrombotic state has been created, by means of agents which inhibit the formation of thrombokinase or neutralize thrombokinase present in the blood. To verify this assumption we used, on the basis of data at our disposal [1], the trypsin inhibitor, obtained from soy beans. The data set forth in Table 5 show that intravenous injection of trypsin inhibitor, 5 min before the injection of thromboplastin, prepared from rat brain tissue, exerted a significant prophylactic effect against thrombus formation, notwithstanding the prethrombotic state, created by the atherogenic diet.

Our experimental findings show that the myocardial infarction occurring in rats kept on the atherogenic diet of Wilgram [16] and Thomas [15] is caused by the prethrombotic state developing in the animals; this state is produced by the inhibition of the physiological anticoagulant system.

The mechanism of the damaging effect exerted by the atherogenic diet upon the functional state of the physiological anticoagulant system is still obscure; it can be assumed, however, that, under the experimental conditions outlined above, pathological changes in the blood vessel wall, caused by the excess animal fat and cholesterol, may lead to a dysfunction of the vascular chemoreceptor apparatus : this may represent one of the possible factors which create the prethrombotic state. If the normal function of the chemoreceptor apparatus is impaired, the appearance of thrombin in the blood in concentrations threatening the animals' life, will fail to elicit the neurohumoral reflex act characteristic for the protective function of the physiological anticoagulant system.

SUMMARY

Experimental investigations carried out in 1958 - 1959 (2-6) have demonstrated that fluid condition of the circulating blood is maintained in the animal body as a result of the neurohumoral anticoagulation system function. In the present work it was established that the diet enriched with animal fat, cholesterol and containing methylthiouracil (given for several months to rats) depresses the function of the physiological anticoagulating system. This phenomenon leads to prethrombotic state which may be followed by a thrombotic condition if thrombin appears in the circulating blood, irrespective of the causes provoking the transformation of the blood plasma prothrombin into thrombin. In experimental prethrombotic state the following changes were detected : a rise of fibrinogen concentration, a marked reduction of the blood fibrinolytic activity and a rise of the plasma tolerance to heparin. The combination of the aforementioned signs may be used for the diagnosis of the prethrombotic state. There is evidence that in this condition caused by depression of the anitcoagulating blood system as a result of atherogenic diet trypsin inhibitor for soy beans a prophilactic action against the thrombus formation provoked by intravenous thromboplastin injection takes place. Experimental data obtained show that development of myocardial infarction in rats on atherogenic diet (Wilgram 10) was caused by the prethrombotic state appearing in the experimental animals, which is the sequence of depression of the physiological anticoagulating system function [2-9].

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