NATURE OF THE CHALONE FRACTION OF ERYTHROCYTE EXTRACTS

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Chalone factors [2, 16], which belong to the group of membrane surface proteins or their fragments [9], are present in extracts of erythrocytes [2, 16]. One of the chalone factors is fraction 1, which has physicochemical properties similar to those of albumin [8]. According to other investigators [13], fraction 1 of erythrocyte extracts consists of a stock of free hemoglobin chains. The nature of this fraction thus remains uncertain.

The aim of this investigation was to study this problem.

EXPERIMENTAL METHOD

Partially purified erythrocytic chalones (EC) were obtained by incubating erhthrocytes, previously washed four times with physiological saline to remove contaminating leukocytes and plasma proteins [8]. Erythrocytes were taken from five healthy blood donors, six patients with polycythemia, and 20 rats weighing 120-150 g. The EC and blood serum were separated by disk electrophoresis. Areas of the gels containing fraction 1 [8] were cut out and it was used to immunize eight rabbits [10]. At each injection 25 μg/g of the chalone fraction 1 was used. In Ouchterlony's test fraction 1 of erythrocyte extracts from blood donors and from patients with polycythemia, standard hemoglobin and human serum albumin preparations, and also blood serum from three patients with hematologic diseases and from 29 rats weighing 80-100 g with posttransfusion polycythemia and with acute blood loss, was investigated. The volume of blood removed and of erythrocytes injected was 2% of the body weight.

EXPERIMENTAL RESULTS

The chalone fraction gave one precipitation line with antiserum, which was observed in a maximal dilution of 1:64. This fraction, obtained from healthy donors' erythrocytes, cross-reacted with the analogous fraction isolated from erythrocytes of patients with polycythemia, and with the standard serum albumin preparation, but did not react in this way with hemoglobin (Fig. 1). Immunologic identify also was observed between the chalone fraction of rat erythrocyte extracts and rat blood serum albumin.

Because of the absence of immunologic similarity between hemoglobin and fraction 1 molecules, it can be concluded that this fraction does not belong to the stock of free hemoglobin chains. This hypothesis is supported by data showing different biological activity of these proteins: Hemoglobin stimulates [6], whereas chalones inhibit erythropoiesis [8].

Despite their immunologic identity fraction 1 of erythrocyte extracts and albumin differed in their electrophoretic mobility (Fig. 2), and this was observed on dilution of the purified preparations also [8]. The molecular weight of the chalone fraction is 13,000 daltons less than that of albumin. The biological (inhibitory) activity of the chalone fraction was exhibited in a dose of 23 μg/g body weight [8], whereas injection of protein fractions of normal blood serum, enriched by 50-81% with albumin, into animals stimulates erythropoiesis [17]. Albumin in a dose of 625 μg/g body weight also has the same action on proliferation of regenerating cells [11]. Data obtained on polycythemic rats also indicates a difference between serum albumin and fraction 1 of erythrocyte extracts. Compared with the control, during posttransfusion polycythemia the content of all protein fractions was increased in the blood serum:

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TABLE 1. Titer of Chalone Fraction Rat Blood Serum after Transfusion of Erythrocytes and Acute Blood Loss

Parameter	Polycythemia					Blood loss
	time of investigation, h					
	0	2½	4	6	24	24
Titer Hematocrit	1:2000 52±2,21	1:2000 53±2,73	1:2000 52±2,7	1:4000 52±2,1	>1:16 000 55±1,3	1:500 24±2,52

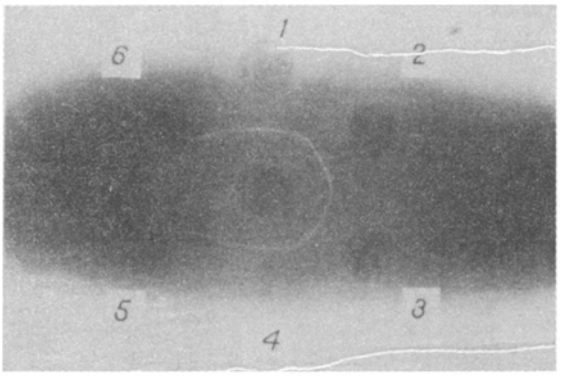

Fig. 1

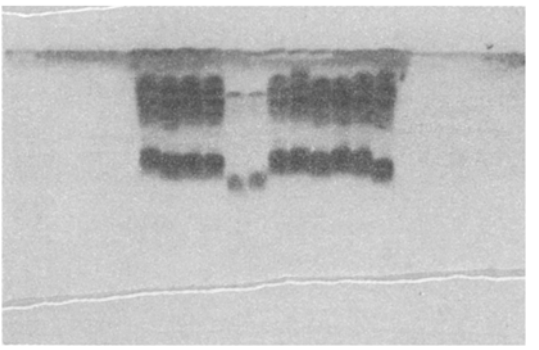

Fig. 2

Fig. 1. Ouchterlony's precipitation test. Central well contains antibodies to chalone fraction; peripheral wells: 1, 4) chalone fraction, 2, 3) albumin, 5, 6) hemoglobin.

Fig. 2. Disk electrophoresis of EC (two columns in center) and of blood serum (four columns on left and six columns on right). Stained with Coomassie.

of prealbumins by four times, of albumins by 1.3 times, of α-globulins by 2.5 times, and of β- and γ-globulins by 1.4 times [7].

The most probable cause of the increase in protein content in the blood of the polycythemic animals is liberation of proteins from the erythrocyte membranes [5, 9]. Besides protein molecules, fragments of them may also evidently be liberated, and may form complexes with serum proteins. Albumin was found to have particularly great ability to form inetermolecular bonds [14]. Immunologic analysis of blood serum of control and polycythemic rats revealed a more than eightfold increase in the titer of the chalone fraction in polycythemia (Table 1), which is difficult to explain purely on account of a change in the albumin content alone.

Thus on the basis of the similarity and differences between albumin and the chalone fraction it can be postulated that different sites exist in the molecules of these proteins: 1) some of them, responsible for antigenicity, are identical, 2) others, on which biological activity depends, are different. Properties of this kind are observed not only with chalones, but also with other biologically active substances, such as fibronectins [3], hormones [2], and also plasma clotting factors and proteins isolated from the surface of platelets [1].

The presence of a cross reaction with the chalone fraction and albumin makes true determination of the level of endogenous inhibitors difficult, although in some cases, for example, in posttransfusion polycythemia and posthemorrhagic anemia, it does allow the state of erythropoiesis to be judged (Table 1).

Inhibition of proliferation of the erythron is found 6 h after transfusion of erythrocytes and it increases in intensity with time [4]. Changes in the chalone titer in the blood of polycythemic animals (Table 1) correlates with these data. The results indicate that an important role in the mechanisms triggering the development of polycythemia is played by chalones, and not by erythropoietin, the quantity of which depends on the level of the hematocrit index [15], and does not change substantially during the first day of investigation [6]. In posthemorrhagic anemia the chalone titer is sharply depressed, but this is accompanied by stimulation of erythropoiesis, and is evidence that chalones are involved, along with erythropoietin, in this process.

Clinical observations are in good agreement with the experimental data. In control patients (five) the serum chalone level varied from 1:16,000 to 1:4000, whereas in three patients with posthemorrhagic anemia it was below 1:250, 1:250, and 1:500, respectively. The erythrocyte counts of these patients varied from 2.96 millions to 3.5 millions per mm^3 blood.

Bleeding is used in the treatment of polycythemias. Investigations showed that both in four rats (six bleedings, each of 0.3% of body weight, on alternate days), and in a patient with polycythemia (four bleedings each of 0.7% of body weight in the course of 1 month), the chalone level in the blood was observed to rise fourfold.

It can thus be postulated on the basis of these results that in certain cases, by determining the chalone levels, it is possible to monitor the state of erythropoiesis in man and in animals. With the aid of many repeated bleedings, the concentration of endogenous inhibitors in the blood can be increased.

LITERATURE CITED

1. V. P. Baluda and V. V. Shiryaev, Usp. Sovrem. Biol., 78, No. 3 (6), 371 (1974).
2. V. A. Beloshevskii, in: Proceedings of the 4th International Symposium on Chalones [in Russian], Moscow (1983), p. 102.
3. S. M. Bychkov, Vopr. Med. Khim., No. 6, 2 (1983).
4. I. I. Gitel'zon, S. F. Sid'ko, N. V. Khripach, et al., Tsitologiya, No. 6, 632 (1977).
5. G. P. Grigor'ev, Vopr. Med. Khim., No. 1, 91 (1981).
6. N. N. Mosyagina, N. A. Fedorov, V. I. Gudim, et al., in: Normal Hematopoiesis and Its Regulation [in Russian], Moscow (1976), p. 341.
7. G. V. Neustroev, Patol. Fiziol., No.4, 69 (1978).
8. G. V. Neustroev, Fiziol. Zh. SSSR, No. 6, 846 (1980).
9. G. V. Neustroev and N. P. Korolev, Byull. Éksp. Biol. Med., No. 9, 345 (1979).
10. L. G. Kovaleva, Gematol. Transfuziol., No. 10, 29 (1984).
11. V. M. Rodionov, N. A. Kuz'micheva, A. V. Pospelova, et al., Vopr. Med. Khim., No. 4, 569 (1981).
12. V. B. Rozen, Fundamentals of Endocrinology [in Russian], Moscow (1980).
13. I. M. Shur'yan, N. F. Starodub, and A. N. Gritsak, Byull. Éksp. Biol. Med., No. 11, 1328 (1976).
14. M. L. Callango, L. H. Caldera, R. Suinaga, et al., Acta Haemat. (Basel), 68, 295 (1982).
15. T. M. Kilbridge, W. Fried, and P. Heller, Blood, 33, 104 (1969).
16. D. J. Perrins and W. A. Jones, Acta Haemat. (Basel), 65, 247 (1981).
17. B. Steinberg, H. Frank, and R. A. Martin, Acta Haemat. (Basel), 33, 279 (1965).