# EXPERIMENTAL DETERMINATION OF THE RESERVE CAPACITY OF ERYTHROPOIESIS

I. I. Gitel'zon, N. B. Khripach,
V. P. Makarov, and E. I. Kalent'eva

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A sharp decrease in the number of erythrocytes in the circulating blood to different levels and the maintenance of these levels subsequently for long periods were observed in rabbits after acute blood loss to the extent of 1.5-2.1% of the body weight.

The intensity of hematopoiesis may be as much as five times higher than normal for up to 245 days. The absence of a positive response to stimulation with cobalt and testosterone suggest that the observed intensity of erythropoiesis was close to the maximum.

* * *

One of the factors determining the effectiveness of control in biological systems is the reserve capacity or "control reserve" [4] of these systems. The reserve capacity of the hematopoietic system can therefore be used as a qualitative index of the mechanism of regulation of the erythrocyte concentration in the blood system. By reserve capacity of erythropoiesis is meant the highest attainable intensity of erythropoiesis, expressed as the degree to which the output of erythropoiesis per unit time exceeds its normal level while the blood system remains in equilibrium.

On a priori grounds, the concept of reserve capacity must evidently be subdivided into peak capacity, developing during an acute disturbance of equilibrium in response to a stimulus acting once and for all, and the stationary reserve capacity, which the system can develop for a long period of time (comparable with the life span of the erythrocytes) in response to a continuous stimulus.

It was assumed during the investigation of the kinetics of erythropoiesis that under experimental conditions the rate of erythropoiesis can be increased to 10 times the normal value [11].

Data on reparative erythropoiesis in the literature apply either to acute blood loss [2, 7], or to chronic losses of relatively small volumes of blood [1, 6, 10, 12-14]. In neither of these cases does the level of hematopoiesis necessarily reach its steady upper limit.

The object of this investigation was to determine the highest rate of hematopoiesis which the erythropoietic system can develop and steadily maintain in long-term experiments.

## EXPERIMENTAL METHOD

A sharp decrease in the erythrocyte count of the circulating blood to various levels was produced in rabbits weighing 2900-3000 g by acute blood loss to the extent of 1.5-2.1% of the body weight, and the erythrocyte count was subsequently maintained for long periods by measured blood losses on alternate days (the volume of blood withdrawn was calculated each time from the erythrocyte concentration in the blood stream).

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TABLE 1. Changes in Hematologic Indices during Prolonged and Repeated Bleedings

| Series of expt. | Erythrocyte count ($10^6$/mm$^3$) | Hemoglobin concentration (in g%) | Reticulocyte count (in %) | Hematocrit index | Circulating blood volume (in ml) | Volume of maintenance of blood loss (in ml) |
|---|---|---|---|---|---|---|
| Control | 4,56±0,23 | 13,1±0,6 | 2,6±0,3 | 38±3,0 | 142±5,8 | — |
| I | 2,74±0,11 | 6,6±1,1 | 21,4±3,6 | 21±2,0 | 139±5,3 | 22,8±4,2 |
| II | 2,08±0,59 | 4,6±0,3 | 28,2±2,0 | 20±2,0 | 144±5,3 | 29,9±3,1 |

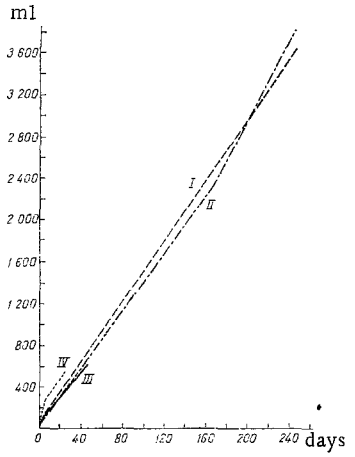


Fig. 1. Increase in total volume of blood withdrawn. 1) Erythrocyte count $2 \cdot 10^6$/mm$^3$; 2) erythrocyte count $3 \cdot 10^6$/mm$^3$; 3) blood loss with simultaneous stimulation by testosterone and cobalt; 4) preliminary injection of testosterone and cobalt followed by blood loss.

Before the beginning of the experiments and during their course the erythrocyte count and hemoglobin concentration were determined in the rabbits by the method of Derviz and Vorob'ev [3, 5], and the reticulocyte count and circulating blood volume by a dye method (using Evan's blue). The value of each of these indices was obtained as the mean of results of measurements on three blood samples, in which the standard deviation of the erythrocyte count and hemoglobin concentration was 1.5-2% and the standard deviation of the reticulocyte count and circulating blood volume was 3-5%.

Two series of experiments were carried out. In series I, the erythrocyte count was reduced to $3 \cdot 10^6$/mm$^3$ in the animals by acute blood loss amounting to 1.5% of the body weight, and it was subsequently maintained at this level for 245 days by measured bleeding on alternate days.

In the experiments of series II, the erythrocyte count was reduced to the level of $2 \cdot 10^6$/mm$^3$ by acute blood loss amounting to 2.1% of the body weight, and this level was maintained until the end of the experiment by measured bleeding on alternate days. The experiment lasted for 239 days, but some of the animals died before this period had ended, without, however, any sign of hypoplasia of the bone marrow or a decrease in the productivity of erythropoiesis until their death.

The degree to which the intensity of erythropoiesis exceeded the normal level was calculated from the formula:

$$n = \frac{r_i E_i}{r_0 E_0} \cdot 100\%,$$

where $r_0$ represents the original percentage of reticulocytes, $E_0$ the original number of erythrocytes per mm$^3$, $r_i$ the mean percentage of reticulocytes during the experiment, and $E_i$ the mean number of erythrocytes per mm$^3$ during the experiment.

The result of calculation by means of this formula agrees well with direct determination of hyperfunction of erythropoiesis based on the total volume of the mass of erythrocytes liberated.

## EXPERIMENTAL RESULTS

In both series of experiments the intensity of erythropoiesis was increased by 510% over normal (Table 1), i.e., a level on the average 5 times higher was reached and maintained steadily for 8 months, or for not less than 4 generations of erythrocytes.

The stability of this process is also demonstrated by the daily variations in the volumes of blood which had to be withdrawn to maintain the stipulated erythrocyte concentration in the circulating blood (Fig. 1).

By the end of the experiment the productivity of hematopoiesis had not fallen. During the first month, in the series with an erythrocyte count of $2 \cdot 10^6$, for instance, 427 ml blood was taken, compared with 456 ml during the last month, when the corresponding reticulocyte counts were 29.3 and 19.9%. The decrease

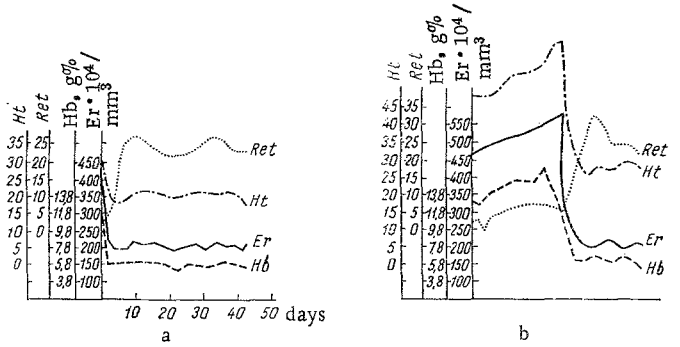


Fig. 2. Changes in red blood indices in rabbits with an experimentally produced erythrocyte count of $2 \cdot 10^6/mm^3$ followed by stimulation with testosterone and cobalt:
A) bleeding and simultaneous injection of stimulants;
B) preliminary injection of stimulants followed by bleeding.

in reticulocyte count while the productivity of erythropoiesis remained the same can evidently be explained either by changes in their maturation time and an increase in the effectiveness of erythropoiesis (through a decrease in output of erythrocytes with a shortened life span), or by an increase in the area of active hematopoiesis. In the series with an erythrocyte count of 2,800,000, in the first month 436 ml blood was taken, and in the last month 559 ml, when the reticulocyte counts were 20.5 and 23.1%, respectively.

To determine whether the level of erythropoiesis attained in the first two series of experiments was at the upper limit, the experiments of series III were carried out, in which hematopoiesis was stimulated by blood losses accompanied by injections of testosterone and cobalt. The basis for the use of these substances to stimulate erythropoiesis is given by results described in [8, 9].

The experiments of series III were of two types. In the first, the animals received subcutaneous injections of testosterone and cobalt simultaneously with bleeding in accordance with the following scheme: testosterone (0.0125 g), followed after 2 days by cobalt (3 mg/kg), followed by a break for 24 h. The injections were subsequently repeated in the same order. In the experiments of the second type, preliminary injections of cobalt and testosterone were given by the same scheme and in the same doses until no further increase in the erythrocyte count could be obtained. Bleedings were then carried out by the scheme specified above. The changes in the hematologic indices in the experiments of these types are shown in Fig. 2, and the increase in the total volume of blood removed, in Fig. 1.

In these experiments, the productivity of erythropoiesis relative to normal was 500% in the first group and 520% in the second group of experiments. In one short experiment, moreover, the erythrocyte count was lowered to $1.5 \cdot 10^6/mm^3$, although in this case the productivity of erythropoiesis likewise was increased by not more than 5 times.

Clearly, therefore, hematopoiesis can persist for a long time at a level 5 times higher than normal.

The absence of a positive response to stimulation by cobalt and testosterone, in the form of a further intensification of erythropoiesis, suggests that in all groups of experiments the intensity of erythropoiesis which developed was close to the upper limit, i.e., the complete reserve of the stationary erythropoietic capacity of the animal to correspond to the aims of the experiment had been mobilized.

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