RNA CONTENT IN ERYTHROCYTES AS A MEASURE OF THE RATE OF ERYTHROPOIESIS

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KEY WORDS: erythropoietin; erythropoiesis; testing.

Mainly two methods are used at the present time to determine the rate of erythropoiesis. The first method is based on counting the relative number of reticulocytes [1], the second on determining incorporation of radioactive iron (^{59}Fe) into newly formed erythrocytes [5]. It is well known, however, that visual counting of reticulocytes is laborious, liable to serious errors, and that no method of automatic counting of reticulocytes has yet been devised. In turn, the use of radioactive iron requires a specially equipped laboratory and special conditions for keeping the animals with incorporated radioactivity, so that the widespread use of this method in clinical and laboratory practice is difficult. Meanwhile, in hematology, there is a need for a convenient method of diagnosis of the state of erythropoiesis in patients with aplastic anemia and other blood diseases.

For the reasons given above intraerythrocytic markers contained in young erythrocytes but absent in mature cells are interesting. These include RNA. The RNA content in reticulocytes is known to be much greater than in mature erythrocytes, and no special difficulties are attached to its quantitative determination.

In the investigation described below correlation between the RNA content in erythrocytes and the rate of erythropoiesis was studied under various conditions, and on the basis of this correlation a method of testing erythropoietic activity is suggested.

EXPERIMENTAL METHOD

Experiments were carried out on female CBA mice weighing 19-22 g and on noninbred rabbits. The rate of erythropoiesis in the rabbits was increased by bleeding (40% of the blood volume). The RNA content in 0.1 ml of erythrocytes was determined by a spectrophotometric method [3] using a conversion factor of 10.5 for RNA. Reticulocytes were counted to 2000 erythrocytes in dried films stained with brilliant cresyl blue. The rate of erythropoiesis in mice was reduced by keeping them in a pressure chamber at 0.5 atm for 20 h daily for 21 days, in order to produce hypoxic polycythemia. Erythropoietin from rabbit plasma was purified by the method in [2] after stage 2 and tested by means of International Standard B by the method in [5].

EXPERIMENTAL RESULTS

The change in the RNA content in the erythrocytes must be influenced primarily by nucleic acids contained in the leukocytes. However, data in the literature [4] for man show that this effect is negligible compared with the RNA content in the erythrocytes. This effect should be even less in animals with a short life span of their erythrocytes, notably in mice and rabbits. In fact, by killing leukocytes by several different methods and then measuring the RNA content in the erythrocytes, no significant decrease in this parameter was found in the presence of leukocytes in a concentration of up to 10^4 cells in 1 mm^3 blood. To use the RNA content as a criterion of the rate of erythropoiesis, correlation was studied between the RNA content in erythrocytes and the number of reticulocytes during stimulation (Fig. 1). It will be clear from Fig. 1 that the character of the change in

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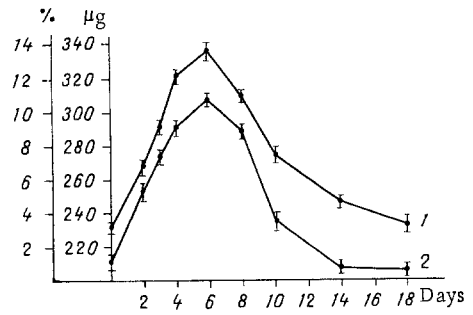

Fig. 1

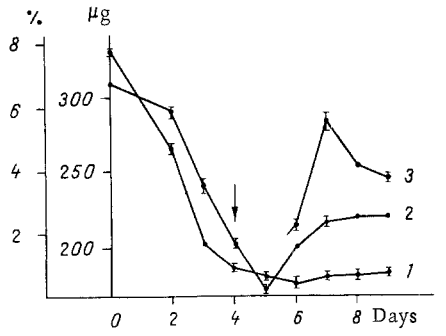

Fig. 2

Fig. 1. Changes in RNA content and reticulocyte count in rabbit after bleeding. Abscissa, time (in days). 1) Reticulocyte count (in %), 2) RNA content (in μg/0.1 ml erythrocytes).

Fig. 2. Changes in RNA content and reticulocyte count in mice with hypoxic polycythemia after exposure in pressure chamber and effect of erythropoietin on RNA content. Abscissa, time (in days). 1) Reticulocyte count (in %), 2) RNA content (in μg/0.1 ml erythrocytes), 3) RNA content (in μg/ml erythrocytes) after administration of erythropoietin. Arrow indicates time of injection of erythropoietin.

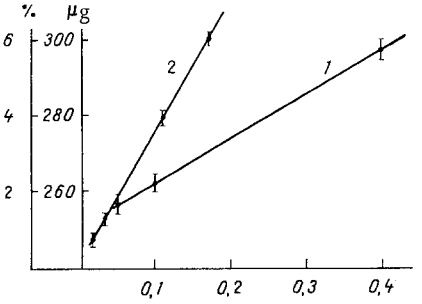

Fig. 3. RNA content in erythrocytes and incorporation of ^{59}Fe into erythrocytes of mice with hypoxic polycythemia depending on dose of erythropoietin B (standard). Abscissa, units of erythropoietin B standard. 1) Incorporation of ^{59}Fe into erythrocytes (in %), 2) RNA content (in μg/0.1 ml erythrocytes).

these values was completely identical, but the relative change in the RNA content was smaller than the change in the number of reticulocytes. Correlation of this nature can be explained on the grounds that RNA in young erythrocytes is degraded more slowly than the reticulocytes can take up the stain. The class of RNA-containing cells is thus probably wider than the class of reticulocytes, but all the latter was included in it. This conclusion is also confirmed by the inhibition of erythropoiesis in mice with hypoxic polycythemia (Fig. 2).

The relationship between the RNA content and rate of erythropoiesis was used to test erythropoietic activity. First the action of one dose of erythropoietin was tested in mice with inhibited erythropoiesis. For this purpose, starting on the 1st day after withdrawal of the mice from hypoxic conditions, the RNA content was measured in the animals of one group (control), whereas animals of the other group (experiment) received an injection of 0.12 unit erythropoietin on the 4th day after the end of their stay in the pressure chamber and measurement of the RNA content continued. As Fig. 2 shows, injection of erythropoietin into mice with hypoxic polycythemia caused a marked increase in the RNA content in the erythrocytes compared with the control.

The method of testing erythropoietic activity based on RNA content in the erythrocytes thus consists of preparing mice with inhibited erythropoiesis, into which 0.5 ml of the test erythropoietic material in 0.9% NaCl was injected on the 4th day after the return to normal conditions. Control mice were given an injection of 0.5 ml

of 0.9% NaCl. The mice were decapitated on the 7th day, blood was collected, and the RNA content in 0.1 ml of erythrocytes was measured. Activity of the test material was determined from a calibration curve obtained with a known dose of erythropoietin (Fig. 3). As Fig. 3 shows, the first point (a dose of 0.05 unit of the erythropoietin standard) for the method with radioactive iron differs significantly from the control (0.49 ± 0.06%, $P < 0.05$). Smaller doses of the erythropoietin standard under the same conditions no longer differ significantly from the control. Consequently, the sensitivity of the method with radioactive iron is 0.05 unit of erythropoietin, in good agreement with data in the literature [5].

For the method of testing based on the RNA content in the erythrocytes, under the same conditions, the first point – a dose of 0.01 unit of erythropoietin – differs significantly under the same conditions from the control (219.8 ± 1.87 μg, $P < 0.01$). When the suggested method is used, sensitivity is thus increased fivefold.

Moreover the main advantage of the suggested method of testing erythropoietic activity is its simplification as a result of doing away with the need to work with a radioactive label, so that the method is capable of wide application in clinical and laboratory practice.

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EFFECT OF MORNING AND EVENING CORTISOL INJECTIONS ON CIRCADIAN RHYTHMS OF 11-HYDROXYCORTICOSTEROID EXCRETION IN RATS

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KEY WORDS: stress; cortisol; injections; circadian rhythms; 11-hydroxycorticosteroids.

In modern life man frequently encounters factors which disturb the circadian rhythm of glucocorticoid secretion. Such factors include night work, flexible shift work, sleep disturbances, flights across time zones, steroid therapy, and so on. Investigations have shown a connection between the therapeutic effects of corticosteroids and the time of their administration [8, 11, 12]. However, the biorhythmologic aspects of the pharmacodynamics of glucocorticoids caused by their long-term administration or stimulated by stress, whose metabolic action depends essentially on relations with rhythms of excretion of other hormones [9], have received insufficient study.

This paper describes a study of circadian rhythms of 11-hydroxycorticosteroid (11-HCS) excretion with the urine during injection stress and administration of cortisol at different times (morning and evening).

EXPERIMENTAL METHOD

Male Wistar rats weighing 200 g were used. The animals were kept five in a cage at a temperature of 24 ± 1°C, with artificial lighting from 8 a.m. to 8 p.m. and with access to food and water ad lib. A microcrystalline suspension of hydrocortisone acetate (from Richter, Hungary) was injected intramuscularly once a day

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