THE EFFECT ON ERYTHROPOIESIS OF SERUM FROM HEALTHY AND ANEMIC SUBJECTS

COMMUNICATION 1. CHANGES IN THE BLOOD AND BONE MARROW

S. Yu. Shekhter and A. Ya. Yaroshevskii

From the Laboratory of the Physiology of Circulation (Head – Professor G. P. Konradi) of the AN SSSR I. P. Pavlov Institute of Physiology and from the Teaching Clinic of Internal Diseases (Head – Active Member of the AMN SSSR M. D. Tushinskii) I. P. Pavlov I Leningrad Medical Institute

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Until the last few years, insufficient attention has been paid to the erythropoietins (hemopoietins) as factors controlling erythropoiesis. However, between 1951-1952 and the present, increasing importance is being attached to those substances on account of their theoretical and physical importance. The conception of erythropoetins in the serum was first advanced in 1906, when Carnot and Deflandre found that serum from animals rendered anemic by bleeding acquires the property of increasing the erythrocyte count and hemoglobin in rabbits into which it was injected. This result led the authors to suppose that the regeneration of the blood is due to the increased formation of a special hemopoietic stimulator which they called hemopoietin. According to them, this substance is present, but in smaller amounts, in healthy animals.

Later Zhibelli showed that the hemopoietic activity of serum appears when the animals are rendered anemic by means of phenylhydrazine. Similar properties are developed in serum in hypoxia [5, 9].

Ya. G. Uzhanskii [2] has made a detailed study of hemopoietic substances occurring in the serum after bleeding. M. G. Kakhetelidze [1] used an original method of tissue culture to demonstrate the erythropoietic activity of normal plasma and its change in different pathological condtions.

Many authors [3, 7, 8, 10] have attempted to find erythropoietins in the serum of patients with different kinds of anemia, but most of them obtained positive results only in the posthemorrhagic and hemolytic anemias.

The most extensive and detailed study of the different hemopoietins has been made by Japanese workers. According to Komyia and his coworkers [7], they are formed in the liver and spleen. Their formation and entry into the blood stream is controlled by the splanchnic and vagus nerves. Once in the blood stream, they act directly on the bone marrow.

In spite of the large amount of work, much of which has been done abroad, until now neither the nature, mode of action, origin, or regulation of the secretion of hemopoietins has yet been established, and very little is known of the frequency of their appearence in clinical forms of anemia.

METHOD

A sample of 10-15 ml of blood was obtained from a vein, and after clotting it was centrifuged at 3000 rev/minute for 10 minutes. Then the serum was sucked off, and 3 ml injected simultaneously into each of two healthy rabbits; during the two previous weeks, the erythrocyte count, hemoglobin, and number of reticulocytes had been determined, and the condition of the bone marrow studied. With the exception of 3 animals, variation in the number of reticulocytes was not more than 1%. After the injection of serum, the blood was examined daily for 7 days, and then once weekly for the next month. The blood samples were taken from an ear vein. The bone

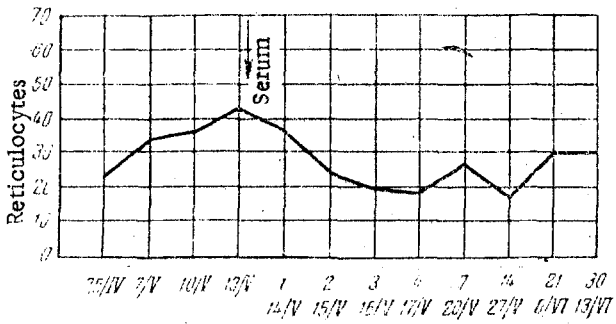

Fig. 1. Change in the erythrocyte count in a rabbit following injection of healthy human serum.

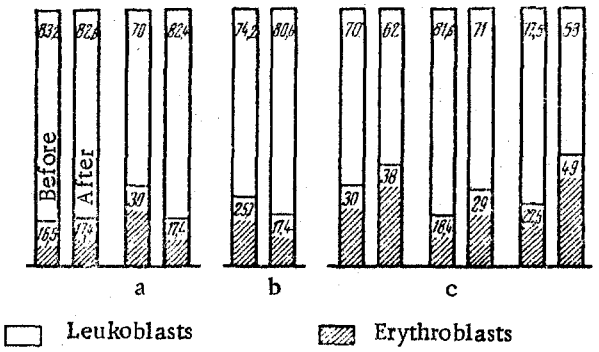

Fig. 2. Change in the ratio of erythroblasts to leukoblasts in the bone marrow (in percentage) in rabbits after injecting serum from healthy and anemic patients. a) Serum from healthy subjects; b) from patients, which does not induce reticulocytosis; c) serum which does induce reticulocytosis.

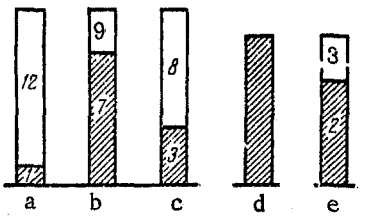

Fig. 3. Incidence of reticulocytogenic properities of the sera of healthy subjects and anemic patients. a) healthy subjects; b) B_{12}-deficient anemic patients; c) iron deficiency anemia; d) acute posthemorrhagic anemia; e) hypoplastic anemia; 1, 7, 3, 2) number of cases when serum caused no reticulocytosis; 12, 9, 8, 3) number of cases when the serum did cause reticulocytosis (numbers over the columns represent total number of subjects investigated).

marrow sample was taken by puncture of the epiphysis of femoral bone 4-5 days after injecting the serum.

In all, the serum was examined in 14 healthy subjects and in 29 patients with different kinds of anemia. Seventy-six rabbits were used and 31 injections given, of serum from 14 healthy human subjects.

RESULTS

The serum of only one of the members of the control group caused an increase in the number of reticulocytes, but in all the remaining cases, the variation in the reticular count was small (Fig. 1).

There was also very little change in the bone marrow after the injection, and the ratio of leukoblasts to erythroblasts remained unaltered (Fig. 2, a).

In no single case was any increase in the hemoglobin or erythrocyte count observed.

The absence of any reticulocytosis following the injection of healthy human serum into rabbits does not constitute a final proof of the absence of any erythropoietic stimulators in it, since the introduction of a foreign serum might by itself mask the action of small amounts of hemopoietin. That this is so is clear from the fact that injection of 3 ml of serum from 1 healthy rabbit into another, on two occasions caused an increase in the number of reticulocytes of 1 1/2-2 times.

Just as when healthy serum was injected, in no single case was there any increase in the hemoglobin or erythrocyte count after injecting anemic serum. At the same time, unlike healthy serum, that from the anemic patients much more frequently caused a marked reticulocytosis (Fig. 3).

The serum from 3 patients with severe posthemorrhagic anemia caused an average increase in the number of reticulocytes of 260% (increase from 2 to 5.2%); the reticulocytosis lasted for 3-4 days. The serum of 7 of the 9 B_{12}-deficient anemic patients caused an increase in the number of reticulocytes. Even by the 1-2nd day after the injection, a reticulocytosis developed and was maintained until the 5th day, representing an average increase of 236% above the original level (increase from 2.2 to 5.2%; Fig. 4). On no occasion did an injection from the other 2 patients cause any increase in the number of reticulocytes. The serum of only 3 of the 8 patients with gastrogenic iron deficiency anemia induced reticulocytosis, and this developed during the first few days after the injection, where the increase was 261% above the initial number of cells (increase from 2.1 to 5.6%). The serum of the remaining 5 patients had no such effect.

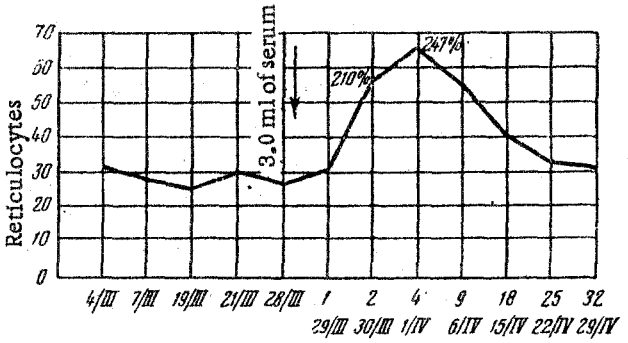

Fig. 4. Change in the number of reticulocytes after injecting serum from a patient with pernicious anemia at the height of the anemia.

The serum from one individual from the group with hypoplastic anemia, who died from this condition, caused not the slightest increase in the number of reticulocytes when injected into rabbits. Nevertheless, the serum from 2 other patients in whom there was a remission, caused an increase from 2.2 to 6.4%, or 287% above the initial level. This reticulocytosis developed on the 5-7th day after the injection, i.e. somewhat later than in the previous cases, and lasted for 3-4 days.

Injection of serum from a patient with a hereditary hemolytic anemia caused an increase in reticulocytes of 350% above the initial level (from 2 to 6 and 7%) in two rabbits into which it was injected. Serum from patients with various secondary anemias also increased the reticulocyte count, while sera from a patient with polycythemia vera and from 2 patients suffering from splenomegaly and cirrhosis of the liver caused no change in the reticulocyte number.

The reaction of the bone marrow to the serum injection was similar to that of the peripheral blood, i.e. the increase in the number of reticulocytes in the latter was accompanied by a reticulocytosis and an increased production of erythroblasts with a shift towards the polychromatophils in the bone marrow (see Fig. 2).

The variable condition of the patients and the fact that only the small number of 29 of them were available made it impossible for us to reach a final conclusion as to the result to be obtained with any particular kind of anemia; nevertheless, the impression was gained that the power to induce reticulocytosis in animals is present in the serum of patients with acute posthemorrhagic anemia (in agreement with published results); the same effect is still better shown in the serum of patients with B_{12}-deficiency anemia, though this result does not agree with that of Komyia [7]. At the same time, the reticulocytogenic effect is not typical for the sera of patients with iron deficiency anemia (see Fig. 3).

We were not able to observe any relationship between the reticulocytogenic effect of the serum and the stage of the desease, degree of the anemia, or number of reticulocytes.

Thus, injection of serum from patients with different kinds of anemia into healthy rabbits frequently caused an increase in the number of reticulocytes; the effect was well shown and appeared to be genuine, since it was accompanied by a corresponding reticulocytosis and erythroblastosis in the bone marrow. Since healthy human serum does not possess this property, it must be supposed that a large amount of some substance which stimulates erythropoiesis is formed in the blood of these patients. It remains to inquire whether these substances are genuine stimulators, or whether they lead to an increased erythropoiesis by bringing about some previous hemolysis; this view seems to us all the more likely because in no case did we observe a polycythemia vera. To find the answer to this and to many other problems concerning the mode of action of the erythropoietins, and their chemical structure, we have carried out further studies including experiments on pigment metabolism, the presence of hemolysins, and the significance of the globulin fraction, etc; the results will form the subject of a further paper.

SUMMARY

A subcutaneous injection of 3 ml of serum of patients suffering from acute posthemorrhagic anemia and from pernicious anemia provokes an increase in the number of reticulocytes in healthy rabbits. Not only is there a peripheral reticulocytosis, but there is also an increase in the number of reticulocytes and erythroblasts in the bone marrow.

The serum of some patients suffering from iron deficiency anemia possesses the same property, but the effect occurs less regularly than in the groups mentioned previously.

Some of the healthy persons has no effect on the number of reticulocytes. Possibly there is an increased production of erythropoetins in some forms of anemia.

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