vessels of the brain, causing lasting dilatation of them. In response to stimulation of the lateral hypothalamic area, zona incerta, and zones H_1 and H_2, the phenomena of edema and swelling of the brain observed against the background of the other changes described above play an important role.

LITERATURE CITED

1. A. I. Arutyunov, Vrach. Delo, No. 2, 101 (1954).
2. N. N. Burdenko, Vest. Akad. Med. Nauk SSSR, No. 4, 74 (1941).
3. Yu. N. Kvitnitskii-Ryzhov, in: Problems in Neurosurgery [in Russian], Vol. 2, Kiev (1955), pp. 223-231.
4. Yu. N. Kvitnitskii-Ryzhov, Vopr. Neirokhir., No. 1, 12 (1964).
5. B. N. Klosovskii, Vopr. Neirokhir., No. 4, 48 (1944).
6. O. A. Kovalev, "The pathogenesis of acute prolapse of the brain after mechanical stimulation of the brain stem," Author's Abstract of Candidate's Dissertation, Leningrad (1962).
7. S. Ischii, H. Tsuii, K. Ozava, et al., in: Brain Edema, New York (1967), pp. 32-66.
8. J. Le Beau and M. Bonvalet, C. R. Soc. Biol., 127, 126 (1938).
9. S. Obrador and J. Pi-Suner, Arch. Neurol. Psychiatr., 49, 826 (1943).
10. F. Reinoso-Suarez, Topographischer Hirnatlas der Katze für experimental-physiologische Untersuchungen, Darmstadt (1961).
11. T. Teraura and J. S. Meuer, Confin. Neurol. (Basel), 37, 296 (1975).
12. C. Vincent, J. Le Beau, and G. Giot, Proc. Roy. Soc. Med., 40, 689 (1947).

EFFECT OF CATIONIC DYES ON BLOOD COAGULATION

A. K. Chepurov and G. I. Lazarenko

UDC 612.115.014.46:547.97

Experiments on rabbits showed that intravenous injection of dyes of the thionine series (toluidine blue, azure A, 1,9-dimethylmethylene blue) causes hypofibrinogenemia, a decrease in the concentration of factors of the prothrombin complex, thrombocytopenia, and a decrease in the index of platelet adhesion against the background of slowing of the time of thrombus formation. The blood heparin tolerance and the free heparin concentration were sharply reduced. It is suggested that the hypocoagulation effect of the cationic dyes on the blood is due to thrombocytopenia and to a decrease in the aggregating activity of the platelets.

KEY WORDS: *cationic dyes; coagulation; platelets.*

Toluidine blue and azure are known to form a complex with the heparin of the blood and they are responsible for its metachromatic staining [4, 5, 8, 14, 15]. Most methods of determining free heparin in the blood are based on this property [4, 5, 11]. There are indications of changes in blood coagulation under the influence of metachromatic dyes [10, 12], although the character of these changes differed in investigations by different workers.

The object of this investigation was to study the effect of various cationic dyes, giving a metachromatic effect, on the coagulating activity of the blood and functional properties of the blood cells.

Institute of Transplantation of Organs and Tissues, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.)

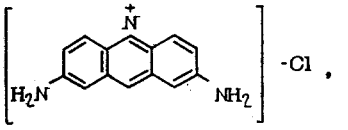
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 9, pp. 279-282, September, 1977. Original article submitted November 18, 1976.

TABLE 1. Effect of Cationic Dyes on Blood Coagulation (M ± m)

Blood coagulation index	Control	Toluidine blue			Azure A		
		time of investigation					
		30 min	1½ h	2½ h	30 min	1½ h	2½ h
Thrombus formation time of blood, sec	390±24	374±18	489±12	600±24	426±18	459±6	510±18
P		>0,1	<0,05	<0,02	<0,05	<0,01	<0,01
Prothrombin complex, sec	41±3	53±2	67±4	79±3	49±4	56±2	72±4
P		>0,05	<0,05	<0,01	>0,1	<0,02	<0,001
Fibrinogen, mg %	226±18	179±10	119±16	148±11	150±9	133±15	88±12
P		>0,05	<0,02	<0,05	<0,02	<0,01	<0,001
Heparin, mg %	1,6±0,3	1,7±0,2	0,6±0,2	0,9±0,3	1,3±0,2	0,9±0,1	1,4±0,2
P		>0,05	<0,001	<0,02	<0,05	<0,001	<0,01
Platelet count, thousands/mm³	296±32	222±17	194±23	176±19	244±12	224±14	200±12
P		>0,05	<0,05	<0,02	>0,05	<0,05	<0,02
Index of platelet adhesion	20,4±3,2	8,2±2	7±1	5±0,5	13±2,5	10±2	11±3
P		<0,001	<0,001	<0,001	<0,02	<0,001	<0,001

EXPERIMENTAL METHOD

Experiments were carried out on 50 rabbits weighing 2.5-3 kg. Toluidine blue, azure A, and 1,9-dimethylmethylene blue, all derivatives of thionine with the general formula



were used. The dyes were injected intravenously as 1, 5, or 10% solutions in a dose of 0.25 ml/kg. Blood samples were taken by cardiac puncture before injection of the dyes and again 30 min and 1.5 h after their injection. The thrombus formation time of the blood [7], thromboelastogram, concentration of factors of the prothrombin complex [2], fibrinogen concentration [1], plasma heparin tolerance [9] (with graphic recording on the coagulograph), the free heparin concentration [4], the platelet count, the index of platelet adhesion [3], and their aggregation were determined.

EXPERIMENTAL RESULTS AND DISCUSSION

The most marked coagulation of the blood was observed after injection of a 10% solution of the dyes; the effect of toluidine blue and azure A was greater than that of 1,9-dimethylmethylene blue. Data on the effects of the first two dyes only are therefore given in Table 1.

The thrombus formation time of the blood was increased after injection of the dyes, especially after 2.5 h. The concentration of factors of the prothrombin complex and of fibrinogen was reduced. These changes again were most marked 2.5 h after injection of the dyes. The platelet count in the blood fell progressively. The index of platelet adhesion fell as early as 30 min after the injection of the dyes and it remained lower than initially throughout the period of investigation. Toluidine blue, in particular, lowered the platelet adhesion index by 75% compared with the control. The maximal amplitude of platelet aggregation was reduced (Fig. 1).

The free heparin level was lowered throughout the period of investigation after injection of the dyes (Table 1). The greatest decrease in the free heparin concentration was observed after 1.5 h, whereas the blood heparin tolerance was lowered most after 2.5 h (Fig. 2).

After injection of cationic dyes of the thionine series marked changes were thus observed in the coagulation properties of the blood. Hypocoagulation changes were recorded, it will be noted, at a time of a marked fall in the free heparin level in the blood. The reduction in blood clotting was evidently due to the thrombocytopenic effect of the cationic dyes and also to the sharp reduction in the adhesive and aggregating properties of the platelets. In these experiments the cationic dyes caused a marked decrease in the ability of platelets to associate into aggregates, so that they spread widely [6].

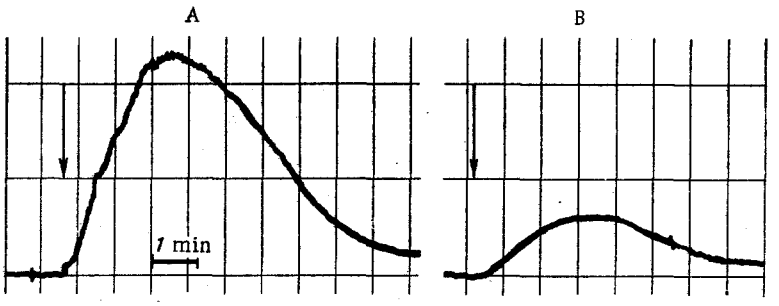


Fig. 1. Effect of azure A on aggregation of platelets induced by ADP. A) Normal aggregation of rabbit platelets; B) aggregation of platelets after intravenous injection of 10% solution of azure A in a dose of 0.25 ml/kg. Arrow indicates time of injection of ADP.

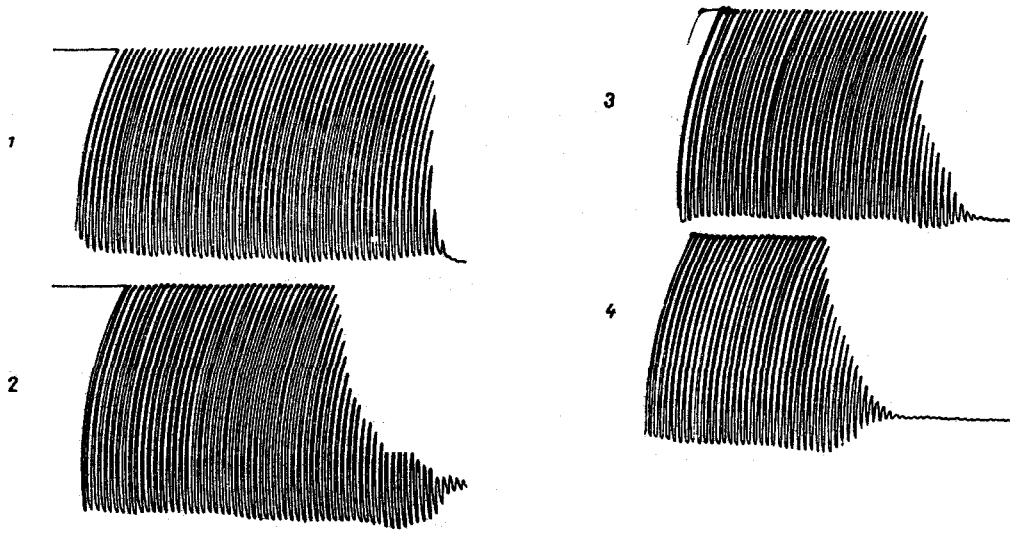


Fig. 2. Blood heparin tolerance after intravenous injection of 10% solution of toluidine blue into rabbits in a dose of 0.25 ml/kg: 1) initially; 2) 30 min, 3) 1.5 h, and 4) 2.5 h after injection of toluidine blue.

Dyes of the thionine series have been shown to be competitive acceptors of electrons [8, 10]; their metachromatic effect is associated with the action of van der Waals forces [13]. These properties of the cationic dyes may play a role in the decrease in the adhesive and aggregating properties of the platelets. Under these circumstances the electrophoretic mobility of the platelets may increase, but this is a matter for special investigation.

The reduction in the heparin concentration after injection of the dyes is evidence that a stable complex of heparin and the cationic dyes is formed not only in vitro, but also in vivo; this may possibly happen both in the circulating blood and also in the mast cells and other heparinocytes. The stability of the resulting complex is demonstrated by the fact that hypoheparinemia was observed for 36 h after injection of the cationic dye, i.e., for the whole time that the dye was present in the circulating blood, in amounts detectable by a nephelometric method.

The results open the way to the practical use of cationic dyes for the reduction of coagulability.

LITERATURE CITED

1. R. A. Rutberg, Lab. Delo, No. 3, 7 (1957).
2. V. A. Tugolukov, Lab. Delo, No. 3, 11 (1953).

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.