# MEASUREMENT OF HEMOGLOBIN CONTENT IN ALBINO RAT ERYTHROCYTES DURING ONTOLOGY

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Systems analysis of the erythron [6] leads to the idea of a functional system existing within the constraint of this morphological concept. The main system-forming factor, namely the result of activity, in this system is an adequate oxygen capacity of the blood, maintained by a definite erythrocyte count and hemoglobin level. From the hierarchical point of view this functional system may be included in the system supplying the body's requirements of oxygen [5]. A central question in the study of this functional system is that of investigation of the oxygen-binding capacity of the erythrocytes. Ontogenetic analysis of transformations of the functional system is an urgent task also from the standpoint of elucidation of the general principles of system formation [1].

The aim of this investigation was to study the hemoglobin concentration in rat erythrocytes in the prenatal and early postnatal period.

## EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats of the following ages: adult (n = 15); 18-day (n = 26), 20-day (n = 21), and 22-day fetuses (n = 15): neonates (n = 29), and young rats aged 5 days (n = 21) 10 days (n = 13), and 15 days (n = 12). Pregnancy was dated by the usual method [3] and the day when spermatozoa were first found was taken to be the first day of pregnancy. Blood was sampled by decapitation, using C-heparinoid ("Spofa") as the anticoagulant. Investigation of the fetuses was carried out by laparotomy and hysterotomy under pentobarbital anesthesia (50 mg/kg). The erythrocyte concentration was measured by counting in Goryaev's chamber and the hemoglobin concentration by the hemoglobincyanide method, using the GF-Ts-04 hemoglobinometer. The hemoglobin concentration in an individual erythrocyte was measured by photometry on the MIF cytophotometer at a wavelength of 415 nm [7]. The results were subjected to statistical analysis by methods of variance, correlation, and regression analysis, using Student's t test.

## EXPERIMENTAL RESULTS

Analysis of the state of the red blood of the albino rats showed the main tendency of transformation of its picture during ontogeny to be a steady rise of the erythrocyte concentration (Table 1). Changes in the hemoglobin concentration were phasic in character. During the last quarter of pregnancy it remained at the 92-94 g/liter level, rising on the first day of life to 111 ± 2.62 g/liter. This parameter subsequently fell, corresponding to the early postnatal anemia described in rats [8], followed by a rise.

The cytophotometric study of the hemoglobin concentration in the erythrocytes showed a progressive fall. It was more than twice as high in 18-day-old fetuses as in adult rats (Table 1). The most rapid fall of the mean optical density of the cell took place during the first 5 days of postnatal development, evidence of an intensive change in the composition of the circulating erythrocyte population, for the parameter studied is constant for each individual cell.

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TABLE 1. Parameters of Erythrocyte Saturation with Hemoglobin in Albino Rats of Different Ages

| Parameter | Adults, n = 15 | Prenatal period | | | Postnatal period | | | |
|---|---|---|---|---|---|---|---|---|
| | | 18 days, n = 7 | 20 days, n = 21 | 22 days, n = 15 | 1 day, n = 29 | 5 days, n = 21 | 10 days, n = 13 | 15 days, n = 12 |
| Erythrocyte concentration, $10^{12}$/liter | 5,85±0,20 | 1,26±0,11 | 1,80±0,05 | 1,81±0,08 | 2,27±0,09 | 2,31±0,11 | 2,75±0,17 | 3,77±0,20 |
| $p_1$ | | <0,001 | <0,001 | <0,001 | <0,001 | <0,001 | <0,001 | <0,001 |
| $p_2$ | | | <0,001 | >0,8 | <0,001 | >0,04 | <0,05 | <0,001 |
| Hemoglobin concentration, g/liter | 129±2,80 | 94±3,70 | 94±2,05 | 92±2,00 | 111±2,62 | 86±2,07 | 90±3,06 | 99±3,77 |
| $p_1$ | | <0,001 | <0,001 | <0,001 | <0,001 | <0,001 | <0,001 | <0,001 |
| $p_2$ | | | >0,8 | >0,04 | <0,001 | <0,001 | >0,2 | >0,05 |
| Mean hemoglobin content per erythrocyte, pg | 22,2±0,57 | — | 53,0±1,58 | 51,7±2,06 | 50,6±2,47 | 39,4±2,13 | 33,4±1,58 | 27,2±1,75 |
| $p_1$ | | | <0,001 | <0,001 | <0,001 | <0,001 | <0,001 | <0,05 |
| $p_2$ | | | | >0,4 | >0,4 | <0,01 | <0,05 | <0,05 |
| Mean optical density of erythrocytes, conventional units | 18,1±0,52 | 43,5±1,51 | 38,3±0,81 | 38,9±0,81 | — | 24,6±1,11 | 25,4±0,60 | 21,0±0,22 |
| $p_1$ | | <0,001 | <0,001 | <0,001 | | <0,001 | 0,001 | <0,001 |
| $p_2$ | | | <0,2 | >0,8 | | <0,001 | >0,8 | <0,001 |

**Legend:** $p_1$) comparison of parameter with level in adult animal; $p_2$) comparison with previous age group.

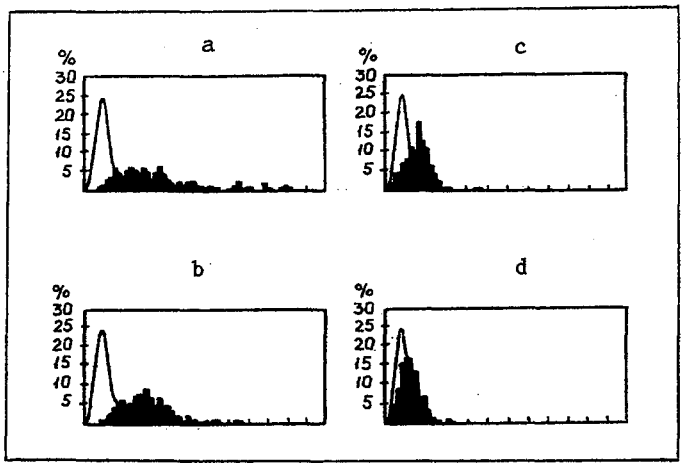


Fig. 1. Histograms of distribution of erythrocytes by hemoglobin content in 18- (a) and 22-day fetuses (b), and in young rats aged 5 (c) and 15 days (d). Line on all figures indicates distribution of erythrocytes of adult rats. Abscissa, optical density of cells (conventional units); ordinate, number of cells (in percent).

Correlation analysis between the mean optical density of the erythrocyte and the mean hemoglobin content showed a high degree of correlation between these parameters ($r = 0.842$; $p < 0.001$). Coefficients of the linear regression equation were calculated, so that optical density units could be converted into absolute units of hemoglobin content:

$$C = -7{,}138 + 1{,}527\ E,$$

where C denotes mean hemoglobin content in erythrocyte (in pg); E denotes optical density of cell (conventional units).

Besides a decrease in the mean hemoglobin content per erythrocyte, marked changes took place in the character of distribution of the cells with respect to this parameter (Fig. 1). A significant decrease in the width of the distribution, a shift of the histogram toward lower values of hemoglobin content, and the appearance of a distinct modal class in the 20 pg region for adult rats were noted.

Analysis of the numerical data shows that the peripheral component of the fetal rat erythron in the later stages of development is characterized by a low erythrocyte concentration, coupled with increased saturation with hemoglobin. Erythrocytes in the blood differ considerably in their hemoglobin content, evidently reflecting the sequence of age-related layers of hematopoiesis and the simultaneous presence of the progeny of erythroid precursor cells, belonging to different layers, in the peripheral blood [2]. With the transition to extrauterine conditions of development, a stepwise increase in the erythrocytes and hemoglobin concentration in the peripheral blood was observed, possibly due to a decrease in the circulating plasma volume as a result of changes in the hemodynamics. Later, against the background of an increase in erythrocyte concentration, a decrease in the hemoglobin concentration was observed (early postnatal anemia).

The changes in values of hemoglobin concentration in the erythrocytes indicate reduction of heterogeneity of the circulating erythrocyte pool, superposed on a decrease in the mean values of these parameters. This last fact may be evidence that precursors cells of the involvement and predominance of precursor cells belonging to the hematopoietic layer of adult animals.

The general tendency for changes in the distribution of rat erythrocytes by hemoglobin content in ontogeny agrees with the principles discovered by the study of age transformations of the human erythron [4] and it confirms that the rat erythron is an appropriate model on which to study the general mechanisms of formation of the erythrocytic system.

## REFERENCES


1. T. A. Adzhimolaev, Functional Systems of the Organism [in Russian], Moscow (1987), p. 395.
2. A. N. Vorob'ev, I. L. Chertkov, and M. D. Brilliant, Textbook of Hematology [in Russian], Vol. 1, Moscow (1987).
3. A. P. Dyban, V. F. Puchkov, V. S. Baranov, et al., Objects of Developmental Biology [in Russian], Moscow (1975), pp. 505-566.
4. V. G. Leonova, Analysis of Erythorcyte Populations in Human Ontogeny [in Russian], Novosibirsk (1987).
5. A. N. Medalyanovskii, Functional Systems of the Organism [in Russian], Moscow (1987), pp. 77-103.
6. K. V. Sudakov, Functional Systems of the Organism [in Russian], Moscow (1987), pp. 26-43.
7. Yu. R. Khrust, L.L. Litinskaya, M. M. Yakobashvili, et al., Tsitologiya, **13**, No. 12, 1525 (1971).
8. T. Sanengen, P. H. Holter, A. Haga, et al., Molecular and Cellular Aspects of Erythropoietin and Erythropoiesis, Bad Windsheim (1987), pp. 187-204.