FACTORS REGULATING THE ERYTHROCYTE PICTURE OF THE BLOOD

Yu. M. Zakharov

UDC 616-005.1-07:616.155.1-007.1

An increase in the concentration of erythropoietin in the blood plasma of rabbits is observed 24 h after blood loss, and after 120 h an inhibitor which depresses maturation of erythroblasts and the release of reticulocytes into the blood stream is found. The daily production of erythrocytes reaches a maximum during the first 24 h after blood loss, but with the appearance of the inhibitor in the blood, erythropoiesis falls off considerably.

The erythropoietic activity of blood plasma rises during the first 24 h after blood loss, but subsequently falls considerably [4, 6], because of increased utilization of erythropoietic factor (EPF). However, substances retarding the hemoglobinization of reticulocytes in vitro have been found in the plasma after blood loss [2]. The role of an inhibitor in the regulation of hematopoiesis has been postulated by several workers [1, 8, 9].

The object of the investigation described below was to study the erythropoietic properties of plasma and the intensity of erythropoiesis at different times after blood loss.

EXPERIMENTAL METHOD

Experiments were carried out on 45 rabbits and 20 rats. In the experiments of series I, the erythropoietic properties of the plasma were investigated in normal recipient rats. Plasma was obtained from rabbits 20, 72, 120, and 168 h after blood loss (2% of the body weight; three rabbits at each time), frozen, and lyophilically dried on the KS-30 apparatus. Before use, the plasma was dissolved in physiological saline. The recipient rats received a subcutaneous injection of 1 ml of the prepared solution, equivalent to 8 ml of native plasma. The reticulocyte count of the rats was determined on the second day.

In the experiments of series II the erythropoietic properties of the rabbits' plasma were studied 20 (five animals) and 120-144 h (eight animals) after massive blood loss by testing their action on the erythroblastic series in a bone marrow culture [7]. Bone marrow was cultivated with the test plasma for 15 min at 38°. Plasma, and also Hanks's solution (control), were added to the bone marrow culture in doses of 0.5 ml. After incubation, differential counts of 200 cells of the erythroid series were made.

TABLE 1. Changes in Reticulocyte Count in Blood of Recipient Rats under the Influence of Concentrated Rabbit Plasma (thousands/mm^3)

Statistical index	Normal plasma	Plasma after blood loss			
		after 20 h	after 72 h	after 120 h	after 168 h
$M \pm m$	159,6±58,0	135,3±38,6	—15,0±3,8	—226,9±33,4	1,9±4,0
n	4	4	4	4	4
P	>0,05	<0,05	>0,05	<0,01	>0,3

Department of Pathological Physiology, Chelyabinsk Medical Institute. Laboratory of Experimental and Clinical Hematology, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad.
(Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 11, pp. 40-43, November, 1970. Original article submitted June 29, 1969.

TABLE 2. Changes in Number of Erythroid Cells of Bone Marrow of Normal Rabbits during Cultivation with Plasma of Normal Rabbits (1), Plasma Obtained 20 h after Blood Loss (2), and Plasma Taken 120-144 h after Blood Loss (3)

Cells of erythroid series		M	$\pm m$	n	P
Erythroblasts	1	—	—	3	>0,05
	2	—1,0	0,44	5	>0,05
	3	0,37	0,58	8	>0,05
Pronormoblasts, basophilic normoblasts	1	—6,6	4,04	3	>0,05
	2	—7,8	3,3	5	>0,05
	3	—3,12	3,5	8	>0,05
Early polychromatophilic normoblasts	1	—4,0	1,0	3	>0,05
	2	—6,0	0,9	5	<0,01
	3	11,2	3,2	8	<0,02
Middle and late polychromatophilic normoblasts	1	—4,1	2,1	3	>0,05
	2	—4,2	1,9	5	>0,05
	3	—7,75	3,23	8	<0,05
Orthochromic normoblasts	1	10,3	2,2	3	<0,05
	2	16,6	3,2	5	<0,01
	3	0,4	3,8	8	>0,05
Mitoses in erythroblasts, pronormoblasts, and basophilic normoblasts	1	0,66	0,54	3	>0,05
	2	1,0	0,5	5	>0,05
	3	0,1	0,2	8	>0,05
Mitoses in polychromatophilic normoblasts	1	1,3	0,25	3	<0,05
	2	2,4	0,5	5	<0,01
	3	—0,37	0,56	8	>0,05

In the experiments of series III the intensity of erythropoiesis was determined in rabbits before and after blood loss. For this purpose, the total daily production of erythrocytes per mm^3 (P) and the number of erythrocytes per mm^3 (N) were determined in five rabbits before blood loss (P_0, N_0), and also at intervals of 24 h (P_1, N_1), 48 h (P_2, N_2), and 120 h (P_3, N_3) thereafter. The intensity of erythropoiesis (P) was determined relative to maturation of reticulocytes in vitro [5], and calculated by the formula:

$$P = N\frac{\Delta r}{4}\cdot\frac{24}{1000},$$

where P represents the daily number of erythrocytes entering the circulating blood (thousands/mm^3), N the number of erythrocytes per mm^3, and Δr the number of reticulocytes maturing during incubation for 4 h.

EXPERIMENTAL RESULTS

Results of the experiments of series I are given in Table 1.

Plasma obtained from rabbits 20 h after blood loss increased the number of reticulocytes in the recipients, but plasma obtained 120 h after blood loss led to reticulocytopenia. Plasma obtained 168 h after blood loss had no definite action on erythropoiesis. During the first 24 h after blood loss, an increase in EPF in the plasma was thus observed, but 120 h after blood loss an inhibitor of erythropoiesis was found in the plasma.

Plasma obtained at different times after blood loss differed in its effect also on cultures of bone marrow in vitro (Table 2).

After the addition of plasma obtained 20 h after blood loss, the differential count revealed a statistically significant decrease in the number of early polychromatophilic normoblasts and an increase in the number of orthochromic normoblasts. When bone marrow was incubated in plasma obtained 120-144 h after blood loss, the number of early polychromatophilic normoblasts was higher, but the number of late polychromatophilic normoblasts was lower than in the control. This indicates delay in maturation of the normoblasts. Plasma obtained during the first day after blood loss not only accelerates maturation of normo-

blasts, but also increases the frequency of their divisions. Plasma obtained on the fifth-sixth day after blood loss, on the other hand, retards maturation of the erythroid branch of the bone marrow but has no action whatever on division of erythroblasts.

The daily production of erythrocytes (in thousands/mm^3), which was 250.2 ± 28.8 before blood loss, differed at different times thereafter. After 24 h it was 454.0 ± 25.9, after 48 h 397.2 ± 46.9, and after 120 h 302.2 ± 25.5, while the erythrocyte counts at these times (in millions/mm^3) were 2.96 ± 0.2, 3.3 ± 0.2, and 3.8 ± 0.25 respectively (compared with 4.56 ± 0.2 before blood loss).

These figures show that the daily production of erythrocytes rose sharply on the first day after blood loss and fell considerably as the deficiency of erythrocytes was made good—by the 120th hour after blood loss.

Comparison of these results suggests that in the late periods after blood loss, a factor inhibiting maturation of normoblasts in the bone marrow appears in the blood plasma, and prevents the release of reticulocytes into the blood stream.

This factor not only retards the maturation of nucleated erythroid cells but also inhibits maturation of reticulocytes in vitro [3]. Consequently, the inhibitor of erythropoiesis may prevent maturation of red blood cells at widely different stages of maturity.

LITERATURE CITED

1. R. A. Dymshits and V. S. Zudin, in: The Pathophysiology of Erythropoiesis [in Russian], Sverdlovsk (1965), p. 24.
2. R. A. Dymshits and Yu. M. Zakharov, in: Problems in the Regulation of Hematopoiesis and Blood Destruction [in Russian], Chelyabinsk (1966), p. 114.
3. Yu. M. Zakharov, The Pathogenesis of Experimental Neurogenic Anemias, Author's Abstract of Candidate's Dissertation, Chelyabinsk (1967).
4. M. G. Kakhetelidze and N. A. Fedorov, Pat. Fiziol., No. 1, 14 (1961).
5. E. N. Mosyagina, The Erythrocyte Balance under Normal and Pathological Conditions [in Russian], Moscow (1962).
6. L. N. Timoshenko, Pat. Fiziol., No. 5, 41 (1964).
7. N. A. Fedorov and M. G. Kakhetelidze, in: The Pathophysiology of Erythropoiesis [in Russian], Sverdlovsk (1965), p. 3.
8. S. Yu. Shekhter, in: The Pathophysiology of Erythropoiesis [in Russian], Sverdlovsk (1965), p. 36.
9. A. Ya. Yaroshevskii, Endogenous Stimulators of Hematopoiesis (Erythropoietins) [in Russian], Moscow-Leningrad (1963).