ERYTHROPOIETIN AND ERYTHROPOIESIS INHIBITOR IN NORMAL AND HYPOXIC NEONATES

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Erythropoietin and erythropoiesis inhibitor were studied in 22 healthy neonates and 31 neonates with transposition of the major arteries (TMA) using polycythemic mice as the model. Erythropoietin could not be detected in the plasma or urine of healthy neonates aged 5-7 days, but erythropoiesis inhibitor was found during this period. The erythropoietin level was raised in neonates with TMA until the 14th day, and lowered from the 14th to the 56th day. In concentrates of the 24-hourly urine sample from neonates with hypoxia aged 3-4 weeks erythropoiesis inhibitor also was found. The role of erythropoiesis inhibitor as a physiological regulator appearing in the blood of healthy neonates and neonates with hypoxia in the normalization of erythropoiesis during the first weeks after birth is discussed. KEY WORDS: erythropoietin; erythropoiesis inhibitor; hypoxic neonates (neonates with transposition of the major arteries); polycythemia.

The results of recent investigations suggest that erythropoiesis under physiological conditions is regulated by two factors – erythropoietin (EP) and erythropoiesis inhibitor (EPI). This hypothesis is based on the fact that an inhibitor has been discovered mainly under conditions of posthypoxia, i.e., its appearance is evidently due to the need to normalize erythropoiesis. The inhibitor has been found in polycythemic posttransfusion plasma of animals [2, 4], in the blood of mice after prolonged hypoxia [13], and in the blood of mountain dwellers and climbers during the period of their descent into the valley [10].

Active erythropoiesis has been observed in neonates during the first 1-2 days after birth; the erythropoietic activity and hematological indices of the red blood decline sharply soon after birth [3]. In some cases anemia has actually developed during the first three months [12]. Under these circumstances the character of the curve and the times of disappearance of recticulocytes from the neonatal blood during the first week were in agreement with experimental observations after cessation of a stimulating effect [3]. There is reason to suppose that, by analogy with other polycythemic states, changes in erythropoiesis are brought about by means of an inhibitor which appears in the blood during this period to normalize the red blood. This hypothesis has been confirmed by a number of investigations: inhibitor was found in the blood of neonates from two to three days after birth until one month, and in concentrations of the 24-hourly urine sample of neonates from the second to the seventh day after birth [5-7].

No reports of a study of the state of erythropoiesis in neonates with hypoxia (with transposition of the major arteries – TMA, the cyanotic syndrome) could be found in the literature.

This paper gives the results of determination of the EP and EPI levels in the blood and urine of healthy neonates and neonates with TMA.

EXPERIMENTAL METHOD

Altogether 53 neonates admitted to the neonatal care department of the Charité Children's Hospital, East Berlin, were investigated. The following groups of infants were studied: 22 healthy neonates aged 5-7 days (control); 31 neonates with TM A at different times after birth: 1-14 days, 14-56 days, and 56 days-1 year. Concentrates of the 24-hourly urine sample were studied from neonates with TMA aged 3-4 weeks (Table 1).

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TABLE 1. Erythropoietic Activity in Plasma and Urine of Healthy Neonates and of Neonates with TMA

	Erythropoietin (^{59}Fe uptake), %	Hematocrit, %	Reticulocytes, ‰	pO_2 (torr)
		Plasma		
Healthy neonates (weight 2500 g; n = 17)	0,65±0,61	49,7±9,2	40.0±18	>80
Neonates with TMA (weight over 2500 g):				
1-14 days (n = 8)	4,40±1,90	48,4±6,3	17,0±13	28±6
14-56 days (n = 12)	0,6±0,40	49,1±3,4	17±5	22±5
over 56 days (n = 8)	5,7±3,6	58±3,6	20±7	32±7
		Urine of healthy neonates		
Be (5 days)	0,19±0,3	52	—	85
		Urine of neonates TMA		
Bu (28 days)	0,1±0,2	63	—	34
Sp (21 days)	0,18±0,2	40	—	25
Gr (21 days)	0,03±0,03	55	—	25

Legend. n) Number of neonates.

TABLE 2. Erythropoiesis Inhibitor in Blood and Urine of Neonates (mean results of analyses of 5-7 polycythemic mice)

Material	Inhibition expressed as ^{59}Fe, %	P
Plasma of heathy neonates	32	<0,05
Urine of healthy neonates:		
Be	74	<0,01
Eng	64	<0,01
Ju	62	<0,01
Urine of neonates with TMA:		
Bu	87	<0,01
Sp	95	<0,01
Gr	97	<0,01

The EP level was determined by a biological test on polycythemic hypertransfused female mice of the Agness Blum strain or noninbred mice weighing 20-25 g by the method of Necas and Neuwirt [9]. The EI level also was determined on polycythemic mice: on the 4th-5th day after transfusion, 5-7 mice received an intraperitoneal injection of 0.5 ml plasma or urine concentrate, followed by a subcutaneous injection of a standard EP preparation in a dose of 0.2 units 30-60 min later. The animals of the control group received physiological saline and the same dose of standard EP. The percentage inhibition of EP in mice of the experimental group compared with the control was calculated.

EP (standard C) was obtained from the urine of patients with aplastic anemia and calibrated in accordance with the international standard B [1]. To study EP and EPI in the urine, the latter was collected in the presence of 1% phenol and kept at −20°C. The urine was concentrated by ultrafiltration on PSAL and PSAC disks (Millipore) or lyophilized and then dialyzed against distilled water for 48 h to remove low-molecular-weight urotoxins. Urine concentrates were injected in equivalent doses, calculated as creatinine (5 mg/ml). Statistical analysis of the results was carried out by Wilcoxon's test [14].

EXPERIMENTAL RESULTS AND DISCUSSION

Virtually no EP could be found in the plasma and urine concentrates of healthy neonates. In the patients with TMA during the first 14 days the blood EP level was clearly raised; from the 14th to the 56th day no EP could be detected in the blood or urine, despite the presence of hypoxia; later, however, elevation of the EP level was observed (Table 1). The curve of the change in the number of blood reticulocytes during the first six weeks after birth generally repeated the curve of the change in erythropoietic activity. The hematocrit index remained constant until the 4th week, then increased as a result of activation of erythropoiesis (Fig. 1).

To study the cause of the low EP level in the neonates with TMA between the 14th and 56th days the presence of EPI in urine concentrates at the age of 3-4 weeks was investigated. EPI in the plasma and in 24-hourly

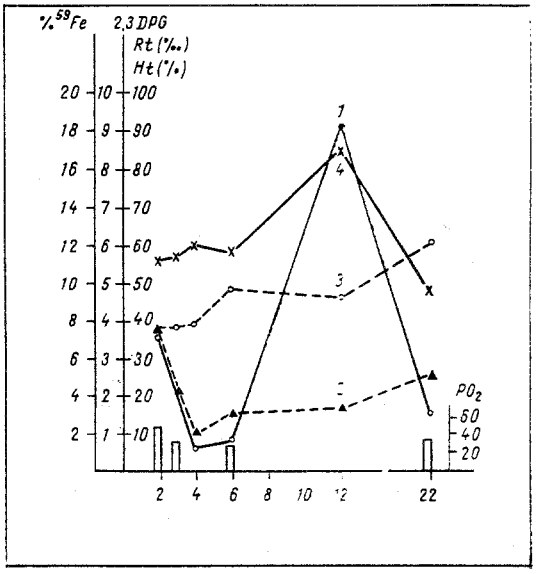

Fig. 1. EP level, in percent ^{59}Fe (1), blood reticulocyte count Rt (2), hematocrit index Ht (3), 2,3-DPG level (4), and PO_2 (torr) in neonates with TMA. Abscissa, age of neonates in weeks.

urine concentrates from healthy neonates aged 5-7 days was determined as the control. EPI was found in the plasma and 24-hourly urine concentrates of healthy neonates aged 5-7 days: EPI also was found in the 24-hourly urine concentrates of neonates with TMA aged 3-4 weeks (Table 2).

The absence or a low blood level of EP in healthy neonates aged 5-7 days conforms with data in the literature [8, 11]. Inhibitor appearing in the blood of neonates during the first days of life evidently depresses erythropoiesis and the EP level to normal. According to the present results and data in the literature [7, 11], EPI was found in the blood and urine of healthy neonates.

The study of erythropoiesis in neonates with TMA showed that the curve of the fall of their EP was shifted to the right with an interval of 10-12 days: whereas in healthy neonates no EP could be detected in the blood after the second to fourth days of life [8, 11], the EP level in the blood of neonates with TMA was clearly raised until the 14th day (Table 1, Fig. 1). The fall in the EP level in neonates with TMA from the 14th to the 56th day, against the background of obvious hypoxia, will be noted. The causes of the fall in the EP level during this period are not clear and, as in healthy neonates, it may probably be due to the appearance of EPI, which was discovered in 24-hourly urine concentrates of neonates aged 3-4 weeks. This indicates that erythropoiesis in neonates with TMA is probably also regulated by means of two factors – EP and EPI. Under these conditions elevation of the blood 2,3-DPG level also is observed (Fig. 1); this shifts the oxygen dissociation curve and facilitates the transfer of oxygen to the tissues, thus compensating to some extent for the tissue hypoxia.

In the present investigations the inhibitor was studied by the polycythemic mice method, so that its effect could be detected at the stage of undifferentiated erythroid precursors. Its action may perhaps be manifested as a disturbance of EP formation or the blocking of its effect. However, the question of the specificity to this factor requires special study.

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LIVER FUNCTION AFTER MASSIVE TRANSFUSIONS OF PACKED RED CELLS

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The liver function was studied in 16 dogs after experimental transfusion of massive doses of packed red cells in order to identify which component of the blood influences the liver function. Transfusion of massive doses of packed red cells was found not to cause any significant changes in the excretory, assimilative, and protein-forming functions or in the content of transaminases. In the control group receiving transfusions of massive doses of whole homologous blood considerable disturbances of liver function were found. The results confirm the view that one cause of disturbances of liver function in the "massive blood transfusion syndrome" is incompatibility of the plasma proteins of the donor and recipient. KEY WORDS: blood transfusion; liver function.

It was shown previously that the "massive transfusion syndrome" is characterized by disturbances of various functions of the body. Changes have been found in the blood [3, 6], circulatory [4, 11], and hemostatic [2] systems, in kidney function [9], and immunologic reactivity, signs of toxemia have been found [8], and changes observed in the composition of the blood proteins [5]. The liver is very sensitive to the action of massive transfusions of homologous blood after acute blood loss [1, 7, 10-12].

To determine which component of blood – plasma or red cells – affects liver function, experiments were carried out in which massive transfusions of these components were given separately. This paper describes the results of an investigation of the liver function after transfusion of massive doses of packed red cells.

EXPERIMENTAL METHOD

Experiments were carried out on 16 dogs. In the experiments of series I (control) repeated losses of small volumes of blood were replaced by an excess of homologous blood to the extent of 150% (seven dogs); in series II massive doses of packed red cells were transfused (nine dogs).

The animals were chosen so that the recipients and donors had identical blood groups for red cell antigens. To exclude the effect of acute blood loss on the liver function, transfusion of fresh homologous blood stabilized with TsOLIPK 12A solution was carried out without any previous acute blood loss in accordance with the following scheme: 50 ml blood was removed and quickly replaced by transfusion of homologous blood in a volume equal to that of the blood loss, and this was repeated until 50% of the circulating blood volume of the recipient dog had been replaced by the adequate volume of homologous blood. An excess of homologous blood was then transfused (at the rate of 25 ml/kg). Packed red cells were obtained from various donors. The blood of donors and recipients was tested for compatibility for red cell group antigens. Packed red cells freed from plasma and suspended in saline were transfused by the same scheme as that used for transfusion of homologous blood. The dogs were investigated in the initial state on the 1st, 2nd, 6th, 9th, and 11th days of the posttransfusion period. The following liver functions were studied: excretion and assimilation by the bromsulphalein test (BSP), protein-formation (Weltmann's test for colloidal stability of proteins), serum transaminase activi-

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