EFFECT OF ERYTHROPOIETIN ON ULTRASTRUCTURE OF BONE MARROW ERYTHROBLASTS

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In polycythemic albino rats erythropoietin stimulates pinocytosis and ropheocytosis. The contents of the numerous pinocytotic vesicles possess peroxidase activity. The number of pores in the nuclear membrane is increased and the nucleoli are sharply hypertrophied. Aggregation of ribosomes into polysomes develops. Swelling is observed in the mitochondria, which later increase sharply in number and in the electron density of their matrix on account of the accumulation of iron-containing material. It is suggested that an important mechanism of the action of erythropoietin is determined by its reactions at the cell-membrane level.

Erythropoietin promotes specialization of polypotent cells of hematopoietic tissue toward erythroid differentiation, and this is accompanied by an increase in the utilization of Fe^{59} and an increase in the absolute number of reticulocytes in the peripheral blood [10, 13, 14]. Erythropoietin is also known to induce increased proliferative activity at the level of all generations of the erythroblastic series and to shorten the length of their stay in the bone marrow by 50% [9]. After injection of erythropoietin-active serum maturation of reticulocytes is accelerated [4, 16]. Cells of the erythroblactic series differing in their potential level and degree of differentiation thus have common structural components capable of reacting to erythropoietin. The level of organization of the cell at which this interaction takes place must be clarified in order to discover the principles governing the regulation of erythropoiesis [7, 12].

No data on the dynamics of the submicroscopic organization of the erythroid cells under the influence of erythropoietin could be found in the literature. References to it are few in number and fragmentary in nature [15].

The object of the present investigation was to study the ultrastructure of cells of the erythroblastic series during their transformation under the influence of erythropoietin.

EXPERIMENTAL METHOD

Experiments were carried out in 30 noninbred male albino rats weighing 150-180 g. After reduction of erythropoiesis by transfusion of red cells, the serum of albino rats taken 20 h after acute blood loss was injected intraperitoneally in a dose of 0.5 ml/100 g body weight. The erythropoietic activity was tested by determining the stathmokinetic index [6]. The material examined under the electron microscope was femoral marrow taken from the albino rats. The material was fixed in 1% OsO_4 solution and embedded in Epon-812. Sections were cut on the LKB-4800 Ultratome, negatively stained with uranyl acetate, and examined in the UÉMB-100 K electron microscope. The cytotopography of the iron-containing material was analyzed with reference to the localization of peroxidase activity [11].

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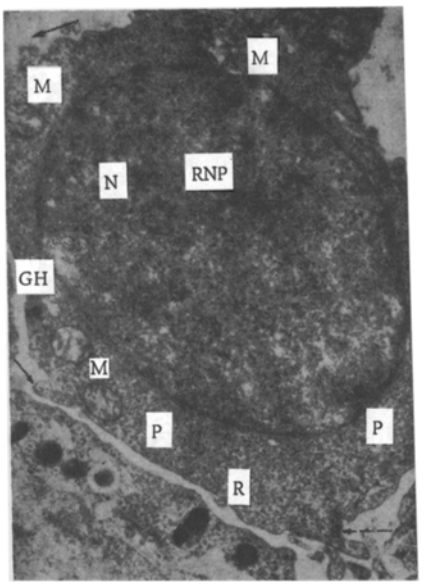

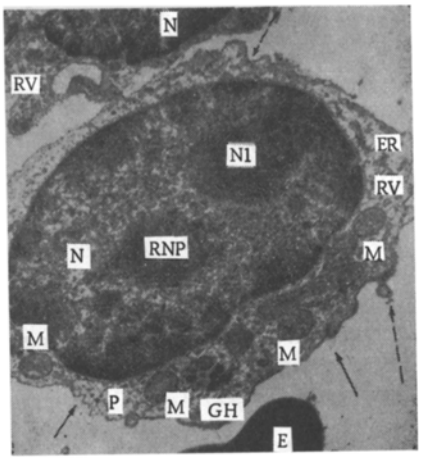

Fig. 1 Fig. 2.

Fig. 1. Erythroblast (12 h after injection of erythropoietin-active serum): areas of pinocytosis (shown here and in other figures respectively by an arrow and a broken arrow) and ropheocytosis, granules of of hemosiderin (GH), numerous ribosomes (R) and groups of polysomes (P); solitary mitochondria (M) are round in shape, their cristae are arranged haphazardly, and the matrix is translucent (20,000 ×).

Fig. 2. Erythroblast (72 h after injection of erythropoietin-active serum): nucleolus (Nl) increased in volume and in contact with inner nuclear membrane; pinocytotic and ropheocytotic vacuoles (RV) and hemosiderin granules (GH) visible in cytoplasm; mitochondria (M) increased in number, cristae tightly packed, matrix osmiophilic (20,000 ×).

EXPERIMENTAL RESULTS

The erythropoietic activity of the blood serum, which was 60 ± 8 in the intact animals, was significantly reduced in the polycythemic albino rats to 18 ± 5 ($P < 0.01$) and it rose sharply 20 h after acute blood loss to reach 195 ± 17 ($P < 0.001$).

The early features of activation of target cells include the formation of cytoplasmic projections which appear 12 h after injection of the erythropoietin-active serum into animals with reduced erythropoiesis and indicate stimulation of micropinocytosis (Fig. 1). The reaction of the cytoplasmic membranes to erythropoietin develops only in stem cells and in all generations of the erythroblastic series, reflecting the selective character of increased permeability, which falls into the category of active transport [2]. Meanwhile numerous invaginations are formed (ropheocytosis) on the surface of the cytoplasm of these same cells, a morphological reflection of another method of active absorption of substances by the cell from the surrounding medium. The end result of the pinocytosis and ropheocytosis is the formation of pinocytotic and ropheocytotic vacuoles (Figs. 2 and 3). Their membrane has high electron density and possesses peroxidase activity. With the normal tempo of hematopoiesis, the erythroblastic cells also characteristically have the same pathways of active iron transport [8], but under the influence of erythropoietin the intensity of these processes rises sharply. The number of cytoplasmic projections and vacuoles with iron-containing material reaches a maximum in the reticulum cells, erythroblasts, and basophilic and polychromatophilic normoblasts, and they are present in smaller numbers in the oxyphilic normoblasts and reticulocytes.

The reaction of the ultrastructure of the nucleus correlates with the early changes in organization of the cytoplasmic membrane. The volume of the nuclei in the target cells rises sharply 12 h after injection

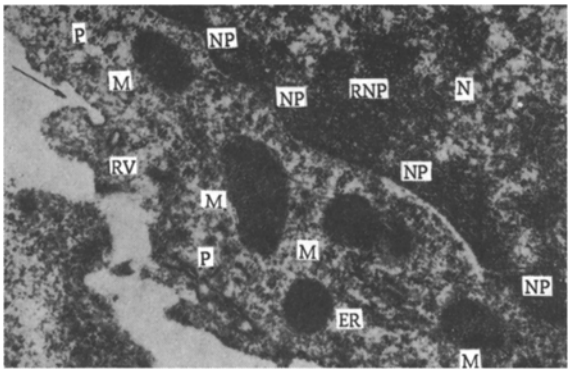

Fig. 3. Basophilic normoblast (96 h after injection of erythropoietin-active serum): ribonucleoprotein granules (RNP) shifted toward inner nuclear membrane in nucleus (N) and concentrated near nuclear pores (NP). Membrane of ropheocytotic vacuoles (RV) and matrix of mitochondria (M) have high electron density. Ribosomes (R) and polysomes (P) lie freely in cytoplasm and also are attached to membranes of endoplasmic reticulum (ER) (50,000×).

of the erythropoietin-active serum. The nucleoli increase considerably in size. The nuclear membrane is perforated by numerous pores which are surrounded on the nuclear side by granules of material with high electron density (Fig. 2). The nucleoli are in contact with the inner nuclear membrane, and this plays an important role in nucleo-cytoplasmic exchange [3, 18, 20].

The period of activation of nucleo-cytoplasmic exchange corresponds to aggregation of ribosomes into polysomes, responsible for hemoglobin synthesis [19]. Dilatation of the cisterns of the endoplasmic reticulum is noted (Figs. 2 and 3).

The regular reaction of the mitochondria to erythropoietin is clearly defined. In the early stage (12 h after injection of the erythropoietin-active serum) the volume of the mitochondria increases but the outer membrane is unchanged. The cristae are few in number, most of them have "straightened-out" folds, the spaces between the cristae are moderately dilated, and the matrix is translucent (Fig. 1). The early stage in the kinetics of the ultrastructural organization of the mitochondria is thus characterized by the development of swelling. This reaction is combined as a rule with intensification of active iron transport and may be osmotic in character. The second stage (48-72 h after injection of erythropoietin-active serum) is characterized by an increase in the number of mitochondria (Figs. 2 and 3). Often the mitochondria are dumbbell-shaped, indicating that they are starting to divide [5]. The presence of these processes is confirmed by the increase in mitochondrial protein in the bone marrow during stimulation of erythropoiesis observed biochemically [1]. In the later stages (96 h after injection of the erythropoietin-active serum) the volume of the mitochondria continues to fall, the cristae are tightly packed, and the matrix strongly osmiophilic (Fig. 3). Reconstruction of the inner membrane is combined with the very intensive formation of tiny granules of electron-dense material which was identified cytochemically with the iron-containing components. The mitochondria are concentrated around the nucleus, where the greatest demand for ATP arises because of the intensive structural metabolism.

Interaction between erythropoietin and cells sensitive to it thus takes place at widely different levels of their submicroscopic organization, including the cell membranes and genetic material. Activation of pinocytosis and ropheocytosis plays an essential role in this interaction. Changes in permeability and active transport in cell membranes are known to give rise to changes in intracellular ion concentrations, which in turn induce proliferative reactions in the cell [2, 17]. The phenomena observed in the nuclei form a group of basic reactions controlling and specializing the structural activity in the erythroblasts and normoblasts, although not to be classified as primary reactions. These extranuclear processes probably precede, and later control nuclear activation.

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