Scanning Electron Microscopy of Rat Erythrocytes during Chronic Alcoholic Intoxication Combined with Protein and Vitamin Deficit

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Electron microscopy revealed morphological changes in erythrocytes from rats with chronic alcoholic intoxication kept on protein- and vitamin B-deficient rations. All animals had anisopoikilocytosis (up to 50%). Most pronounced changes in erythrocyte population attesting to accelerated erythrocyte aging (stomato- and microcytosis, discocyte swelling, and spontaneous hemolysis) were found in alcohol-fed rats kept on deficient ration.

Key Words: *erythrocyte; chronic alcoholic intoxication; food ration; scanning electron microscopy*

Chemical composition of the food and activity of its components are important factors affecting quantitative parameters and surface architectonics of blood cells [1,14]. Qualitative and quantitative protein composition of the ration is important for the maintenance of constant erythrocyte and hemoglobin levels [7]. Protein deficiency leads to a decrease in blood erythropoietin concentration and impaired utilization of iron by erythrocytes, which result in decreased hemoglobin content [9]. Vitamin deficiency impairs many physiological functions [4]. We found only few reports on the effect of vitamin deficiency on hemopoiesis under conditions of alcohol intoxication [10]; the effects of vitamin B_{12} and folic acid deficiency on hemopoiesis are most studied [11]. Since direct examination of the reaction of the blood system to chronic alcoholic intoxication (CAI) against the background of isolated or combined alimentary insufficiency is impossible, we studied erythrocyte morphology in the experiment. We studied peculiarities of surface architectonics of erythrocytes in laboratory animals kept on protein- and vitamin B-deficient diet and exposed to long-term ethanol intoxication.

MATERIALS AND METHODS

Albino male rats weighing 160-200 g (n=120) were used in the experiments. Group I rats were kept on a vitamin-deficient ration with normal protein content; group II rats were fed a protein-deficient ration with normal vitamin concent; group III rats received a protein-vitamin-deficient ration. Control rats received standard vivarium chow.

Protein content was decreased and water-soluble vitamins were extracted as described elsewhere [5,6]. The animals were kept in cages with wire floor to exclude coprophagy and additional consumption of vitamins synthesized by gut microflora.

Alcohol (40% ethanol, 7 g/kg body weight) was administered to 50% animals in each group via a gastric tube for 21 or 42 days, other animals received distilled water.

The animals were decapitated on the next day after alcohol treatment in accordance with Principles of Humane Attitude to Laboratory Animals [2].

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For electron microscopy, erythrocytes were fixed in 2.5% glutaraldehyde on isotonic phosphate buffer (pH 7.2) for 1 h at 4-6°C [12] and dehydrated in ascending ethanol concentrations. The preparations were coated with gold film (about 20 nm) by gold-particle bombardment on an Eiko device and examined under a Hitachi S-500 scanning electron microscope at accelerating voltage of 25 kV.

Erythrocyte count was determined on a Picoscal analyser, the mean erythrocyte life-span was calculated as described elsewhere [3].

The data were processed statistically using Student *t* test.

RESULTS

Electron microscopy of erythrocytes from the control rats not subjected to CAI showed normal state of these cells and optimal content of discocytes, stomatocytes, and echinocytes throughout the experiment.

In animals exposed to CAI for 42 days the number of swollen macro-, micro-, and discocytes in the peripheral blood moderately increased (10-30%). Stomatocytosis (about 20% of all forms), spherocytosis, and solitary abnormal erythrocyte forms were revealed against the background of unchanged number of echinocytes.

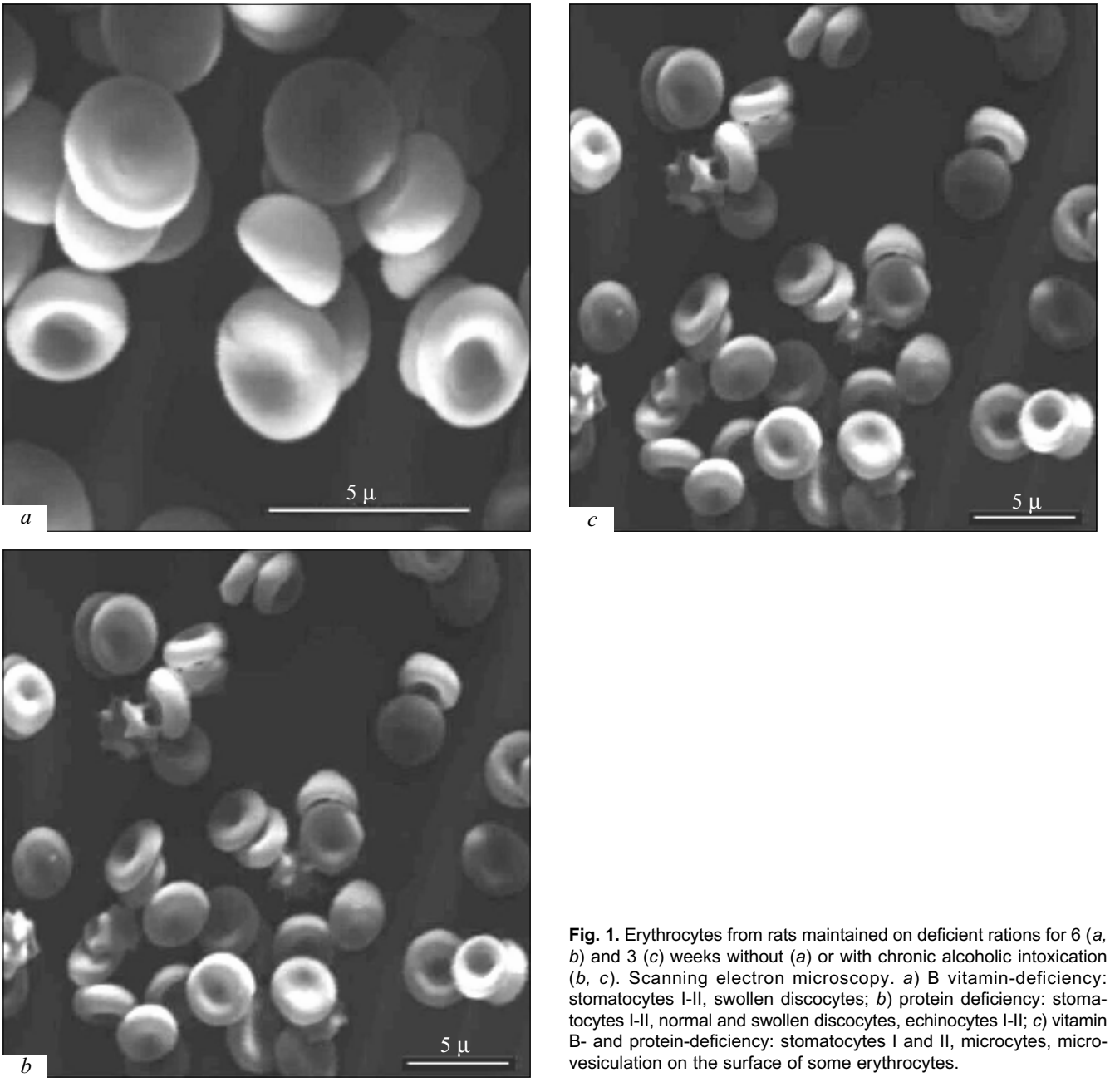
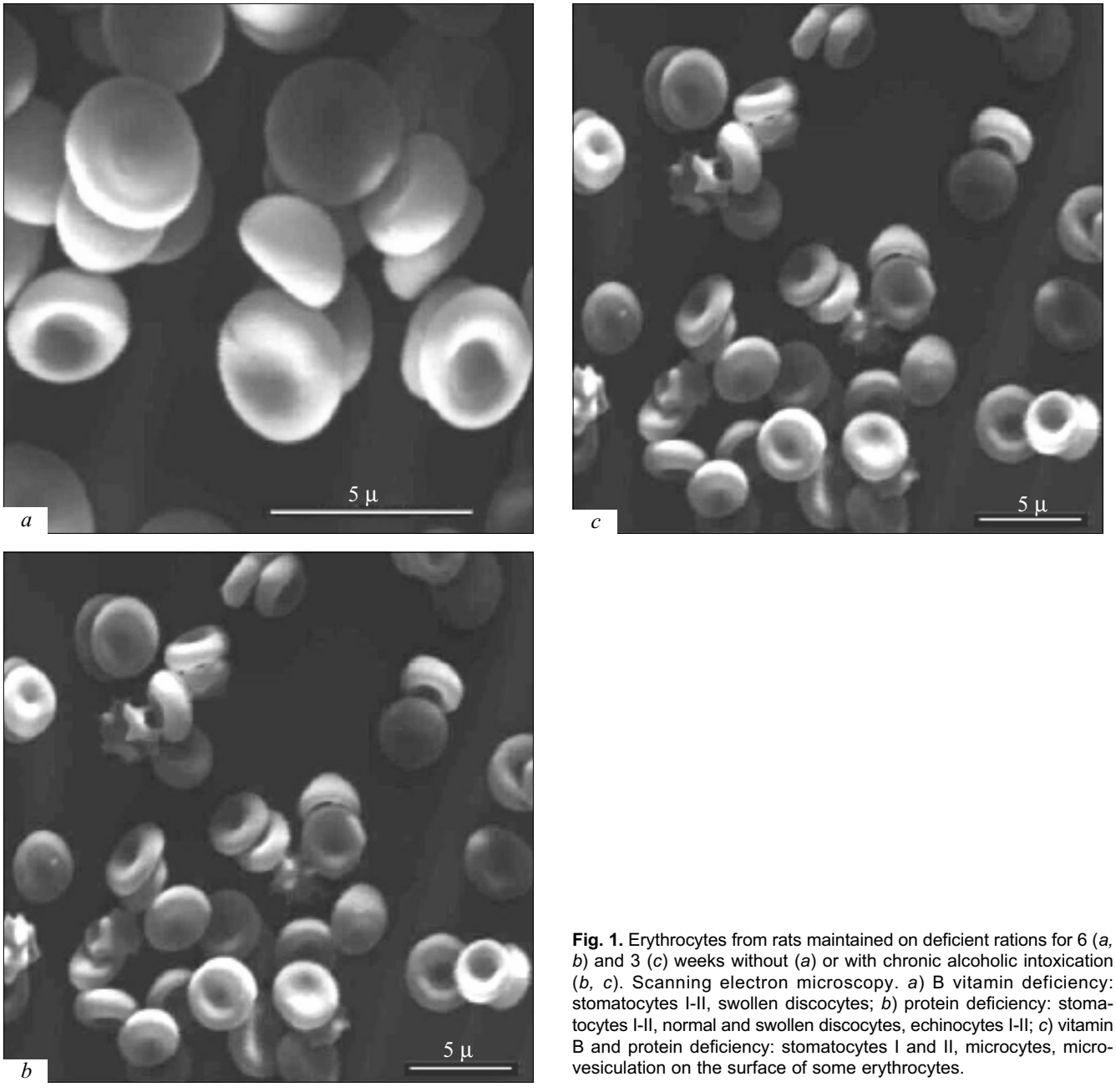


Fig. 1. Erythrocytes from rats maintained on deficient rations for 6 (*a, b*) and 3 (*c*) weeks without (*a*) or with chronic alcoholic intoxication (*b, c*). Scanning electron microscopy. *a*) B vitamin-deficiency: stomatocytes I-II, swollen discocytes; *b*) protein deficiency: stomatocytes I-II, normal and swollen discocytes, echinocytes I-II; *c*) vitamin B- and protein-deficiency: stomatocytes I and II, microcytes, microvesiculation on the surface of some erythrocytes.



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In vitamin B-deficient rats the number of swollen discocytes on days 21 and 42 of the experiment increased by 10-30% and 30-50%, respectively. Stomatocytosis progressed from moderate to pronounced, while echinocytes almost completely disappeared, which probably reflects transition to the stomatocytic pathway of erythrocyte aging (Fig. 1, *a*). The number of microcytes increased to 30%; solitary macrocytes were noted.

Similar morphological changes in erythrocytes were observed in vitamin B-deficient rats subjected to CAI: disappearance of echinocytes, microvesiculation in discocytes and stomatocytes, and delayed spontaneous hemolysis.

Microvesicularization and sharp decrease in the number of echinocytes in this group attest to stomatocytic way of normocyte-discocyte transformation. This type of aging is characterized by predominance of endomicrovesiculation processes at the initial stages [8]; microvesicles are then released from erythrocytes due to hemolysis (observed in our experiments on day 42) and exomicrovesiculation.

In rats maintained on a protein-deficient diet the number of echinocytes was normal throughout the ex-

periment, while microcytes constituted 10-20% cells. In the CAI group the number of swollen discocytes increased to 50% on weeks 3 after the start of treatment, moderate stomatocytosis, pronounced microcytosis, and a tendency to echinocytosis (above 10%) persisted throughout the entire experimental period (Fig. 1, *b*). Echinocytosis in these animals suggests that apart from stomatocytic transformation, echinocytic transformation associated with changes in membrane cytoskeleton also contributes to erythrocyte aging. Appearance of solitary abnormal erythrocyte in the first half of the experiment, spontaneous hemolysis on week 6 of the experiment, microvesucularization of discocytes, and stomatocytes were also noted in CAI group.

In group III rats the observed changes in erythrocyte architectonics were similar to those in group I. Echinocytes almost completely disappeared, stomatocytosis, microcytosis, and spontaneous hemolysis were minor. In the CAI group, the number of swollen discocytes increased, microvesiculation of stomatocytes was also noted (Fig. 1, *c*).

Stomatocytosis in this group suggests that in CAI combined with protein and vitamin deficiency erythrocyte aging proceeds by the stomatocytic pathway [13]; alcohol-induced pathology of the liver can also contribute to this process. Exovesiculation and exomicrovesiculation indicate irreversibility of this process.

Complex examination of the red blood in animals maintained on different rations demonstrated that CAI combined with protein and vitamin deficiency was associated with maximum strain in the erythron system. The presence of many forms of aging erythrocytes revealed by scanning microscopy agrees with the observed intensification of erythropoiesis (349.3±13.01×10^7 *vs.* 235.0±3.88×10^7 erythrocytes/μl in the control group, $p<0.001$) and reduced erythrocyte lifespan (19.0±0.71 *vs.* 27.3±0.42 days in the control, $p<0.001$).

Thus, the observed changes in erythrocyte surface architectonics induced by CAI combined with inadequate nutrition point to disorganization of the red blood system. Accelerated cell aging triggers the impairment of erythrocyte homeostasis associated with overstrain of compensatory systems culminating in cell exhaustion and functional insufficiency.

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