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CHANGES IN ERYTHROCYTES AFTER INJECTION OF EXCESSIVE DOSES OF RETINOIDS

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Evidence that removal of damaged and old erythrocytes from the blood stream is effected by means of lymphocytes and macrophages has been published [1]. Vitamin A and retinoids have the property of stimulating immune defense nonspecifically [18]. Bearing in mind the detergent properties of compounds of this group [18] it can be tentatively suggested that injury to the erythrocyte membrane may be one stage in the mechanism of the adjuvant action of retinoids, similar to what is found during autohemotherapy.

Now that it is possible to use synthetic analogs of vitamin A (retinoids [15]) clinically, the investigator is faced with the problem of devising methods of detecting signs of overdosage of the compounds of this group. The most convenient tests for practical purposes could be those determining blood parameters, for the blood cells are among the first to be exposed to the action of retinoids. The plasma concentrations of vitamin A and retinoids also reflects to a definite extent the degree of saturation of the body with these substances.

Natural forms of vitamin A in large doses have been shown to have a damaging action on erythrocytes [19], although no methods have yet been devised for the study of erythrocytes as a test object for determination of the degree of hypervitaminosis A. As regards changes in the erythrocytes after administration of excessive doses of retinoids and the role of these changes in the mechanism of the adjuvant action of these compounds, these topics have not been discussed in the literature.

The aim of this investigation was to analyze morphological and functional changes in the erythrocytes of mice receiving large doses of retinoids.

TABLE 1. Effect of Retinoids on Number of Erythrocytes in 1 mm^3, Hemoglobin Concentration, and Osmotic Resistance of Erythrocytes in C57BL/6 Mice on the 40th Day of the Experiment (M ± m, n = 8)

Procedure	Erythrocytes, millions	Hemoglobin, g%	Osmotic resistance of erythrocytes, %	
			limit of minimal resistance	limit of maximal resistance
Oily solution (control)	8,47±0,19	15,0±0,2	0,65±0,01	0,48±0,01
MR	5,42±0,17*	13,3±0,1*	0,70±0,01*	0,51±0,01
13-CMR	4,97±0,18*	12,8±0,1*	0,69±0,02	0,52±0,01*
RC_{15}	5,93±0,16*	14,8±0,1	0,67±0,01	0,49±0,01

Legend. Here and in Table 3, *P < 0.05 compared with the control.

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TABLE 2. Effect of MR on Number of Erythrocytes in 1 mm^3, Hemoglobin Concentration and Erythropoiesis in Red Marrow of AKR Mice on 87th Day of Experiment (M ± m, n = 7-8)

Procedure	Erythrocytes, millions	Hemoglobin, g%	Blast form of cells, %	Basophilic erythroblasts, %	Polychromatophilic erythroblasts, %	Erythroid mitotic index, ‰
Oily solution (control)	6,55±0,10	12,9±0,1	0,10±0,03	9,7±0,7	17,4±1,0	2,5
MR	5,07±0,11	10,5±0,3	3,5±0,3	3,5±0,1	8,8±0,9	6,0

Legend. *P < 0.001 compared with control. [As in Russian original – Publisher.]

EXPERIMENTAL METHOD

Experiments were carried out on young sexually mature C57BL/6 and AKR mice. The retinoids tested included all-trans-methylretinoate (MR), 13-cis-methylretinoate (13-CMR), and retinoid C_{15} (RC_{15}). The compounds were obtained from the Laboratory of Chemistry of Polyene Compounds (Head, Professor G. I. Samokhvalov) of the "Vitaminy" Scientific-Production Combine. The effect of the retinoids was studied in four groups of animals, and the numerical composition of the groups is shown in the tables.* The retinoids were injected intraperitoneally into the animals in the form of 0.5% oily solutions in doses of 0.3 ml once every 5 days. Blood for investigation was taken from the caudal vessels between the 37th and 40th days of the experiment. The dose of MR for AKR mice was 0.15 ml of the 0.07% solution. The substance was injected intraperitoneally once a week. The duration of the experiment was 87 days. Animals receiving persic oil (solvent) intraperitoneally in the same volume as the retinoids served as the control.

Methods of determining the number of erythrocytes in the blood and of cells of the erythroid series in the red marrow, the hemoglobin concentration, and osmotic resistance of the erythrocytes were used. A method of determining different types of erythrocytes, based on their ability to change their staining properties relative to paraldehyde fuchsine (PAF), depending on changes in the functional state of man and animals in various diseases and experimental situations, also was used [9, 10, 13].

The number of erythrocytes in 1 mm^3 and the hemoglobin concentration in the blood were determined by the usual methods. In addition, in the case of AKR mice, the myelogram was counted in red marrow films obtained from the femur and stained with azure II-eosin; and particular attention was paid to cells of the erythroid series. To determine osmotic resistance a series of successive dilutions of NaCl at pH 4.8 was made from 0.78 to 0.36% at intervals of 0.03%. The resulting solutions were transferred in a volume of 1 ml into centrifuge tubes. By means of a capillary tube samples of 0.02 ml of blood were taken from the caudal vessels of the mice and added to the contents of each tube. After 30-40 min the samples were centrifuged at 2000 rpm for 5 min. The results were read immediately after centrifugation. The limit of minimal resistance of the erythrocytes was taken to be the NaCl concentration at which the first signs of hemolysis appeared. The limit of maximal resistance was taken to be the NaCl concentration at which single whole erythrocytes could be identified in the supernatant under the microscope.

To analyze the distribution of erythrocytes among the different types [9] blood films were fixed and stained with PAF [11]. The solution was made up by Halmi's method [17]. Fractions of unstained, partially stained, and completely stained erythrocytes were determined. From each animal 1000 cells were counted and the results were expressed in promille.

The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Injection of all three retinoids gave rise to moderately severe anemia in the animals, as shown by a fall in the number of erythrocytes and their osmotic resistance and a decrease in the hemoglobin concentration (Table 1). The anemia which developed in the AKR mice was

*This does not seem to be the case – Translator.

TABLE 3. Effect of Retinoids on Staining Properties of Erythrocytes with PAF in C57BL/6 Mice on 37th-40th Day of Experiment (M ± m, n = 8)

Procedure	Number of erythrocytes, ‰		
	unstained	partially stained	completely stained
Oily solution (control)	355,7±44,1	456,7±28,7	187,5±19,9
MR	295,7±38,2	380,6±18,1*	323,6±52,9*
13-CMR	704,4±30,2*	131,5±22,9*	164,1±15,0
RC_{15}	700,5±27,9*	100,4±12,6*	199,1±17,5

accompanied by intensification of formation of new erythrocytes (Table 2). Injection of 13-CMR and RC_{15} into the animals led in both cases to a significant increase in the fraction of unstained cells and a simultaneous decrease in the fraction of partially stained cells. There was no significant change in the number of completely stained erythrocytes. After injection of MR into the animals the number of completely stained cells increased by 1.5 times (Table 3).

PAF is known to have affinity for aldehydes formed during oxidation of glycol groups of glycoproteins [17, 20]. One such glycoprotein may be glycophorin, which is the predominant protein in the erythrocyte membrane [12, 16]. It is also known that retinoic acid is carried in the blood in the form of a complex with a carrier protein [5]. The opinion is held that injection of excessive doses of retinoic acid, and also of retinoids that differ in the structure of their molecule from vitamin A leads to the appearance of these substances in the blood stream not bound with the transport protein. In this case the substances exhibit their membrane-active properties [5, 19] and injury to the membranes of erythrocytes, as the most numerous blood cells, may reflect this process. With this information in mind it can be tentatively suggested that lowering of the osmotic resistance of the erythrocytes, a decrease in their number, a decrease in their hemoglobin content, and an increase in the number of cells not staining with PAF are evidence that the erythrocytes have suffered damage from injection of the retinoids. This correlation could be seen with respect to 13-CMR and to RC_{15}. The anemia induced by injection of MR was unaccompanied by accumulation of PAF-unstained cells. MR evidently induces gross lesions in erythrocytes, leading to their rapid hemolysis, as a result of which no PAF-unstained forms accumulate in the blood. These observations suggest that parameters of the morphological and functional state of erythrocytes may provide a basis for the development of methods for detecting signs of retinoid overdosage.

The present investigations showed that inhibition of growth of a transplantable tumor under conditions of hypervitaminosis A is accompanied by increased infiltration of the periphery of the tumor nodule with lymphocytes and macrophages [7]. If retinoid and carcinogen are applied simultaneously to the skin, carcinogenesis is delayed and lymphoid infiltration of the dermis is intensified [4]. Injection of vitamin A and retinoid is accompanied by increased lymphopoiesis in the red marrow, an increase in the number of blast forms of lymphocytes in the blood, an increase in the volume of the paracortical zone of the lymph node, the formation of new splenic lymphoid follicles, and activation of the ability of spleen cells to undergo spontaneous and stimulated rosette formation [14]. Stimulation of immune defense by triple immunization with BCG vaccine potentiates the carcino-protective properties of vitamin A [6]. Injection of vitamin A and retinoids into animals is accompanied by an increase in the number of macrophages and by stimulation of their phagocytic activity [2, 3]. The present investigation showed that under conditions of hypervitaminosis A and after injection of excessive doses of retinoids, the number of erythrocytes in the blood, the hemoglobin concentration, and the osmotic resistance of the erythrocytes all decrease and the number of erythrocytes with a modified cell surface increases. This suggests that after injection of excessive doses of vitamin A and retinoids the outer membranes of cells of the blood and other tissues and organs, and in particular, erythrocyte membranes are injured. Lymphocytes and macrophages are responsible for removal of the injured erythrocytes from the blood stream, and the number of these cells in the blood and lymphoid organs increases with an increase in the number of injured erythrocytes. Accumulating effectors may also destroy tumor cells nonspecifically.

Injection of excessive quantities of retinoids into mice thus gives rise to anemia, expressed as intensification of erythropoiesis in the red marrow, a decrease in the number of erythrocytes and hemoglobin concentration in the blood, and a decrease in the osmotic resistance of the erythrocytes. Injection of RC_{15} and 13-CMF into animals was accompanied by an increase in the number of erythrocytes not stained by PAF in the blood.

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PROTECTIVE EFFECT OF HEPARIN AND PHOSPHATIDYLSERINE IN EXOGENOUS THROMBOPLASTINEMIA

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KEY WORDS: thromboplastinemia; tolerance to thromboplastin; heparin; phosphatidylserine.

On entering the blood stream thromboplastin temporarily increases the coagulability of the blood and evokes a protective reaction leading to a reduction in coagulability through consumption of clotting factors [8] and, chiefly, through an increase in the anticlotting potential [5]. The frequency of death of animals receiving a dose of thromboplastin excessive for the animal's powers of compensation may be regarded as an indicator of tolerance to the coagulating agent, whereas the effect of anticoagulants on the mortality rate under these conditions can be used to estimate their effectiveness.

In the investigation described below heparin and phosphatidylserine-containing anticoagulant (PSA), the anticlotting principle of which is phosphatidylserine, an inhibitor of thrombin production and fibrin formation [3, 4], were studied from this aspect.

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