# EFFECT OF ERYTHROCYTIC POLYPEPTIDE ON THE ERYTHRON SYSTEM IN EXPERIMENTAL ANEMIA

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Interaction between the structural elements of tissues in a multicellular organism is an important problem. Several different investigators have repeatedly suggested the existence of information molecules, maintaining communication during cellular activity. For instance, Morozov and Khavinson (1974) suggested that in the body there are biological regulators, which transmit biological information necessary for normal functioning, development, and interaction of cell populations, and they formulated the concept of a new class of informative molecules, known as cytomedins. Cytomedins have now been obtained from virtually all organs and tissues and from blood cells. Their nature, the predominant character of their activity, and also additional side effects have been established. It has been shown that different cytomedins, regardless of the organs, tissues, or cells from which they were isolated, affect cellular and humoral immunity, the state of the hemostasis and complement system, lipid peroxidation, phagocytosis, and other defensive reactions of the organism [2, 3, 5]. Many investigations have been devoted to the study of self-regulation of the erythron. It has been shown that breakdown products of erythrocytes are among the chief regulators of erythropoiesis [1, 4]. However, until now no regulators of erythropoiesis have been isolated from erythrocytes.

The aim of this investigation was to study the effect of erythrocytic polypeptides on the state of the erythron system in animals with induced anemias.

## EXPERIMENTAL METHOD

Polypeptides were obtained from erythrocytes by an original method of our own design, based on acetic acid extraction of erythrocytes in the presence of zinc ions, followed by precipitation of the extract, purification, and lyophilization. The following data are evidence in support of the polypeptide nature of the preparation, conventionally named "erythrocytin":

1. The preparation gives a positive biuret reaction.
2. The maximum of the absorption spectrum of a solution of the preparation lies between 220 and 280 nm.
3. On gel filtration erythrocytin splits up into five polypeptide fractions, with mol. wt. of between 3000 and 8000 D. Experiments were carried out on noninbred female albino rats weighing 180-200 g. Before the experiment began, the following parameters were determined in all the animals: erythrocyte, leukocyte, and reticulocyte counts (counting chamber, microscopy of blood films stained with brilliant cresyl blue), hemoglobin (by photoelectric colorimetry), and hematocrit (by the usual method). Toxic anemia was induced in the experimental animals by injection of phenylhydrazine hydrochloride in a dose of 3 mg/100 g body weight intramuscularly for 2 days [6]. Starting with the 5th day after the last injection of phenylhydrazine (the peak of the toxic anemia) the rats were divided into three groups: 30 animals received injections of erythrocytin, dissolved in isotonic sodium chloride solu-

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TABLE 1. Parameters of Hemogram in Rats with Toxic Anemia (M ± m)

| Parameter of hemogram | Intact animals | | Control (physiological saline) | | | Experiment 1 (erythrocytin) | | Experiment 2 (vitamin $B_{12}$ + Ferrum Lek) | |
|---|---|---|---|---|---|---|---|---|---|
| | background | 14th day | bkgrnd. | 7th day | 14th day | 7th day | 14th day | 7th day | 14th day |
| Hemoglobin, g/ℓ | 154,3±3,30 | 152,6±3,50 | 94,6±2,60 | 96,0±5,50 | 96,4±4,50 | 100,1±2,10 | 100,2±3,50* | 90,6±2,30 | 97,8±3,10 |
| Erythrocytes, × $10^{12}$/liter | 7,20±0,12 | 7,01±0,10 | 3,00±0,18 | 3,22±0,11 | 3,54±0,14 | 3,68±0,10 | 5,35±0,15** | 3,41±0,12 | 3,99±0,20 |
| Reticulocytes, % | 20,1±3,50 | 21,4±4,30 | 7,02±1,80 | 8,02±1,60 | 7,03±1,20 | 16,4±2,20** | 20,7±2,80** | 9,32±1,91* | 10,8±2,60 |
| Leukocytes, × $10^{9}$/liter | 6,95±0,11 | 7,80±0,15 | 3,20±0,10 | 3,42±0,12 | 4,18±0,09 | 4,21±0,08** | 5,31±0,09* | 3,93±0,10 | 4,86±0,11* |

**Legend.** *$p < 0.05$ indicates statistically significant difference between toxic anemia and background, **$p < 0.001$ for statistically significant difference compared with background.

tion, during the next 5 days in a dose of 1 mg/kg (experiment 1); 30 rats received vitamin $B_{12}$ in accordance with the same schedule in a dose of 1 $\mu$g/kg, together with "Ferrum Lek" in a dose of 0.1 ml/kg (experiment 2); the control group, consisting of 30 animals, received daily intramuscular injections of 0.2 ml physiological saline. Determination of these parameters was repeated on the 7th and 14th days after the beginning of "treatment." The rats were killed on the 15th day and their femoral bone marrow was studied. The results were subjected to statistical analysis by Student's test.

## EXPERIMENTAL RESULTS

The data given in Table 1 show that in all groups of animals the initial parameters studied were lower than in intact rats. In animals of the control group injection of physiological saline caused no significant changes in the parameters studied, except a tendency for the leukocyte count to rise; red blood parameters in this group of animals indicated the presence of marked anemia. After injection of erythrocytin (experiment 1) the erythrocyte count of the rats with anemia rose on average by 78.3%. Meanwhile their hemoglobin level rose ($p < 0.001$) and their reticulocyte count returned to normal, evidence of stimulation of erythropoiesis in the bone marrow. In animals receiving vitamin $B_{12}$ and the iron preparation, a small and not significant ($p > 0.05$) increase in the erythrocyte count was observed. Unlike erythrocytin, vitamin $B_{12}$ and Ferrum Lek had no normalizing effect on the hemoglobin level and reticulocyte count. In all experimental groups a very small nonspecific increase in the leukocyte count was observed, but it was not significant.

In animals with phenylhydrazine-induced anemia, histological preparations of the bone marrow revealed marked inhibition of erythropoiesis, as shown by a decrease in the number of primarily differentiated precursors – pronormocytes and basophilic normocytes; among the polychromatophilic and oxyphilic normocytes, degeneration of the nuclei was delayed, and irregular oxyphilia occurred in the cytoplasm. The number of cells in the final stages of erythropoiesis was sharply reduced.

In rats receiving vitamin $B_{12}$ and Ferrum Lek no significant time course of the parameters of erythropoiesis could be seen compared with the controls.

By contrast, animals receiving erythrocytin showed a distinct increase in the intensity of erythropoiesis. The numbers of all forms of precursors of erythrocytes were increased in these animals and signs of delay of nuclear degeneration were abolished, so that normal maturation of the erythrocytes was facilitated.

Clinical observations on anemic rats receiving erythrocytin showed that their body weight exceeded that of the controls, and became very close to that of intact rats. These animals had a better appetite, they were distinguished by greater motor activity, and they did not lose as much hair as rats of the control group.

Polypeptides obtained from erythrocytes can thus restore the regenerative potential of the erythron in toxic anemia, so that it can be classed as a possible regulator of erythropoiesis.

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# $\alpha_1$-ADRENERGIC RECEPTORS IN THE LIVER PARENCHYMA IN CHILDREN: CHANGES ASSOCIATED WITH CIRRHOSIS

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An important role is nowadays ascribed to adrenergic systems in the development of several liver diseases [7, 9-12]. $\beta$- and $\alpha$-adrenergic receptors and their agents are involved in the pathogenesis of severe forms of cirrhosis of the liver, hepatomas, and certain forms of portal hypertension [6, 8, 13, 15, 16]. However, receptors of the liver parenchyma in children have virtually not been studied. In preliminary experiments a very small increase was found in the number of $\alpha_1$-adrenergic receptors in the liver parenchyma of children with chronic hepatitis, possibly due to the participation of these receptors in the regulation of hepatocyte growth during regeneration of the liver [4, 5].

The aim of this investigation was to study $\alpha_1$-adrenergic receptors of the liver parenchyma in children with cirrhosis and to establish the role of these receptors in the development of parenchymatous liver damage.

## EXPERIMENTAL METHOD

Specimens were obtained during surgical biopsy of the liver by the marginal resection method, performed on children aged from 2 to 14 years with an extrahepatic form of portal hypertension (EHPH) without parenchymatous damage (control group, n = 7) and with parenchymatous damage (group of patients with cirrhosis, n = 8).

The diagnosis was based on morphological and electron-microscopic analysis, together with consideration of clinical, biochemical, virological, and immunological data. After removal the specimens were quickly frozen in liquid nitrogen and stored at −70°C. The liver membranes were isolated by the method in [3]. The tissue was homogenized in buffer containing 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 8.0, in a "Polytron" homogenizer (USA). The homogenate was centrifuged ("Beckman I2-21," USA) for 20 min at 2000*g*, and the resulting supernatant was centrifuged for 60 min at 32,000*g*. The residue was resuspended in 40 ml of buffer containing 50 mM Tris-HCl, pH

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