EFFECT OF STIMULATION OF ERYTHROPOIETIC ACTIVITY OF THE BLOOD SERUM ON THE SIALIC ACID CONCENTRATION IN THE SERUM AND ORGANS OF RABBITS

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The concentration of sialic acids was determined in the blood serum, spleen, liver, and renal cortex and medulla of rabbits exposed to hypoxic hypoxia or blood loss and rabbits with phenylhydrazine anemia, and a decrease in their concentration was found in the renal medulla of all the experimental animals. The sialic acid concentration in the blood serum and other organs and tissues of animals with hypoxic hypoxia and phenylhydrazine anemia also was increased, but not by the same degree as in the renal medulla. The sialic acid concentration in animals after blood loss was increased only in the renal medulla, it was reduced in the spleen and was unchanged in the renal cortex, liver, and blood serum, possibly on account of hypovolemia. A hypothetical scheme for the regulation of erythropoiesis is suggested.

It has recently been suggested that a renal factor (erythrogenin) acts on a serum substrate with the formation of active erythropoietin [4, 8, 10, 13]. These observations have been confirmed by the work of Lewis et al. [9], who found two factors in the urine of anemic patients, one of which, bound with glycoproteins and containing sialic acid, is inactive while the second acts as its activator. The biological activity of erythropoietin obtained from human urine also is readily abolished by neuraminidase through the specific removal of the sialic acid [14].

Since sialic acid is an inseparable part of erythropoietin [6] it was interesting to study the sialic acid concentration in the blood serum, spleen, liver, and renal cortex and medulla during stimulation of erythropoiesis in various ways.

EXPERIMENTAL METHOD

Experiments were carried out on male rabbits divided into four groups. The 15 rabbits of group 1 were exposed to hypoxic hypoxia by keeping them for 6 h in an atmosphere containing 10-11% oxygen. Blood was taken from the animals after 24 h by cardiac puncture; some of it was used for the determination of sialic acid [1] and the rest for testing of its erythropoietic activity in mice with polycythemia (developing after the animals had been kept in a low atmospheric pressure). The erythropoietic activity was assessed on the basis of the number of reticulocytes in the animals' blood after two injections of 1 ml of the test serum. Several modifications were made to the technique of determining sialic acids in organs [2]: instead of two drops of 66% sulfuric acid and 4 ml cyclohexanone, 4 ml of acid butanol (200 ml butanol + 10 ml 12 N HCl) was added to the aqueous phase after removal of the deoxiribose from it.

Similar experiments were carried out on 10 rabbits of group 2 after a single bleeding to the extent of 2.5% of the body weight. The erythropoietic properties of the serum and the sialic acid concentration

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TABLE 1. Changes in Sialic Acid Concentration in Blood Serum and Organs of Rabbits after Stimulation of Erythropoietic Activity of the Blood Serum in Various Ways (in mg %)

Substrate tested	Hypoxic hypoxia			Phenylhydrazine anemia			Blood loss		
	n	*M*±*m*	*P*	*n*	*M*±*m*	*P*	*n*	*M*±*m*	*P*
Blood serum	35	+2,40±0,54	<0,01	26	+2,00±0,84	<0,05	30	0	
Renal cortex	20	+0,36±0,12	<0,01	19	+0.51±0,11	<0,01	20	−0,09±0,11	>0,05
Renal medulla	20	+1,35±0,06	<0,01	19	+1,11±0,11	<0,01	21	+0,58±0,09	<0,01
Spleen	20	+0,77±0,10	<0,01	19	+0,50±0,10	<0,01	19	−0,23±0,10	<0,05
Liver	20	+1,06±0,08	<0,01	19	+0,20±0,09	<0,05	20	+0,09±0,06	>0,05

were studied in these animals 24 h after blood loss. The six rabbits of group 3 received two injections of 5% aqueous solution of phenylhydrazine at intervals of 48 h in doses of 30 mg/kg body weight. The animals of this group were killed 3-4 days after the second injection of phenylhydrazine, at a time when the hemoglobin percentage and red-cell count in the peripheral blood were lowered on the average by 50%. The control group (4) consisted of 20 rabbits.

EXPERIMENTAL RESULTS AND DISCUSSION

Although there is no doubt about the role of the kidneys in the production of the humoral stimulator of erythropoiesis, the precise place where erythropoietin (erythrogenin) is produced remains uncertain. It is postulated that it is a product of the cells of the loop of Henle [3], the renal glomeruli [5], or the renal cortex [11, 15] or medulla [7, 12].

The results obtained (Table 1) showed that with all methods of stimulation of erythropoietic activity of the rabbits a marked increase in the sialic acid concentration was observed in their renal medulla. To judge from the change in its sialic acid concentration, the role of the spleen is essentially to act as a store of the stimulator, as reflected in an increase in its content in the animals with hypoxic hypoxia and phenylhydrazine anemia and a decrease after blood loss. However, after blood loss, by contrast with the animals with hypoxic hypoxia and phenylhydrazine anemia, the sialic acid level was lowered in all the organs studied (except the renal medulla) and also in the blood serum, possibly as the result of hypovolemia.

The blood serum of the animals of all experimental groups had a high erythropoietin titer; this was expressed as a marked increase in the reticulocyte count in the peripheral blood of the recipient mice (by 2-3 times above normal; $p < 0.01$).

The view is held that the renal erythropoietic factor (erythrogenin) acts on a serum substrate and converts it into active erythropoietin. However, the analysis of the present experimental results suggests a different sequence of events. Erythrogenin, containing sialic acid in its molecule, accumulates or, more probably, is produced in the kidneys, for an increase in the sialic acid concentration was observed in the renal medulla. The blood serum evidently contains an activator of erythrogenin, possibly an α-globulin. The activation process may take place in the kidneys or within the blood stream. However, the possibility cannot be ruled out that one of the organs studied in this investigation participates in this process.

It can be concluded from the analysis of these experimental results and of data in the literature that the humoral regulation of erythropoiesis under normal and pathological conditions takes place in accordance with the following scheme: as a result of hemolysis of the red cells activators on erythrogenin, α-globulins by nature, accumulate in the blood. For instance, in the present experiments in situations leading to destruction of red cells (phenylhydrazine anemia, blood loss) the concentration of α-globulins in the blood rose sharply by comparison with its level in animals with hypoxic hypoxia. Meanwhile hypoxia, through a change in metabolism in the tissues, induces erythrogenin to accumulate in the kidneys, where under the influence of the activator it is converted into active erythropoietin. The ability of the red cells to secrete an erythrogenin activator may be acquired in the process of differentiation of the erythroid cells in the bone marrow under the influence of erythropoietin itself; the red cells may also acquire this ability in the spleen during the reticulocyte stage.

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