Injection of excessive quantities of retinoids into mice thus gives rise to anemia, expressed as intensification of erythropoiesis in the red marrow, a decrease in the number of erythrocytes and hemoglobin concentration in the blood, and a decrease in the osmotic resistance of the erythrocytes. Injection of RC_{15} and 13-CMF into animals was accompanied by an increase in the number of erythrocytes not stained by PAF in the blood.

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PROTECTIVE EFFECT OF HEPARIN AND PHOSPHATIDYLSERINE IN EXOGENOUS THROMBOPLASTINEMIA

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On entering the blood stream thromboplastin temporarily increases the coagulability of the blood and evokes a protective reaction leading to a reduction in coagulability through consumption of clotting factors [8] and, chiefly, through an increase in the anticlotting potential [5]. The frequency of death of animals receiving a dose of thromboplastin excessive for the animal's powers of compensation may be regarded as an indicator of tolerance to the coagulating agent, whereas the effect of anticoagulants on the mortality rate under these conditions can be used to estimate their effectiveness.

In the investigation described below heparin and phosphatidylserine-containing anticoagulant (PSA), the anticlotting principle of which is phosphatidylserine, an inhibitor of thrombin production and fibrin formation [3, 4], were studied from this aspect.

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TABLE 1. Death of Animals (in %) Receiving Injection of Thromboplastin (58 mg/kg) 1 h after Injection of Heparin (2.6 mg/kg) or PSA (80 mg/kg) or a Mixture of Both (in the same doses)

Experimental conditions	Number of rats	Death rate	
		during 4 h	during 12 h
Control (no anticoagulant injected)	60	67±12	70±13
Heparin	40	55±12 (0,18)*	80±36 (0,0)
PSA	40	14±1,4 (0,78)*	25±3,3 (0,64)*
PSA + heparin	40	0,00 (1,00)*	0,00 (1,0)*

Legend. Values of effectiveness of inhibition (i) shown in parentheses; asterisk indicates significantly different values.

TABLE 2. State of Blood Clotting after Injection of Thromboplastin (2.6 mg/kg)

Parameter tested	Group	Intervals between injection and taking blood samples				
		5 min	1 h	3 h	6 h	12 h
Recalcification time, sec	Control	70±3	59±9	62±3	47±2	51±1
	Experiment	540±60*	195±15*	92±8*	62±7*	51±1
Thromboplastin time, sec	Control	21±1	19±1	21±1	23±1	21±2
	Experiment	117±3*	47±3*	37±3*	32±3*	23±2
Thrombin time, sec	Control	27±3	19±2	20±3	19±2	19±2
	Experiment	197±13*	102±3*	37±3*	32±3*	21±2
Fibrinolysis activity, mm	Control	68±19	44±4	64±7	56±8	39±3
	Experiment	53±24	127±18*	72±10	61±11	38±3
Fibrinogen concentration, mg%	Control	258±76	290±91	269±71	335±96	328±63
	Experiment	7±1*	57±4*	207±38*	173±29*	234±37*

Legend. Here and in Table 3, 8-9 animals were used in each stage of the experiment and control; asterisk indicates significantly different values.

EXPERIMENTAL METHOD

A suspension of thromboplastin in 0.14 M sodium chloride solution was injected into the jugular vein, and this was followed by injection of heparin, PSA, or a mixture of both into the same vein. Blood samples were taken for determination of the recalcification time [12], the thromboplastin and thrombin time of the plasma [14, 9], the concentration of thrombin-precipitated fibrinogen [2], and fibrinolysis activity [11], and for the autocoagulation test [13], on the basis of which the autocoagulogram (ACG) was constructed to assess the state of the following parameters: activity at the 2nd minute (A), maximal activity (MA), the time taken to reach A (T_1) and MA (T_2), the time taken for MA to fall by half (F), and the thrombin-prothrombinase inactivation index (TII). Experiments were carried out on sexually mature albino rats. PSA was obtained as described in [7]. Commercial preparations of thromboplastin, thrombin, and fibrinogen (Kaunas) and of crystalline heparin (from Reanal, Hungary) were used. The experimental conditions and number of animals are indicated below.

EXPERIMENTAL RESULTS

Injection of thromboplastin (56 mg/kg) caused death of animals of the control group mainly during the first 4 h. Between 4 and 12 h mortality was very low (3%) and after 12 h no deaths were observed in any group (Table 1). Heparin reduced the mortality of the animals in the first 4 h, but by 12 h mortality was close to the control level. The death rate after administration of PSA was 4.5 and 2.8 times lower than after heparin, after intervals of 4 and 12 h respectively. No deaths were observed after combined injection of heparin and PSA.

TABLE 3. State of Blood Clotting 1 h after Injection of Thromboplastin (2.6 mg/kg) and Heparin or PSA, Separately or Together (2.6 and 80 mg/kg)

Experimental conditions	Recalcification time, sec	Thromboplastin time, sec	Thrombin time, sec	Fibrinolysis activity, mm	Fibrinogen concentration, mg%
Control	66±5	24±1	23±1	53±12	317±42
Thromboplastin	127±24*	80±25*	100±30*	70±12*	155±16*
Thromboplastin + heparin	173±17*	66±9*	206±59*	80±20*	190±21*
Thromboplastin + PSA	307±54	134±35*	456±147*	150±38*	192±19*
Thromboplastin + heparin + PSA	766±324*	155±32*	709±210*	191±37*	316±48

Legend. Asterisk indicates significantly different values.

TABLE 4. Parameters of ACG 1 h after Injection of Thromboplastin, Heparin, and PSA (animals from experiments referred to in Table 3)

Experimental conditions	A, %	MA, %	T_1, min	T_2, min	F, min	TII
Control	36	50	1,5	15	50	1,78
Thromboplastin	24	38	1,5	6,7	37,5	2,50
Thromboplastin + heparin	0,15	4,7	25,5	30,0	37,0	12,10
Thromboplastin + PSA	0,0014	0,36	9,5	11,0	18,0	30,00
Thromboplastin + heparin + PSA	$25 \cdot 10^{-8}$	$34 \cdot 10^{-4}$				

Legend. Parameters T_1, T_2, F, and TII were not determined after combined administration of heparin and PSA because of the low values of A and MA.

To determine whether the protective effects of heparin and PSA undergo summation or potentiation, the formula $i_{1,2} \gtrless i_1 + i_2 - i_{1,2}$ was used, where $i_{1,2}$ denotes the effectiveness of inhibition by their combined administration; i_1 and i_2 the effectiveness of inhibition by heparin and PSA separately. If the left hand side of the equation is equal to the right hand side, it is the result of potentiation [10]. In the cases described below potentiation was found for 4 h and, in particular, for 12 h: $1 > 0.18 + 0.78 - 1$ and $1 > 0.0 + 0.64 - 1$.

To study disturbances of blood clotting in thromboplastinemia, a dose not causing death of the rats (2.6 mg/kg) was used. Only 5 min after injection of thromboplastin the recalcification time was increased, but later it decreased and reached the control level after 12 h (Table 2). The same remarks also apply to the plasma thromboplastin and thrombin times. The concentration of thrombin-precipitated fibrinogen fell sharply until the 5th minute, then rose gradually to reach the control level after 12 h. Activation of fibrinolysis was observed only 1 h after injection of thromboplastin.

The results are in agreement with those of many other investigations and enable the time when the largest number of parameters was changed to be determined — 1 h after injection. Accordingly, in the next series of experiments heparin and PSA were injected immediately after injection of thromboplastin and blood samples were taken 1 h later. It was found (Table 3) that the anticoagulants intensified the changes induced by thromboplastin: changes in the plasma recalcification, thromboplastin, and thrombin time and in fibrinolysis activity were considerably increased after injection of heparin and, in particular, of PSA. Only the degree of fall of the fibrinogenemia was reduced. After simultaneous injection of heparin and PSA the fibrinogen concentration was completely unchanged. Calculation shows that the effects of heparin and PSA were potentiated in this case also.

Heparin and PSA thus intensified the anticlotting reaction evoked by thromboplastin, but the mechanism of development of the reduced coagulability of the blood in this case differed. Thromboplastin depresses clotting activity by activating anticlotting mechanisms (the protective anticlotting reaction [5]) and by causing partial utilization of the clotting factors, including fibrinogen [1, 6]. Consequently, in thromboplastinemia, the hypofibrinogenemia is due both to the conversion of fibrinogen into a refractory form and also to its consumption. Heparin and PSA, by blocking thrombin production and the thrombin–fibrinogen reaction, prevents fibrinogen consumption. This is confirmed by the changes in the ACG (Table 4).

The initial stages of formation of prothrombinase and thrombin in hemolysate of a calcium mixture from the blood of animals treated with thromboplastin are inhibited, and maximal activity (the parameters A and MA) is depressed, the time taken to reach MA (T_2) was increaed, inactivation of prothrombinase and thrombin (the parameter F) was accelerated, and the degree of inactivation (the parameter TII) was increased. This confirms that injection of thromboplastin induces a powerful protective reaction, limiting the fibrin concentration.

Heparin potentiates the fall in A and MA by 160 and 8.1 times respectively, lengthens T_1 and T_2 by 17 and 4.5 times, and increases TII by 4.8 times. PSA acts in the same direction but more strongly: the parameters A and MA were reduced by 17,142 and 139 times respectively, although the changes in the T_1 and T_2 parameters were less than those due to heparin. TII was increased by 12 times and F doubled.

After combined injection of heparin and PSA the parameters A and MA were reduced by 0.96×10^8 and 0.68×10^4 times. The remaining parameters of the ACG in this experiment could not be determined, for it was impossible to construct the graph because the difference between individual components of the coagulogram amounted to 5 or 6 orders of magnitude.

Heparin, interacting with antithrombin III, takes part in the inactivation of factors Xa and IIa. This, with the formation of complexes of heparin with various procoagulants [5], evidently explains the considerable anticoagulant effect of heparin in conjuction with thromboplastin. PSA, in the dose tested, inhibits clotting activity by a greater degree than heparin (Table 3, 1 h after injection). Accordingly changes in the parameters of the ACG were more marked under the influence of PSA. It was shown previously that inhibition of fibrin self-assembly by phosphatidylserine increases proportionally to the decrease in the concentration of fibrin monomer [4]. Potentiation of the effects of heparin and PSA may evidently be due in particular to the fact that heparin (and PSA) inhibits thrombin formation [3] and, consequently, lowers the concentration of fibrin monomer, resulting in more marked inhibition of self-assembly of phosphatdiylserine. The possibility of interaction between phosphatidylserine and heparin at the molecular level likewise cannot be ruled out, and this is a matter for special investigation.

It can be concluded from the data described above that heparin and PSA increased resistance to thromboplastinema, and that in the case of their combined administration, the protective effect of each is potentiated. This protective action is manifested not only as a fall in the death rate of the animals due to injection of thromboplastin into the blood stream, but also as prevention of the development of hypofibrinogenemia on account of fibrinogen consumption.

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