mitosis in OE, the maximal effect was observed during interaction of peritoneal macrophages and T lymphocytes activated by anemia factors in the early periods after hemorrhage. Our data indicate the presence of T-cell-macrophage interaction but not their sum effect, because the deficit of eliminated T cells was compensated for during transfer of up to 10^7 cells by macrophages.

The data indicate that the PC population is an extremely important component of the regulatory system, because the transfer of these cells to intact recipients with a normal level of erythropoiesis is capable of upsetting stable erythropoiesis and triggering repair erythropoiesis. This makes it easier to understand why the development of peritonitis in rats after blood loss selectively blocked the macrocytic regeneration [5] despite a high level of erythropoietin production. The usefulness of a regulatory system of rapid response to blood loss in the organism becomes clear if we bear in mind that peritoneal macrophages control the "reserve erythropoiesis" which ensures survival at the early period of blood recovery.

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The Phenomenon of Mouse Death after Parenteral Administration of Mink Blood Serum

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Parenterally administered mink blood serum is found to kill mice. The lethal factor of the serum is shown to be thermolabile, not dialyzed, stable during prolonged storage at -20°C and repeated freezing-thawing, destroyed by trypsin, and detected in globulin fractions after fractionation of the serum with ammonium sulfate.

Key Words: *mink blood serum; mice; protein*

The object of this research was to study the rapid death of mice after parenteral administration of low amounts of mink blood serum (MBS).

MATERIALS AND METHODS

Hybrid mice (CBA×C57B1/6)F_1 and outbred male mice weighing 16-18 g were used in the study. Blood was collected from minks of both sexes weighing 1-1.5 kg. Blood was taken from the heart after the animals had been sacrificed under ether anesthesia. MBS was injected to mice intraperitoneally or under ether into the retroorbital venous plexus. The volume of material injected was brought to 0.5 ml with PBS. MBS was treated with trypsin in 0.05 M phosphate buffer, pH 8.0, for 2 h at 37°C, with 5 mg trypsin (Serva) added per 2 ml MBS. After incubation, 4 mg of soybean trypsin inhibitor (Spofa) as a 1% solution in the aforesaid buffer

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TABLE 1. Death of Mice after Intravenous Injection of Pooled MBS

MBS, ml	Group	Experiment		
		1st	2nd	3rd
0.25	(CBA×C57Bl/6)F_1	5/5	5/5	5/5
0.05	As above	0/5	0/5	0/5
0.25	Outbred	5/5	5/5	–
0.05	As above	0/5	0/5	–

Note. Here and in Tables 2 and 3: the numerator is the number of dead mice and the denominator the number of animals in the group.

was added to the samples, after which they were incubated for 30 min at 37°C. MBS thus treated was intravenously injected in a dose of 0.3 ml.

RESULTS

Intravenous injection of a pool of sera from 15 minks to mice in a dose of 0.25 ml led to rapid death of all animals, whereas a dose of 0.05 ml was not lethal (Table 1). Mortality was also observed after the injection of sera obtained from individual minks (Table 2).

After intravenous injection of 0.3 ml MBS the mice became dull as soon as 7 to 10 min postinjection, and after 20-30 min their sluggishness noticeably increased; they hardly moved and made no attempt to run away when touched. After 50-60 min the animals died.

MBS in a dose of 0.15 ml caused the death of the majority of animals after 1-3 h, although some animals survived 20-24 h. The mice became sluggish 50-60 min after the injection of 0.075 ml MBS, but after 3 to 4 h the torpidity was overcome, and the majority of animals survived. Some mice died after 20-24 h.

It is worthy of note that the relationship between mouse death and the dose of MBS injected was, first, similar for the sera of all the 5 minks examined and, second, had a jumpwise pattern: mortality did not occur if the MBS dose was reduced just twofold (from 0.15 to 0.075 ml). The lethal effect of MBS on mice was more pronounced after intravenous (LD_{50}=0.10 ml) than after intraperitoneal injection (LD_{50}=0.25 ml) ($p<0.05$).

The lethal action of MBS for mice is completely destroyed after heating at 56°C for 15 min or after trypsin treatment, but is retained after seven freezings and thawings, after dialysis against phosphate buffered saline (48 h at +4°C), or after storage at -20°C for 8 months.

These data indicate that the lethal activity of MBS for mice is associated with proteins.

In order to identify the class of this protein, we fractionated MBS with ammonium sulfate. The resultant protein fractions were sedimented at 30%, from 30 to 50%, and from 50 to 70% saturation with ammonium sulfate.

TABLE 2. Mouse Death after Injection of MBS

MBS No.	MBS dose, ml		
	0.3	0.15	0.075
1	5/5	4/5	0/5
2	5/5	4/5	0/5
3	5/5	5/5	1/5
4	5/5	5/5	1/5
5	5/5	4/5	0/5

After dialysis against phosphate buffered saline the volumes of fractions were brought to the volume of the initial serum and intravenously injected to mice in a dose of 0.5 ml. The entire toxic material was distributed in the protein fractions sedimented at 30 and 30-50% saturation with ammonium sulfate, that is, under conditions of sedimentation of α- and β-, and β- and γ-globulins, respectively. The fractions obtained at saturation with 50 to 70% ammonium sulfate (corresponding to albumins) did not cause animal death (Table 3). The results suggest that MBS contains a factor which kills mice. Apparently, it is a thermolabile protein which is detected in the globulin fraction upon fractionation of the serum with ammonium sulfate. This protein may be an enzyme to which the mouse organism does not contain an inhibitor in sufficient amounts. Mortality may be caused by activation of the blood clotting system. We have been unable to find in the literature reports about the manifest toxicity of sera of other animals for mice.

TABLE 3. Mouse Death after Intravenous Administration of Fractions of Pooled MBS

Fraction No.	Ammonium sulfate, %	Experiment	
		1st	2nd
1	30	1/4	2/4
2	30–50	3/4	3/4
3	50–70	0/4	0/4