THE ACTION OF SERA OF HEALTHY SUBJECTS AND ANEMIC PATIENTS ON ERYTHROPOIESIS

II. THE MECHANISM OF THE ERYTHROPOIETIC ACTION OF SERUM

S. Yu. Shekhter

Laboratory of the Physiology of Circulation and Respiration (Head – Professor G. P. Konradi), I. P. Pavlov Institute of Physiology of the AN SSSR and Clinic of Propedeutic Therapy (Head – Active Member AMN SSSR M. D. Tushinskii) I. P. Pavlov Leningrad Medical Institute

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 39-42, January, 1960

Original article submitted June 20, 1959

The erythropoietic influence of the serum of anemic animals and human subjects on rabbit blood has been demonstrated many times. It is sometimes thought that this effect is due to the appearance in the serum and plasma of so-called erythropoietins, but the evidence concerning the chemical nature and mode of action of the latter is very contradictory. Carnot and Deflandre [4], V. N. Shreder [2], Hodgson and Toha [10], and Rambach [13] think that their presence is associated with proteins. At the same time Borsook and his co-workers [3], Erslev and his co-workers [6, 7] Linman and Bethell [12], Gordon and his co-workers [9], and Fried and his associates [7] who obtained an erythropoietic effect after injecting a protein-free plasma extract, maintain that erythropoietins are not proteins. Korst and Bethell [11] and Gley and Delor [8] think that there are two erythropoietins one of which is a protien and the other not.

Finally, some workers [1, 2, 14, 15] deny the existence of special erythropoietins. Zih [14] ascribed the hemopoietic effect of the serum of anemic animals to the presence in it of dissolved hemoglobin. Ya. G. Uzhanskii [1] considers that stimulation of erythropoiesis in rabbits occurs secondarily through a hemolysis brought about by erythrodieretins which are present in large amounts in the serum of animals rendered anemic through bleeding.

At the suggestion of A. Ya. Yaroshevskii we have made a special study of the substances responsible for the erythropoietic effects of serum, and have tried to find whether the effect is primary or whether it results from the hemolytic action of the serum. The latter test was particularly important, since in our experimental work we never observed a single case where there was an increase in the amount of hemoglobin or of erythrocytes, in spite of the appearance of a reticulocytic crisis following the injection of many of the sera.

METHOD

The serum was freed from the globulin fraction by electrodialysis, the method being based on the property of globulin to precipitate in a salt-free medium. Distilled water was added to 3 ml of the serum to bring the volume to 10-12 ml, and the solution then poured into an ebonite chamber so that it lay in the space between two layers of parchment. A constant electrical current of maximum strength 150 ma from a 150-200 v source was continued until the current fell to zero through deposition of the electrolyte (after $1\frac{1}{2}$-$2\frac{1}{2}$ hours). The dialysate was filtered from the precipitated globulins, and after adding NaCl was injected into the rabbit at the same time that the serum of the same patient was injected into a second animal, as explained in a previous communication.

The presence in the serum and the titre of hemolysins was determined using the method of Thomson as modified by A. D. Zaslavskii. One drop of a 20% suspension of rabbit erythrocytes was added to 6 drops of whole or diluted plasma. The hemolysis was estimated visually after maintaining the mixture at 38° for 45 minutes.

Pigment metabolism was studied in rabbits using the usual method of Terven. Since no stercobilin was found in the feces, even after intravenous injection of distilled water which is known to be a hemolytic agent, we could determine only the amount of urobilin in the urine, although the presence of this pigment may result not only from an increased hemolysis, but also from liver dysfunction. The urobilin was estimated 1-2 days before and 3-7 days after injecting the serum.

RESULTS

In all, we carried out an electrodialysis of 20 sera from 15 anemic patients. Without giving detailed clinical descriptions of their condition, it may be mentioned that in 18 cases out of the 20 the effect of the injected dialysate was the same as that of the serum injected simultaneously; in 11 cases neither the serum nor the dialysate caused any reticulocytosis, in 7 both the serum and the dialysate caused an increase in the number of reticulocytes. Only in two cases had the serum treated by electrodialysis lost its reticulocytogenic properties. Both sera were taken from patients with secondary anemias (associated with cancer of the stomach and rheumatic endocarditis).

The fact that electrodialysis in most cases does not eliminate the reticulocytogenic properties of the serum indicates that the part played by the globulin in the hemopoietic action is secondary. The maintenance of the power of the serum to cause a reticulocytosis when the globulin fraction is removed indicates indirectly that the hemolytic action of the serum is not primary, because chemically the hemolysins are related to the globulins. Nevertheless we thought it necessary to make a special investigation of this point.

We therefore made a study in vitro to determine the presence of hemolysins acting on the erythrocytes of the patient or those of the rabbit. In 9 cases, not a single hemolysin active against the erythrocytes of the blood itself was found. The determination of hemolysins which acted on rabbit erythrocytes was carried out for 35 sera from 4 healthy human subjects and 25 patients. In all 35 cases, the serum completely dissolved rabbit erythrocytes, a result which accords with published results. At the same time, reticulocytosis occurred only with the injection of 13 out of 35 sera. We had to consider whether the reticulocytosis which occurs only in certain cases is due to the high content of hemolysins in the sera of the subjects. We therefore determined the titre of hemolysins in 19 sera from 16 patients. In 11 out of the 19 cases the value obtained was 1: 4, and reticulocytosis occurred or was absent in the same number of cases irrespective of the titre.

Serum treated by electrodialysis lost the power of dissolving rabbit erythrocytes in vitro. Of the 10 dialysates studied by us of which 7 were taken from patients and 3 from healthy subjects, hemolysins were absent in all cases, whereas all the sera were reticulocytogenic.

Since the effect of the serum is independent of the titre of the hemolysins, and does not disappear when they are removed, we may explain the observed action of the serum only by assuming it is due to the introduction into the rabbit of hemolysins already elaborated.

We studied pigment metabolism * in 27 rabbits, into 17 of which injections from 4 healthy subjects and 12 anemic patients were injected. It was found that in all animals, before the serum injection, urobilin was either absent from the urine or present only in traces. Injection of healthy human serum never caused any increase in the amount of urobilin in the urine. At the same time, of the 17 injections of the serum of anemic patients, in 5 urobilinuria occurred.

However, this urobilinuria was never accompanied by a reticulocytosis, and in two instances the reticulocytosis actually occurred before urobilin was formed in the urine. The effect on urine urobilin of injecting dialysates was studied in 10 rabbits, and only in one animal was there an increase in the amount ofurobilin, although there was no reticulocytosis. At the same time we made obsevations on six rabbits in which the reticulocytosis caused by the injection of dialysate (2 cases) or serum (4 cases) was not accompanied by the formation of urobilin in the urine. The relationship between the amount of urinary urobilin and a reticulocytosis is shown in the table.

Relationship Between the Urobilinuria and Reticulocytosis

Number of rabbit	Amount of urobilin, mg	Effect of serum or dialysate.
56	63,3	+
69	21,6	—
58	32	Reticulocytosis preceded urobilinuria
68	79,8	—
94	20	Reticulocytosis preceded urobilinuria
67	294	—
75	—	+
79	—	+

The study of pigment metabolism has therefore shown that in some cases the serum of anemic patients has the property of causing urobilin to appear in the urine, but the fact that the urobilinuria occurs only occasionally and independently of the reticulocytosis shows that the reticulocytogenic effect of the serum cannot be ascribed to the increased hemolysis. It is possible that urobilinuria is a consequence of the damage to the liver occurring after the injection of the foreign serum.

The negative results obtained with the globulin fraction, with the introduction of complete hemolysins, and in studying hemolysis in the animal itself show that a primary hemopoietic effect is caused by injecting animals with serum from anemic patients. The absence of any parallelism between the increase in hemoglobin and erythrocytes when the number of reticulocytes increases may have been due to the small amounts of serum which we were compelled to use.

* A large amount of the work involved in observations on pigment metabolism was carried out by V. Ya. Plotkin, a student at the first I. P. Pavlov Leningrad Medical Institute.

SUMMARY

Experiments were performed on rabbits treated with serum from anemic patients. The nature of substances possessing erythropoietic properties was studied. It was found that the erythropoietic effect of the patients' blood was not related to the globulin fraction and also that it acted independently of any hemolytic agents present.

LITERATURE CITED

[1] Ya. G. Uzhanskii, The Part Played by Erythrocytes in the Regeneration of Blood [in Russian] (Leningrad, 1949).

[2] V. N. Shreder, Trudy inst. tsitologii, gistologii i ėmbriologii 3, 1, 83 (1948).

[3] H. Borsook, A. Graybiel, G. Keighley, and others. Blood, 1954, v. 9, p. 734.

[4] P. Carnot and Cl. Deflandre, Compt. rend. Acad. sc., 1906, v. 143, p. 384.

[5] P. Carnot, Compt. rend. biol., 1906, v. 58, p. 344.

[6] A. J. Erslev and P. H. Lavietes, Blood, 1954, v. 9, p. 1055

[7] W. L. Fried, L. Pizak, L. O. Jacobson, and others, Proc. Soc. Exper. Biol. and Med. 1956, v. 92, p. 203.

[8] P. Gley and J. Delor, Compt. rend. Soc. biol. 1955, v. 149, p. 635.

[9] A. S. Gordon, S. J. Pilliero, W. Kleinberg and others, Proc. Soc. Exper. Biol. and Med. 1954, v. 86, p. 255.

[10] G. Hodgson and J. Toha, Blood, 1954, v. 9, p. 299.

[11] D. R. Korst and F. H. Bethell, Clin. Res. Proc. 1957, v. 5, p. 142.

[12] J. W. Linman and F. H. Bethell, Blood, 1956, v. 2, p. 310.

[13] W. A. Rambach, H. L. Alt, and J. A. D. Cooper, Blood, 1957, v. 12, p. 1101.

[14] A. Zih, Arch. ges. Physiol., 1930, Bd. 225, S. 613.