with intraperitoneal injection of glutathione in a dose of 38 mg/100 g body weight and unithiol in a dose of 25 mg/100 g body weight showed that Na,K-ATPase can be reactivated in the preclinical period of poisoning, but only by unithiol. Each preparation was injected immediately after intraperitoneal injection of the toxin.

The results of these experiments thus indicate inhibition by BT of active transport of monovalent cations through erythrocyte membranes and that under certain conditions this effect can be abolished by donors of sulfhydryl groups.

LITERATURE CITED

1. A. T. Ivashchenko, Biokhimiya, No. 6, 1086 (1978).
2. V. K. Lishko, M. K. Malysheva, and T. I. Grevizirskaya, Biokhimiya, No. 1, 60 (1974).
3. V. V. Mikhailov and V. V. Mikhailov, Byull. Éksp. Biol. Med., No. 11, 21 (1975).
4. V. V. Mikhailov and G. N. Barashkov, Byull. Éksp. Biol. Med., No. 6, 651 (1977).
5. V. V. Morrison, "The mechanism of changes in the functional state of striated muscle fibers in poisoning with botulinum and tetanus toxins," Candidate's Dissertation, Saratov (1971).
6. E. A. Chernitskii and A. V. Vorobei, Structure and Function of Erythrocyte Membranes [in Russian], Minsk (1981).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).

ELIMINATION OF AUTOLOGOUS CLOTTING FACTOR Xa FROM THE BLOOD STREAM OF INTACT AND THYMECTOMIZED RATS

N. N. Tsybikov, O. I. Tersenev, A. Sh. Byshevskii, B. I. Kuznik, S. G. Shmerkin, S. L. Galyan, and A. V. Kradenov

UDC 612.115.12-06:612.438

KEY WORDS: blood stream; intact and thymectomized rats; exogenous factor Xa.

The intensity of elimination of active enzymes from the blood stream is determined by various mechanisms: the state of the reticuloendothelial system, the velocity of interaction with substrates and inhibitors, and also the immune potential of the body. This last factor is confirmed by the writers' previous investigations, which demonstrated disinhibition of enzyme activity in the hemostasis system of thymectomized rats [2, 3, 6].

The object of the present investigation was to determine the rate of elimination of an active enzyme (factor Xa) from the blood stream of intact and thymectomized rats.

EXPERIMENTAL METHOD

Stuart–Prower factor (blood coagulation factor Xa) was isolated from pooled plasma obtained from 110 rats. The plasma was coagulated by the addition of thromboplastin (from Kaunas) and 1% $CaCl_2$ solution. The fibrin thus formed was removed and the enzyme isolated from the serum by ion-exchange chromatography on DEAE-Sephadex A-50 (from Pharmacia, Sweden), using stepwise elution gradients of phosphate buffer (0.05 M, pH 7.0; 0.2 M, pH 7.0; 0.45 M, pH 8.0). Fractions obtained with the last gradient of buffer were pooled and rechromatographed. The isolated preparation was desalted on Acrilex P-60 (from Reanal, Hungary) and lyophilized. The enzyme possessed BAME-esterase activity, converted prothrombin into thrombin in a prothrombin-factor Xa–Ca^{++} system, and was homogeneous on electrophoresis in polyacrylamide gel. Prothrombin was isolated from pooled bovine plasma by the method in [7]. Factor Xa was labeled with ^{131}I by the method described previously [5]. The specific radioactivity was 0.05 μCi/ml. The preparation completely preserved its enzymic properties for

Department of Normal Physiology, Chita Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.)

Translated from Byulleten' Éksperimental'noi i Meditsiny, Vol. 94, No. 9, pp. 31-33, September, 1982. Original article submitted March 18, 1982.

0007-4888/82/9409-1196$07.50

TABLE 1. Time Course of Disappearance of ^{131}I-Factor Xa from Blood Stream of Intact and Thymectomized Rats (in %)

Time of investigation of blood	Radioactivity of blood taken from caudal vein of rats	
	intact rats	thymectomized rats
5 min	100	100
1 h	65±6,0	55,9±5,8*
6 h	38,8±3,9	36,3±3,9
12 h	23,9±4,6	23,9±3,3
18 h	20,1±3,1	14,2±2,2*

Legend. Here and in Table 2, *P < 0.05.

TABLE 2. State of Blood Coagulation after Injection of Factor Xa into Intact and Thymectomized Rats

Parameter studied	Intact rats		Thymectomized rats	
	initial value	after injection of factor Xa	initial value	after injection of factor Xa
Clotting time, sec	236,8±13,8	168±10,2*	175,8±6,2	195,0±8,6*
Recalcification time, sec	60,3±5,6	56,4±4,1	52,6±3,2	69,1±2,2*
Thrombin time, sec	28,5±2,0	29,8±1,8	29,6±2,1	30,6±1,9
Total antithrombin activity, sec	17,5±1,0	19,5±1,2*	18,0±1,8	22,7±2,4*
Kaolin time, sec	36,0±3,2	33,4±2,1	33,0±1,7	36,4±2,0*
Cephalin time, sec	44,2±2,4	41,7±2,8*	41,8±13,9	44,6±2,6
Fibrinogen concentration, mg/ml	9,8±0,8	12,3±1,6*	12,6±0,7	10,2±0,7*

several hours after labeling. The ^{131}I-factor Xa was injected into the jugular vein of intact (n = 10) and thymectomized (n = 10) rats in a dose of 0.1 ml per animal. Blood was taken in a volume of 0.02 ml from the caudal vein after 5 min and 1, 6, 12, and 18 h and its radioactivity was determined. The content of ^{131}I-factor Xa 5 min after injection was taken as 100%. Parallel with the main experiment, labeled factor Xa was injected (0.1 mg per animal) into the jugular vein of intact (n = 10) and thymectomized (n = 10) rats of another group, and the state of blood coagulation was determined 10 min later. In the control group, physiological saline was injected into intact (n = 10) and thymectomized (n = 10) rats. At the end of the experiment (after 18 h) the rats were killed and radioactivity determined in extracts from the liver, kidney, spleen, brain, skeletal muscle, and aorta of the intact and thymectomized rats. Thymectomy was performed on the animals of the experimental group at the age of 4 weeks and they were used in the experiments 4 months after the operation. The numerical data were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The time course of disappearance of labeled factor Xa from the blood stream of intact and thymectomized rats is shown in Table 1. The concentration of factor Xa only 1 h after the beginning of the experiment was significantly lower in the blood stream of the immunodeficient rats. For the next 12 h the blood radioactivity level remained about the same in rats of the two groups, but toward the end of the experiment a second fall in the concentration of factor Xa was observed in the thymectomized animals.

The results were explained by a study of blood coagulation (Table 2). Intravenous injection of highly purified factor Xa into intact rats caused the development of hypercoagulation. Their blood and plasma clotting times and kaolin and cephalin times were reduced and their fibrinogen concentration increased a little.

Unlike intact animals, hypercoagulation was observed in the thymectomized rats. In these experiments the blood clotting, plasma recalcification, kaolin, and cephalin times were all increased, antithrombin activity was significantly enhanced, and the fibrinogen concentration was reduced. The difference in the blood coagulation parameters, in the writer's view, can be explained as follows. In thymectomized rats in the initial state marked hypercoagulation was observed [6]. Stimulation of blood coagulation under these conditions led to the development of secondary hypocoagulation. The facts described above largely explain the rapid disappearance of factor Xa from the blood stream of the thymectomized rats. The exogenous enzyme was "used up" on its own substrate (prothrombin) and incorporated into the thrombi. At the same time, in our view [3] and on the basis of data obtained by other workers [1, 4], there is a definite class of immunoglobulins which take part in inhibition and elimination of enzymes.

In a special series of experiments the distribution of ^{131}I-factor Xa was studied in various organs of intact and thymectomized rats (Fig. 1).

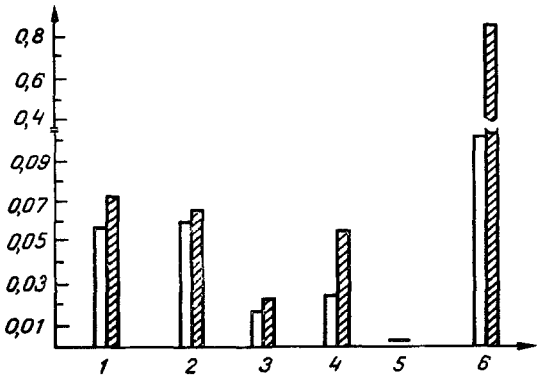

Fig. 1. Distribution of factor Xa in organs of intact (unshaded colums) and thymectomized (shaded columns) rats. Abscissa: 1) liver, 2) kidney, 3) spleen, 4) brain, 5) muscle, 6) aorta; ordinate, specific radioactivity (in counts/mg).

These experiments showed that factor Xa accumulates most of all in the aorta. The content of enzyme in the wall of this vessel was an order of magnitude higher than in other organs. Factor Xa could not be found in skeletal muscle. The fact was noted that the labeled enzyme accumulated more intensively in the tissues of the immunodeficient animals.

Depression of cellular immunity by thymectomy is thus accompanied not only by hypercoagulation, but also by disturbance of elimination of spent factors from the circulation.

LITERATURE CITED

1. A. S. Zaks, A. A. Bykova, and M. L. Susnina, Farmakol. Toksikol., No. 2, 190 (1977).
2. B. I. Kuznik, N. N. Tsybikov, and V. L. Medvedev, in: The Physiology and Pathology of Hemostasis [in Russian], Chita (1980), pp. 30-31.
3. B. I. Kuznik and N. N. Tsybikov, Usp. Sovrem. Biol., No. 2 (5), 243 (1981).
4. V. A. Protsenko, Byull. Éksp. Biol. Med., No. 7, 30 (1980).
5. V. M. Radionov and Yu. P. Reshetko, in: Modern Methods in Biochemistry [in Russian], Moscow (1968), pp. 326-346.
6. N. N. Tsybikov, Patol. Fiziol., No. 6, 65 (1980).
7. W. H. Seegers, Prothrombin, Cambridge (1962).