INVESTIGATION OF CARBOHYDRATE METABOLISM IN ERYTHROID AND MYELOID CELLS OF RABBIT BONE MARROW

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Experiments on rabbits showed that myelokaryocytes, consisting mainly of cells of the erythroblastic series, have higher respiratory activity and a relatively low rate of glucose utilization, glucokinase activity, and intensity of hydrolysis compared with myelokaryocytes, consisting mainly of cells of the myeloid series. The energy metabolism in the cells of the erythroid series is evidently more efficient. This is shown by the much higher concentration of high-energy phosphorus compounds in them than in the myeloid cells.

During the development of erythroid hyperplasia of the bone marrow, the glucokinase activity of its myelokaryocytes falls [1]. Changes in the intensity of respiration and glycolysis in the bone marrow cells during hyperplasia as the result of anemization were observed by Warren [17] and by Wehara et al. [16]. Because of the absence of methods of separating bone marrow cells, investigations of the biochemical properties of the bone marrow cells published in the past have disregarded their heterogeneity, and this has made the results difficult to interpret.

In the present investigation some aspects of the energy metabolism of erythroid and myeloid cells of the bone marrow, isolated with relatively slight contamination by cells of the other type, were investigated.

EXPERIMENTAL METHOD

To obtain erythroblastic cells, bone marrow in a state of erythroid hyperplasia was used, and to obtain myeloid cells, bone marrow with reduced erythropoiesis was used. After isolation of the myelokaryocytes, their oxygen absorption and glucose utilization, hexokinase activity, intensity of glycolysis, content of readily hydrolyzed phosphorus of high-energy compounds, and glycogen were determined.

Experiments were carried out on Chinchilla rabbits weighing 2-3.5 kg. Hyperplasia of the erythroid tissue was induced by three or four successive bleedings (17-20% of the circulating blood volume determined with the aid of Evans' blue dye [4]). To prevent increased regeneration of myeloid tissue, the leukocytes were separated from the blood thus taken [3], resuspended in physiological saline, and returned to the blood stream. To suppress erythroid growth, erythrocytes of intact rabbits, washed and suspended in physiological saline, were injected intravenously into the experimental rabbits in doses of $2 \cdot 10^{10}$–$4 \cdot 10^{10}$ cells/kg body weight three or four times in the course of 12-14 days. Before the experiment and every 3-4 days thereafter, the hemoglobin concentration, the erythrocyte and leukocyte counts, and the hematocrit index were determined for the blood of the experimental animals. The myelogram of some of the animals was studied.

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TABLE 1. Indices of Carbohydrate Metabolism and Morphological Composition of Myelokaryocytes Isolated from the Bone Marrow of Intact Rabbits, Marrow in a State of Erythroid Hyperplasia, and Marrow with Reduced Erythropoiesis

Source of myelokaryocytes	Statistical index	Erythroblasts – normoblasts (in %)	Myeloblasts – myelocytes (in %)	Polymorphs – metamyelocytes (in %)	Glucose absorption (in μg/mg protein)	Glucokinase activity (in μg glucose/mg protein)	Increase in lactic acid (in μg/mg protein)	Absorption of oxygen (in μl/10^9 cells)	Readily hydrolyzed P of high-energy compds. (μg/mg protein)	Glycogen (in mg/10^9 cells)
Bone marrow of intact rabbits (control)	M ± m n	21±4,2 10	27±4,6 10	38±5,8 10	383±49 20	204±20 24	15,4±3,6 6	637±14,9 15	15±1,2 13	2,3±0,17 16
Bone marrow in state of erythroid hyperplasia	M ± m n P	60±4,4 7 <0,001	9±2,9 7 0,005	22±4,2 7 0,04	138±18 25 <0,001	80±8,6 34 <0,001	5,85±0,38 9 <0,02	702±14,4 18 0,006	25±1,9 21 <0,001	1,6±0,08 29 <0,001
Bone marrow with reduced erythropoiesis	M ± m n P P_1	5±0,99 14 0,002 <0,001	57±4,5 14 <0,001 <0,001	20±5,2 14 0,03	283±50 18 0,17 0,01	124±10,7 18 0,001 0,002	10,1±1,06 7 >0,25 <0,002	425±2,3 14 <0,001 <0,001	14±1,3 7	2,4±0,3 23 >0,8 0,016

Note. P is the index of significance of the differences with the control; P_1 is the index of significance of the difference between results of investigation of bone marrow with reduced erythropoiesis and bone marrow in a state of erythroid hyperplasia.

The bone marrow was obtained after the development of hyper- or hypoplasia of the erythroid tissue. Myelokaryocytes were separated by the hemolytic method [3] at 0-2°C. Some of the myelokaryocytes were suspended in a mixture of borate buffer (pH 8.55) and 0.5 M NaCl solution in the ratio of 7:3, disintegrated by repeated freezing and thawing, and then homogenized on ice for 7-8 min. The homogenate was centrifuged at 2000 rpm for 5 min at 2°C. The extract thus obtained was used for the determination of hexokinase activity by Long's method [11], the phosphate buffer [2] being replaced by borate. For the investigation of glucose absorption, the myelokaryocytes (final concentration 6000-8000 cells/mm^3) were incubated in autologous plasma obtained from rabbits before any procedures had been carried out. The oxygen demand of the cells and the rate of glycolysis were determined under anaerobic conditions in the presence of glucose, using cells in a concentration of 35,000-40,000/mm^3 incubation mixture consisting of Krebs–Ringer-phosphate solution (pH 7.4) and blood serum mixed in the proportion of 1:1. The oxygen demand was determined manometrically in a Warburg apparatus and the lactic acid concentration by the method of Barker and Summerson [6]. Incubation was carried out at 37°C, and its duration for investigation of hexokinase activity and glucose utilization was 1 h, while for the investigation of oxygen absorption and the intensity of glycolysis it was 30 min. Protein was precipitated by Somogyi's method [15], the glucose concentration was determined by Nelson's method [13], and the protein concentration by Lowry's method [12]. Inorganic phosphorus was determined in trichloroacetic supernatants of myelokaryocytes [10], and the readily hydrolyzed phosphorus of the high-energy compounds was determined after hydrolysis for 10 min in 1 N HCl solution at 100°C. Glycogen was isolated by Good's method [9] and determined quantitatively as glucose with thymol sulfate reagent [14]. For the statistical analysis of the results, the constant method of indirect differences [5] was used.

EXPERIMENTAL RESULTS

The results are given in Table 1. They show that myelokaryocytes which consisted mainly (77%) of cells of the myeloid series absorbed more than twice as much glucose than myelokaryocytes consisting mainly of erythroid cells (60%). They also possessed much higher glucokinase activity. These findings correspond to the greater intensity of glycolysis observed dur-

ing incubation of myeloid than of erythroid cells. The glycogen content in the myelokaryocytes consisting of cells of the myeloid series was higher than in myelokaryocytes consisting mainly of erythroblastic cells. Glycogen is known to be absent from cells of the erythroid series [7]. The discovery of a small quantity of glycogen in myelokaryocytes consisting mainly of erythroblastic cells can evidently be attributed to contamination by mature forms of granulocytes.

On the other hand, the myelocytes consisting mainly of cells of the erythroblastic series absorbed oxygen twice as fast, i.e., they had a higher intensity of respiration. It can evidently be concluded that glycolytic processes are predominant in myeloid cells. Erythroblastic cells have a higher intensity of respiration then myeloid cells, and a much lower intensity of glycolysis. In all probability they have a higher level of energy metabolism, because respiration, which is more marked in the erythroblastic cells, is several times more efficient from the energy point of view than glycolysis. The higher concentration of readily hydrolyzed phosphorus of high-energy compounds observed in the cells of the erythroblastic series confirms this hypothesis. An increase in the ATP content was observed by Stepanova [8] in erythroid hyperplasia of the bone marrow.

The results of these investigations are naturally of relative importance. However, comparison of the results obtained for the energy metabolism in myelokaryocytes consisting mainly of erythroid or myeloid cells is facilitated to some extent by the fact that the degree of contamination with mature forms of granulocytes was equal in the two cases compared, yet the difference between the indices of energy metabolism which were studied were considerable and were statistically highly significant.

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