3. A. K. Chepurov and G. A. Yakunin, in: The Simulation, Methods of Study, and Experimental Treatment of Pathological Processes [in Russian], Moscow (1973), pp. 114-116.
4. V. A. Shestakov, Grud. Khir., No. 2, 41 (1975).
5. M. Bassione, J. Clin. Pathol., 6, 39 (1953).
6. K. Breddin, Blut, 18, 84 (1968).
7. A. Chandler, Lab. Invest., 7, 110 (1958).
8. R. Lillie, Histopathologic Technic and Practical Histochemistry, McGraw-Hill, New York (1965).
9. R. Marbet and A. Winterstein, Ärztl. Forsch., 9, 1 (1955).
10. L. Michaelis, Cold Spring Harbor Symp. Quant. Biol., 12, 142 (1947).
11. A. G. E. Pearse, Histochemistry, Theoretical and Applied, Little, Brown, Boston (1960).
12. E. Perlick, Antikoagulanten, Leipzig (1964).
13. B. Sylven, Acta Histochem. (Jena), 6, 134 (1959).
14. K. Taylor, J. Histochem. Cytochem., 8, 248 (1960).
15. D. Witterkind, V. Kretschmer, and W. Löhr, Blut, 32, 70 (1976).

ANTITHROMBIN ACTIVITY AFTER BLOCKAGE OF THE RETICULOENDOTHELIAL SYSTEM, SPLENECTOMY, AND PARTIAL HEPATECTOMY

V. P. Babich

UDC 612.115.33-06:[612.42+612.35

After destruction or removal of part of the liver in rats the levels of antithrombins II, III, and IV fell proportionally to the extent of the interference. Destruction of the spleen led to depression, but splenectomy led to activation of antithrombin IV. Blockade of the reticuloendothelial system caused a smaller decrease in the antithrombin level than partial hepatectomy. It is suggested that the spleen produces an inhibitor of antithrombin IV.

KEY WORDS: *antithrombins; reticuloendothelial system; liver; spleen.*

Antithrombins can be regarded as a humoral factor of the anticlotting system [2, 3, 6]. No information is available on the site of their production in the body. Since the antithrombins belong to the globulin fraction, which contains antibodies whose production is connected with the reticuloendothelial system (RES), it can be postulated that antithrombins are also produced by the cells of that system.

This investigation was undertaken to examine the role of the RES in antithrombin formation.

EXPERIMENTAL METHOD

Activity of the antithrombins was determined [1] in albino rats (weight 150-250 g, total number 273) which were given an intravenous injection of a 0.5% solution of trypan blue in 0.85% sodium chloride solution (1 ml/100 g body weight). Control animals received the corresponding dose of the solvent. Experiments have shown [4] that if a dye blocks the RES, a prethrombotic state develops by the third day after its injection. Blood samples were taken 5 and 12 days after administration of the trypan blue. The role of the liver and spleen in maintaining antithrombin activity was studied in experiments in which these organs were injured. The left anterior lobe of the liver or the whole of the spleen was destroyed in the animals (by crushing with Pean's forceps). The control rats underwent laparotomy with mobil-

Department of Biochemistry, Zaporozh'e Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 84, No. 9, pp. 282-284, September, 1977. Original article submitted April 26, 1977.

TABLE 1. Serum Antithrombin Activity (in sec) after Removal of Two-Thirds of Liver Tissue (M ± m)

Activity		Day after operation			
		2nd	4th	6th	12th
Total antithrombin	Control	58,0±5,7 (n=7)	48,0±5,3 (n=7)	46,0±3,8 (n=6)	43,0±2,8 (n=6)
	Experiment	26,0±2,1 (n=7)	23,0±1,9 (n=7)	25,0±2,5 (n=7)	28,0±2,8 (n=7)
	P	<0,05	<0,05	<0,05	<0,05
Antithrombin II	Control	14,0±6,2	8,0±3,2	7,0±1,7	5,0±1,7
	Experiment	7,0±1,7	3,0±1,4	3,0±0,6	3,0±1,2
	P	>0,05	>0,05	>0,05	>0,05
Antithrombin III	Control	15,0±2,0	12,0±1,9	15,0±1,6	14,0±1,9
	Experiment	7,0±1,9	2,0±0,9	7,0±1,9	8,0±1,7
	P	<0,05	<0,05	<0,05	<0,05
Antithrombin IV	Control	29,0±2,2	27,0±1,5	24,0±2,7	23,0±1,5
	Experiment	12,0±1,3	18,0±1,9	14,0±1,7	16,0±1,5
	P	<0,05	<0,05	<0,05	<0,05

TABLE 2. Serum Antithrombin Activity (in sec) on Second Day after Removal of Two-Thirds of the Liver and Total Splenectomy (M ± m)

Activity	Control (n=7)	Experiment (n=7)	P
Total antithrombin	58,0±5,7	25,0±1,1	<0,05
Antithrombin II	14,0±6,2	4,0±1,1	>0,05
Antithrombin III	15,0±2,0	6,0±1,7	<0,05
Antithrombin IV	29,0±2,2	15,0±1,1	<0,05

ization of the corresponding organ, but without injury to it. On the second, fourth, and sixth days after the operation the serum antithrombin activity was determined. In some experiments partial hepatectomy or total splenomectomy was performed.

EXPERIMENTAL RESULTS AND DISCUSSION

On the fifth day after injection a small decrease in the total serum antithrombin activity was found (from 100.0 ± 4.2 to 88.0 ± 11.0 sec) on account of inhibition of antithrombin III (from 29.0 ± 3.9 to 18.0 ± 3.2 sec). On the twelfth day of the experiment (when removal of the dye from the blood stream by cells of the RES was almost complete) the decrease in the total antithrombin activity was more obvious (control 102.0 ± 5.2; experiment 72.0 ± 11.0 sec). This took place on account of inhibition of antithrombin II (control 16.0 ± 2.4, experiment 7.0 ± 0.5 sec) and of antithrombin IV (control 60.0 ± 2.7, experiment 46.0 ± 6.2 sec).

Destruction of the spleen was followed by a reduction in total antithrombin activity, lasting 6 days, on account of antithrombin II (control 14.0 ± 1.0 sec, experiment 11.0 ± 1.5 sec) and antithrombin IV (control 27.0 ± 1.7 sec, experiment 22.0 ± 1.1 sec). After destruction of one lobe of the liver the level of antithrombin activity was below the control only on the second day, on account of inhibition of antithrombin III (control 9.0 ± 1.5 sec, experiment 4.0 ± 0.9 sec) and of antithrombin IV (control 27.0 ± 1.7 sec, experiment 18.0 ± 1.0 sec). Later the differences between antithrombin activity in the control and experimental series disappeared because of the fall in the level of inhibitors in the control animals.

The fact that destruction of a lobe of the liver leads only to temporary inhibition of antithrombin activity can be attributed (assuming that the liver participates in their production) to the rapid compensatory activation of their biosynthesis in the uninjured regions of the organ. To rule out the possible effect of tissue breakdown products of the liver or spleen on antithrombin activity experiments were carried out in which the spleen or the corresponding lobe of the liver was removed.

After partial hepatectomy the same changes developed as after destruction of a lobe of the liver, but they were rather more prolonged. By the sixth day of observation the total antithrombin activity no longer differed from the control because of the tendency toward activation of antithrombin IV (control 27.0 ± 3.7 sec, experiment 33.0 ± 5.2 sec). The prolonged fall in antithrombin IV activity after destruction of the lobe of the liver was evidently the result not only of its reduced production, but also of liberation of its inhibitor from the destroyed tissue [5].

After splenectomy, by contrast with destruction of the spleen, activation of antithrombin IV was observed instead of depression (control 27.0 ± 3.7 sec, experiment 41.0 ± 2.6 sec). This suggests that the spleen contains an inhibitor of antithrombin IV or produces a substance which inhibits its synthesis in the liver.

After removal of two-thirds of the liver a marked decrease in antithrombin III and IV activity and a tendency toward a decrease in antithrombin II activity were observed in the animals (Table 1), reflecting the important role of the liver in maintenance of the antithrombin level in the blood stream, and the limited importance of structures of the RES in this process. Blockade of RES in fact gave rise to a smaller decrease in the antithrombin level than removal of only two-thirds of one of the organs containing elements of the RES.

Removal of the spleen simultaneously with two-thirds of the liver led to a sharp decrease in the antithrombin level by the second day (Table 2).

Changes in the antithrombin level after destruction or removal of a lobe of the liver were similar in character, whereas destruction of the spleen led to inhibition, and its removal to activation of antithrombin IV. The degree of the decrease in the antithrombin IV level after removal of two-thirds of the liver was reduced by simultaneous splenectomy. These findings suggest that the spleen plays a role in the production of the inhibitor of antithrombin IV.

LITERATURE CITED

1. V. P. Babich and A. Sh. Byshevskii, Lab. Delo, No. 8, 467 (1972).
2. T. N. Gorshkova and A. A. Markosyan, in: Heparin. Physiology, Biochemistry, Pharmacology, and Clinical Application [in Russian], Leningrad (1969), pp. 62-64.
3. L. V. Mikhailova and A. Sh. Byshevskii, Byull. Éksp. Biol. Med., No. 5, 16 (1969).
4. V. E. Pastorova et al., Byull. Éksp. Biol. Med., No. 11, 23 (1961).
5. H. H. Henstell et al., Proc. Soc. Exp. Biol. (New York), 122, 247 (1966).
6. L. T. Mann et al., Science, 166, 517 (1969).