EFFECT OF HYPOXIC HYPOXIA ON THE SHAPE AND SURFACE OF ERYTHROCYTES (OBSERVATIONS IN TRANSMISSION AND SCANNING ELECTRON MICROSCOPES)

A. A. Mozhina, E. S. Chertkova, Z. G. Tsagareli, and L. D. Krymskii

UDC 612.111-06:612.273.2/-086.3

Changes in the surface and intracellular structure of erythrocytes in conjunction with the general morphological picture of the blood were studied experimentally. In hypoxic hypoxia changes take place in mature erythrocytes and in bone-marrow tissue erythroblasts. In the early stages of hypoxia (first to fifth days) the number of erythrocytes, their respiratory surface, and their hemoglobin content all increase. These changes are adaptive. In the later stages of hypoxia (10th-15th days) there is no increase in the number of erythrocytes, the number of reticulocytes falls, and erythroblasts disappear, i.e., the erythroblasts capacity of the bone marrow is exhausted and signs of its decompensation appear. The increase in the number of erythrocytes and the hemoglobin concentration in the blood are the result of stimulation of the function of the erythroid series of the bone marrow, as is shown by an increase in the number of reticulocytes in the peripheral blood and the appearance of erythroblasts.

KEY WORDS: hypoxic hypoxia; erythrocytes; scanning electron microscopy; transmission electron microscopy.

The effect of hypoxia on the morphological and functional state of the erythrocytes has been studied extensively and from many different aspects. Meanwhile several problems concerning the regulation of erythropoiesis in hypoxia have not yet been adequately studied, the nature of the subcellular reorganization of the blood cells during the period of adaptation to hypoxia has not been completely explained, and information on changes in the erythrocyte surface is contradictory and fragmentary.

The development of modern methods of morphological investigations, namely transmission and scanning electron microscopy, has enabled a fresh look at the state of the subcellular structures and surface of the blood cells in health and disease.

The object of the present investigation was to study changes in the surface and intracellular structure of the erythrocytes experimentally, taking into account the general morphological blood picture.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male rats weighing 180-200 g. Hypoxic hypoxia was produced by the rebreathing method, whereby the quantity of the gases in the inspired air can be accurately fixed. The degree of hypoxia was monitored as the level of the acid-base balance by the micro-Astrup method. The animals were killed on the 1st, 5th, 10th, and 15th days of hypoxia. A residue of erythrocytes was obtained for electron-microscopic investigation and treated in the usual way by Palade's method. Sections were cut on a Reichert Om-U2 ultratome and examined in the Tesla Bs-500 transmission electron microscope (TEM). Material for scanning electron microscopy was fixed by Hattori's method [4] in 1% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.4. The samples were sprayed with a thin layer of carbon and silver, and then studied in the SSM-S1 scanning electron microscope (SEM) with an accelerating voltage of 10 kV. Changes in the peripheral blood due to hypoxia were characterized by

---

Laboratory of Pathological Anatomy and Postmortem Examination, A. N. Bakulev Institute of Cardiovascular Surgery, Academy of Medical Sciences of the USSR, Moscow. Department of Cytology, A. N. Natishvili Institute of Experimental Morphology, Academy of Sciences of the Georgian SSR, Tbilisi. (Presented by Academician of the Academy of Medical Sciences of the USSR M. N. Solov'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 10, pp. 502-506, October, 1978. Original article submitted January 5, 1978.

 0007-4888/78/8610-1408$07.50

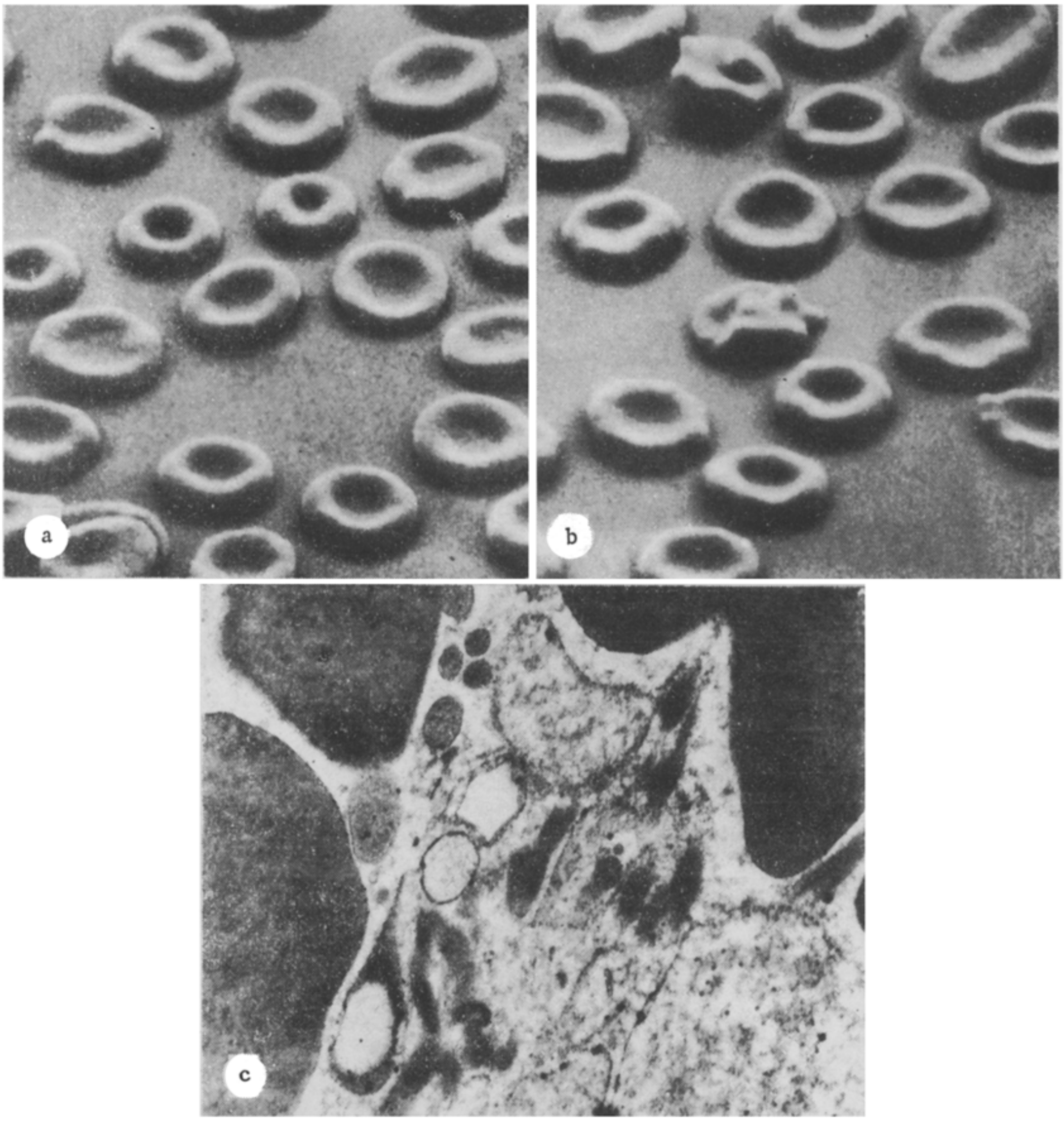

Fig. 1. Stereoultrastructure of erythrocytes on first day of hypoxia: a) semiflattened erythrocytes, SEM, 3000×; b) deformed erythrocytes, SEM, 3000×; c) anisocytosis of erythrocytes, reticulocyte in middle, TEM, 22,000×.

determination of the hemoglobin concentration and erythrocyte and reticulocyte counts, and by examination of blood films.

EXPERIMENTAL RESULTS

The oxygen deficit of the body in hypoxic hypoxia was covered by the development of polycythemia, anisocytosis (mainly macrocytosis), and reticulocytosis. These changes were accompanied by an increase in the hemoglobin concentration and in the circulating blood volume [1, 3, 5, 6].

Examination of the animals' blood on the first day of the experiments revealed an increase in the erythrocyte count to 6,000,000 cells/μl. The erythrocytosis, initially redistributive, was later maintained by the increasing hematopoietic function of the bone marrow. Polycythemia and the appearance of macro-erythrocytes with an increased respiratory surface and, consequently, with an increased hemoglobin concentration, evidently contributed to the increase in their functional activity. This was confirmed by the increase in size of the erythrocytes observed during investigation in the TEM and SEM.

By means of the SEM, besides normal erythrocytes up to 40% of erythrocytes were found to have the same biconcave surface, but the depression in the center of the cell was much

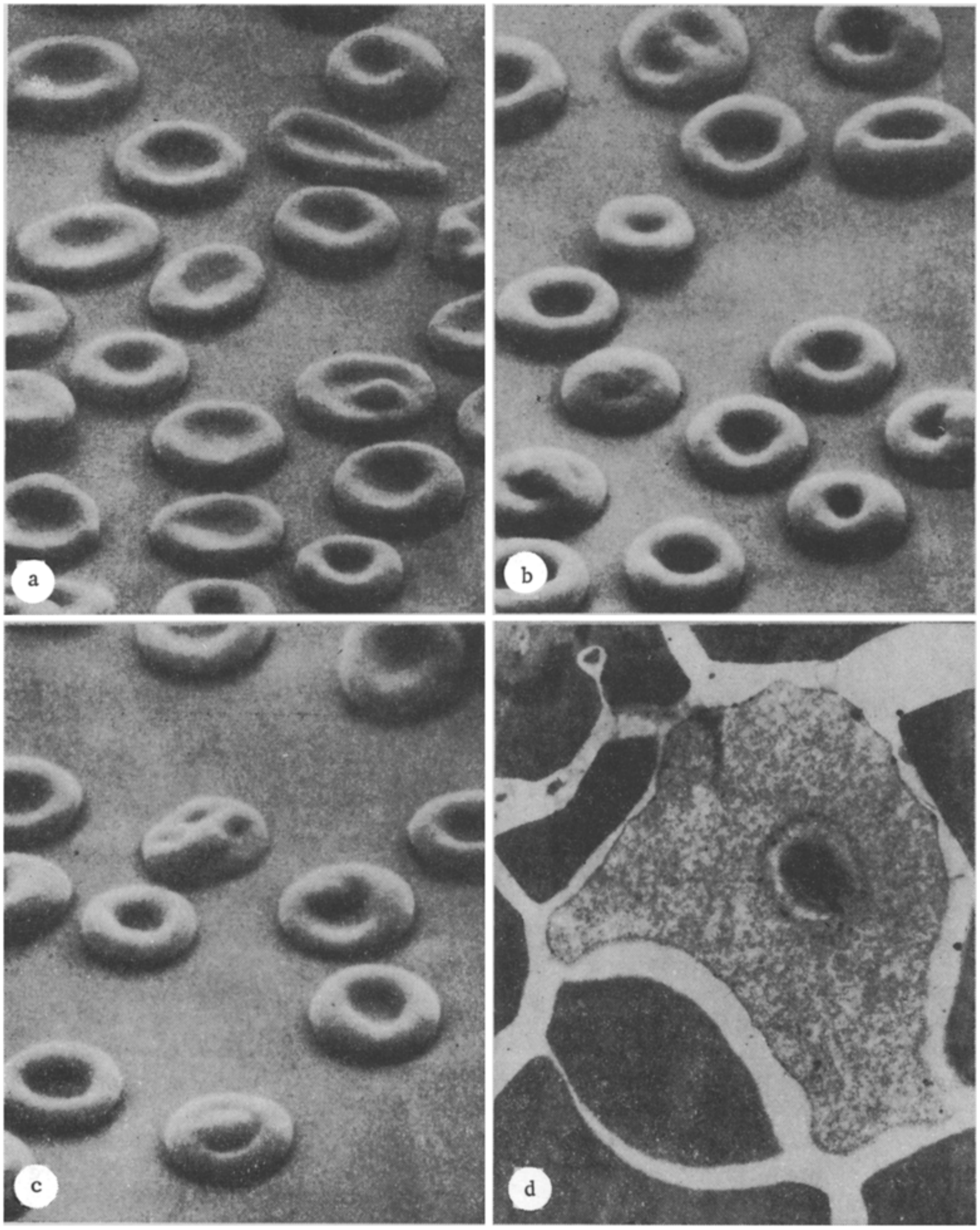

Fig. 2. Stereoultrastructure of erythrocytes on fifth day of hypoxia: a) flattened erythrocytes and ovalocytes, SEM, 3000×; b) swollen and double-pitted erythrocytes, SEM, 3000×; c) vacuolated erythrocytes and reticulocyte, SEM, 3000×; d) erythrocyte with varied electron density, TEM, 12,000×.

wider and the edges more sloping than those of normocytes (semiflattened erythrocytes). The surface area of these erythrocytes was more than twice that of normocytes (Fig. 1). Meanwhile large numbers (40%) of deformed erythrocytes were found, but some of them still had a biconcave surface (Fig. 1b). The reticulocyte count was increased (over 30%). On examination in the TEM, the reticulocytes appeared as cells with small gaps in the cytolemma and containing residues of membranous structures of the mitochondria and endoplasmic reticulum (Fig. 1c).

On the fifth day of the experiment the erythrocyte count in the animals' blood was increased (6,500,000), and this was combined with an increase in the hemoglobin concentration, reticulocytosis, and macrocytosis. Poikilocytosis of the erythrocytes was revealed on investigation in the SEM as flattening of the erythrocytes and the appearance of ovalocytes with pointed poles (Fig. 2a). The increase in size of the erythrocytes was noteworthy. Compared with erythrocytes observed on the first day of the experiment, the cell borders at this time of investigation were considerably raised, as if swollen (Fig. 2b), and the number of vacuo-

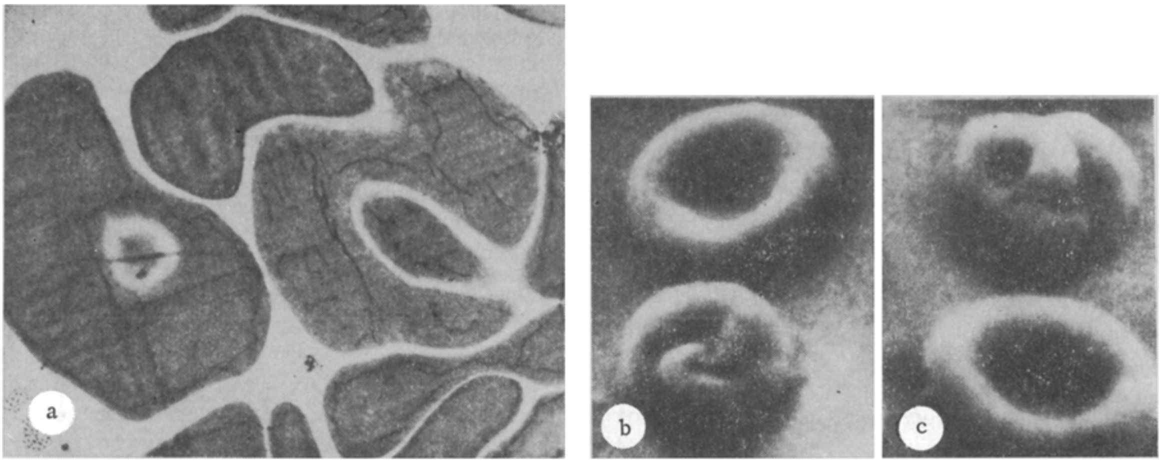

Fig. 3. Stereoultrastructure of erythrocytes on 10th day of hypoxia: a) poikilocytosis of erythrocytes, TEM, 12,000×; b, c) reticulocytes of different shapes, SEM, 3000×.

lated, swollen erythrocytes and reticulocytes was increased (Fig. 2c). The TEM revealed erythrocytes which differed not only in shape, but also in electron density. Besides hyperosmiophilic cells, erythrocytes with pale areas of cytoplasm also were observed, and as the hypoxia increased in severity, these pale areas widened and approached the outer membrane (Fig. 2d). The appearance of young, polychromatophilic erythrocytes, not fully hemoglobinized, with translucent cytoplasm points to intensified regeneration of bone marrow toward the erythroid series.

A further increase in hypoxia (10th day) was accompanied by increased erythroblasts with a corresponding increase in the hemoglobin concentration, and by poikilocytosis of the erythrocytes (Fig. 3a) and the appearance of young forms of reticulocytes, i.e., coil and dense network forms, and also the appearance of erythroblasts — nucleated erythrocytes. Their ultrasturcture was characterized by a large nucleus, a clearly defined nuclear membrane and perinuclear space, and loosely and irregularly distributed chromatin. The cisterns and channels of the endoplasmic reticulum of the erythroblasts were dilated, the internal structure of the mitochondria was disturbed, and the cristae were shortened and had lost their parallel arrangement. Reticulocytes of different shapes were clearly demonstrated by examination in the SEM (Fig. 3b, c). At this time of the investigation, compared with the 5th day of the experiment, the number of swollen erythrocytes and of erythrocytes with raised edges and with reduced concavity increased. The total number of modified erythrocytes (flattened, vacuolated, double-pitted, swollen) was 70-80%.

A deepening of the hypoxic state (15th day of the experiment) was marked by a certain stabilization of the changes described above. The reticulocyte count fell, young forms of reticulocytes disappeared, and no erythroblasts were found. No sharp increase took place in the erythrocyte count and there was likewise no increase in the hemoglobin concentration. Changes in the erythrocytes identical with those observed on the first day of the experiment were detected by the SEM.

The changes in the erythrocytes in hypoxic hypoxia can be explained by a quantitative increase in the young forms, which differ from normocytes in their higher osmotic resistance, more efficient glycolysis [2], and lower hemoglobin content.

LITERATURE CITED

1. Z. I. Barbashova, G. I. Grigor'eva, L. N. Simanovskii, et al., in: Mountains and the Blood System [in Russian], Frunze (1969), pp. 15-17.
2. L. N. Simanovskii, Vopr. Med. Khimii, No. 3, 227 (1971).
3. Ya. G. Uzhanskii, in: Problems of Reactivity in Pathology [in Russian], Moscow (1968), p. 72.
4. J. Hattori, Acta Haemat. Jpn., 35, 7 (1972).
5. H. Cavusoglu, New Istanbul Contrib. Clin. Sci., 10, 79 (1972).
6. B. Kubanek, L. Ferrari, W. Tyler, et al., Blood, 32, 586 (1968).