LITERATURE CITED

1. F. B. Babchinskii and I. P. Savateev, Kosm. Biol., No. 4, 70 (1967).
2. S. N. Efuni (editor), Hyperbaric Oxygenation [in Russian], Moscow (1980).
3. A. G. Zhironkin, Oxygen. Physiological and Toxicological Action [in Russian], Leningrad (1972).
4. A. A. Krichevskaya, A. I. Lukash, and Z. G. Bronovitskaya, Biochemical Mechanisms of Oxygen Poisoning [in Russian], Rostov-on-Don (1980).
5. B. V. Petrovskii and S. N. Efuni, Principles of Hyperbaric Oxygenation [in Russian], Moscow (1976).
6. L. L. Prilipko, O. N. Orlov, S. I. Ivanova, et al., Dokl. Akad. Nauk SSSR, 265, No. 4, 1010 (1982).
7. C. J. Dillard, E. E. Dumelin, and A. L. Tappel, Lipids, 12, 109 (1977).
8. G. D. Lawrence and G. Cohen, Anal. Biochem., 122, 283 (1982).
9. R. E. Litov, C. J. Dillard, J. Downey, et al., Fed. Proc., 37, 306 (1978).
10. C. A. Riely, G. Cohen, and M. Lieberman, Science, 183, 208 (1974).
11. A. Zlatkis, H. Lichtenstein, A. Tishbee, et al., Anal. Chem., 45, 463 (1973).

EFFECT OF PREGNANCY ON STATE OF THE HEMOSTASIS SYSTEM IN INTACT AND THYMECTOMIZED RATS

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Hypercoagulation develops in women during pregnancy and fibrinolysis is inhibited [8, 9, 11]. Immunologic conflict between mother and fetus also frequently develops during pregnancy, and this is bound to aggravate disturbances in the hemostasis system [14].

For the reasons given above, the study of the role of the thymus, the central organ of cellular immunity, in the mechanism of development of coagulopathy during pregnancy is a matter of considerable interest, and the investigation described below was devoted to it.

EXPERIMENTAL METHOD

Experiments were carried out on 48 female rats. The thymus was removed from 24 of them at the age of 2 months. Mating of both thymectomized and intact females took place at the age of 4 months. All investigations were carried out in the last stage of pregnancy according to the following scheme: blood was taken simultaneously from nonpregnant intact (control 1) and thymectomized (control 2) rats and from pregnant intact (experiment 1) and thymectomized (experiment 2) rats.

The following parameters of the hemostasis system were determined: the blood clotting time and plasma recalcification time, the kaolin and cephalin times, prothrombin and thrombin times, total antithrombin activity, fibrinogen concentration, euglobulin fibrinolysis, and the ethanol test. The total platelet, leukocyte, and erythrocyte counts also were determined and the aggregometer tracing recorded. All the methods listed above are described in a recent textbook on investigation of the hemostasis system [1].

The numerical results were subjected to statistical analysis.

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TABLE 1. Blood Clotting and Fibrinolysis in Pregnant Intact and Thymectomized Rats (M ± m, n = 12)

Parameter	Group of animals			
	control 1	experiment 1	control 2	experiment 2
Clotting time	194,0±11,4	228,0±11,4*	153,5±7,9**	239,0±16,5***
Recalcification time	93,0±3,6	110,0±6,6*	75,0±4,7**	112,0±6,7***
Prothrombin time	15,0±0,4	14,0±0,3*	14,0±0,3	14,0±0,3
Kaolin time	53,0±2,6	57,0±2,0	46,0±2,1	60,0±1,9
Cephalin time	62,0±2,5	66,0±3,1	54,0±1,9**	76,0±5,1***
Thrombin time	30,0±0,7	28,5±8,1	25,0±1,1**	28,5±2,4
Total antithrombin activity	11,0±0,9	10,0±1,4	6,4±1,3**	9,0±1,4
Ethanol test	—	+в 55%	+ в 70%	+в 55%
Fibrinogen, mg%	35,0±17,5	435,5±33,2*	410,0±29,7	415,0±20,7
Euglobulin fibrinolysis, m	106,0±14,4	239,0±40,5*	153,0±29,6	199,0±22,4
Factor 3, sec	30,0±2,1	27,0±1,9	22,0±3,0**	29,0±2,4

Legend. *P < 0.05 between control 1 and experiment 1 groups, **P < 0.05 between control 1 and control 2 groups, ***P < 0.05 between control 2 and experiment 2 groups.

TABLE 2. Blood Cells in Pregnant Intact and Thymectomized Rats (M ± m, n = 12)

Blood cells	Groups of animals			
	control 1	experiment 1	control 2	experiment 2
Erythrocytes	5 799 200±178 900	4 995 000±194 500*	5 088 000±195 900**	4 842 700±265 400
Leukocytes	9 089±808	6 279±770	6 539±683	5 923±819
Platelets	1 017 000±145 000	11 110 000±111 000	1 423 000±148 000	1 286 000±111 000

Legend. *P < 0.05 between control 1 and experiment 1 groups, **P < 0.05 between control 1 and control 2 groups.

TABLE 3. Aggregometer Tracing of Pregnant Intact and Thymectomized Rats (M ± m, n = 12)

Parameter of aggregometer tracing	Control 1	Experiment 1	Control 2	Experiment 2
Angle of aggregation α	79,67±1,18	80,8±1,33	80,7±1,04	76,82±1,64**
Aggregation time, sec	195,25±16,28	166,65±11,99	192,5±11,66	188,65±20,52
Amplitude of aggregation, mm	94,75±6,01	92,27±10,38	102,60±10,05	85±9,74
Disaggregation time, sec	656,53±35,53	400,29±41,25*	444,95±72,11	463,49±49,06
Amplitude of disaggregation, mm	90,75±8,13	52,89±11,38*	94,5±10,11	54,6±7,3**
Disaggregation angle α	21,11±2,77	20,11±4,19	22,94±2,15	23,09±2,4

Legend. *P < 0.05 between control 1 and experiment 1 groups, **P < 0.05 between control 1 and control 2 groups.

EXPERIMENTAL RESULTS

Rats developed hypercoagulation 3 months after thymectomy and fibrinolysis was inhibited (Table 1); these changes were reflected in shortening of the blood clotting and plasma recalcification times, the kaolin and cephalin times, and the prothrombin and thrombin times, a fall in the antithrombin level, elevation of the fibrinogen concentration, slowing of euglobulin fibrinolysis, and the appearance of a positive ethanol test.

In the pregnant intact (experiment 1) and thymectomized (experiment 2) rats marked hypocoagulation and further inhibition of fibrinolysis were observed. No differences could be found in the hemostasis system in pregnant thymectomized and intact rats (Table 1).

In the thymectomized rats the erythrocyte and leukocyte counts were fairly sharply reduced but the platelet count was increased. In pregnant rats, both intact and thymectomized, the erythrocyte and leukocyte counts remained low, but the platelet count was a little higher than in the intact nonpregnant animals (Table 2).

Disaggregation began sooner in the thymectomized rats. No difference in platelet aggregation could be detected between pregnant intact (experiment 1) and thymectomized (experiment 2) rats. Meanwhile, in both groups of animals the amplitude of disaggregation was considerably reduced (Table 3).

Just as in the writers' previous investigations [2, 4, 6, 7, 12] 3 months after thymectomy hypercoagulation developed in the rats and fibrinolysis was inhibited.

The genesis of the changes observed is a difficult problem. We know that the thymus is very closely linked with the functions of other glands of internal secretion. The imbalance in the concentrations of individual hormones (of the adrenals, thyroid, gonads, etc.), taking place after its removal, may be reflected in the hemostasis system. However, it seems that this is not the whole story.

The thymus is the central organ of cellular immunity. After its removal a defect of the immune system develops. This system, as we know, is very closely linked with the hemostasis system [6, 13]. The possibility cannot be ruled out that disturbances in the cellular component of immunogenesis lead to disinhibition of the hemostasis system [13] as was observed in the present experiments. Support for this view is given by previous investigations which showed that injection of the low-molecular-weight polypeptide thymus factor thymalin into thymectomized animals not only restored the normal state of immunogenesis, but also led to normalization of blood coagulability and fibrinolysis [2, 4].

As the data given above show, after thymectomy the leukocyte and erythrocyte counts of pregnant and nonpregnant rats decreased but the platelet count increased. This response was due to a disturbance of the regulatory role of the thymus in hematopoiesis. Hematopoiesis is controlled by a special population of T lymphocytes [10]. Meanwhile platelets contain an antifibrinolytic factor and fibrinase, which cause inhibition of fibrinolysis. The same effect results from a decrease in the number of leukocytes, the principal suppliers of plasminogen and of fibrinolysis activators [3, 5].

Pregnant rats, whether thymectomized or not, develop hypocoagulation, unlike pregnant women. Meanwhile, in both pregnant rats and pregnant women, fibrinolysis is inhibited. There is no doubt that intensification of blood clotting in pregnant women is a protective reaction aimed at preventing blood loss. Inhibition of fibrinolysis serves the same purpose. However, the development of hypercoagulation and reduction of fibrinolytic activity may play another role. Injury to the placenta is known to expose antigenic determinants, potentiating immunologic conflict between mother and fetus. Rapid "patching" of the injured surface with a layer of fibrin helps to prevent immunologic conflict.

Why, then, is it that pregnant rats, whether intact or thymectomized, unlike women, do not develop hypercoagulation? No unequivocal answer can be given to this question.

The presence of a positive ethanol test in thymectomized rats is evidence of intravascular blood clotting taking place in the circulation of pregnant animals. The ethanol test was positive in an equal percentage of cases in pregnant rats, whether thymectomized or intact. It may accordingly be postulated that the development of hypocoagulation in rats during pregnancy is secondary in character, due to the appearance of secondary anticoagulants.

Finally, the question arises why no significant differences are observed in coagulability of the blood and fibrinolysis in pregnant intact and thymectomized rats.

The investigations described above showed that in fetuses of thymectomized rats the weight of the thymus is considerably increased: 167.4 ± 10.0 mg in the control, 267.1 ± 12.0 mg in the experiment ($P < 0.01$). Probably hormones and cytomedins produced by the fetal thymus in increased amounts enter the blood stream of the pregnant thymectomized rat, where they neutralize changes developing in the hemostasis system.

LITERATURE CITED

1. V. P. Baluda, Z. S. Barkagan, E. D. Gol'dberg, et al., Laboratory Methods of Investigation of the Hemostasis System [in Russian], Tomsk (1980).
2. B. I. Kuznik, D. P. Budazhapova, L. A. Zagrebina, et al., Farmakol Toksikol., No. 4, 422 (1981).
3. B. I. Kuznik and Ya. D. Krasik, Ter. Arkh., No. 9, 25 (1979).
4. B. I. Kuznik, V. G. Morozov, L. I. Pisarevskaya, et al., Byull. Éksp. Biol. Med., No. 9, 264 (1981).
5. B. I. Kuznik and V. P. Skipetrov, Blood Cells, Vessel Wall, Hemostasis, and Thrombosis [in Russian], Moscow (1974).
6. B. I. Kuznik and N. N. Tsybikov, Usp. Sovrem. Biol., 92, No. 5, 243 (1981).
7. B. I. Kuznik, N. N. Tsybikov, and V. M. Medvedev, in: Physiology and Pathology of the Hemostasis System [in Russian], Chita (1980), pp. 30-31.
8. A. D. Makatsariya, Current Problems in Hemostasiology [in Russian], Moscow (1981), pp. 49-73.

9. M. S. Machabeli, Coagulopathic Syndromes [in Russian], Moscow (1970).
10. R. V. Petrov, R. M. Khaitov, V. M. Man'ko, et al., Control and Regulation of the Immune Response [in Russian], Leningrad (1981).
11. V. P. Skipetrov and B. I. Kuznik, The Obstetric Thrombohemorrhagic Syndrome [in Russian], Irkutsk (1973).
12. N. N. Tsybikov, Patol. Fiziol., No. 6, 65 (1980).
13. N. N. Tsybikov and B. I. Kuznik, Byull. Éksp. Biol. Med., No. 6, 8 (1982).
14. N. I. Tsyrel'nikov, Histophysiology of the Human Placenta [in Russian], Novosibirsk (1980).

EFFECT OF WATER-SOLUBLE ANTIOXIDANTS ON LYSOSOMAL MEMBRANE PERMEABILITY AND ON STRUCTURE OF THE LIVER IN RATS WITH THERMAL BURNS

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The view is held that destructive processes developing in various organs and tissues in pathological states and, in particular, in burns depend on the intensification of lipid peroxidation in these organs and tissues [5, 6]. This dependence is evidently due to the fact that lipid peroxides, formed in excess after burns [3, 11], possess membrane-toxic properties, which are particularly marked in relation to lysosomal membranes [1, 14]. Disturbance of their permeability leads to release of lysosomal hydrolases, stimulation of autolysis, and necrobiosis of the cells. The facts described above served as the basis for the use of inhibitors of peroxidation, i.e., antioxidants, as membrane protectors in various states characterized by intensification of catabolic and destructive processes [7], including in burns [5, 9]. Most membrane-stabilizing natural and synthetic antioxidants (α-tocopherol, ionol) are substances soluble in lipid base. Their effectiveness has been demonstrated experimentally, but it is quite evident that the clinical use of lipid-soluble preparations must be limited to external or peroral administration, and in that case the general membrane-protective action will be dependent on the character of absorption of the substance from the wound surface and mucous membrane of the gastrointestinal tract.

To obtain a preparation suitable for parenteral injection a number of water-soluble antioxidants have been synthesized. Their interaction with the hydrophobic structure of cell membranes has been very closely studied [2], but the action of water-soluble antioxidants in pathological states has been studied extremely inadequately.

The aim of the present investigation was to study water-soluble antioxidants: the water-soluble form of the widely used antioxidant ionol [7] and the antioxidant phenozan, as possible membrane-protectors in relation to lysosomal membranes of liver cells in thermal burns. Ionol in a water-soluble form was generously provided for the experiments by Professor E. G. Nifant'ev (Moscow City Pedagogic Institute), and phenozan by Professor V. V. Ershov (Institute of Chemical Physics, Academy of Sciences of the USSR), to whom the present writers are deeply grateful.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 150-200 g, kept on the ordinary animal house diet. Under superficial ether anesthesia a burn of the IIIB degree was inflicted on the rats (15-20% of the body surface). The investigation began 24 h after burning. De-

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