# THE BLOOD LIPIDS IN EXPERIMENTAL LEUKEMIA

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During the development of experimental erythromyelosis in rats the content of total lipids and phospholipids per ml packed red cells is increased; the fraction of cholesterol and the cholesterol–phospholipid ratio of the red cells are reduced. From the 12th to the 18th after transplantation of the leukemia the absorption of lipids of the red cells in the ultraviolet region at $\lambda$ =233 nm is almost trebled. The initial and terminal stages of development of leukemia are characterized by marked hyperlipidemia. Diene conjugation (the content of conjugated double bonds) of the plasma lipids is increased to a maximum on the 1st-8th day and reduced to 60% of its initial level on the 11th-18th day of the disease.

The constancy of the lipid composition of the membrane is an essential condition for the stability and normal function of the red cells. A decrease in the concentration of arachidic acid in the lipids of red cells and plasma of rats with experimental leukemia has previously been described [4], i.e., the composition of the lipids of the red cell membranes is changed with an increase in the concentration of saturated compounds.

In the investigation described below the content of total lipids, phospholipids, and total cholesterol in the red cells and plasma of rats with Svec leukemia was investigated.

## EXPERIMENTAL METHOD

The intraperitoneal tumor of the donor animals was removed and minced, and a suspension of tumor cells in isotonic NaCl solution containing $22 \cdot 10^6$ cells in 0.3 ml was injected subcutaneously into the recipient animals. At certain time intervals after transplantation the mean diameter of the tumor [3] was measured in the animals, and the number of red cells in 1 $mm^3$ blood was counted; the rats were anesthetized, and blood taken by direct puncture from the heart. Lipids were extracted from the red cells by Rose's method [1] and the plasma lipids by Folch's method. Graphs were plotted for which each point represented the mean of three experiments, in each of which pooled plasma or red cells from 3 to 9 rats were used. The results of measurement of the mean diameter of the tumor and the red cell count were subjected to statistical analysis; the confidence limits with a 95% level of significance were determined for each point. An increase in the absorption at $\lambda$ =233 nm (adsorption spectrophotometry in the UV region is a measure [6, 7] of the intensity of lipid peroxidation.

## EXPERIMENTAL RESULTS AND DISCUSSION

It will be clear from Fig. 1 that the content of total lipids and phospholipids of the red cells, calculated per ml packed red cells increased particularly in the terminal stage of the disease – from the 12th to the 18th day of development of leukemia. In this period the lipid content was 10-30% higher and the phospholipid content 45-85% higher than originally. Meanwhile the fraction of cholesterol in the lipids of the red cells fell to 90% of its initial value. The lipolytic coefficient of the red cells (the ratio between cholesterol and phospholipids by weight) was low throughout the experiment. On the 18th day after transplantation the top

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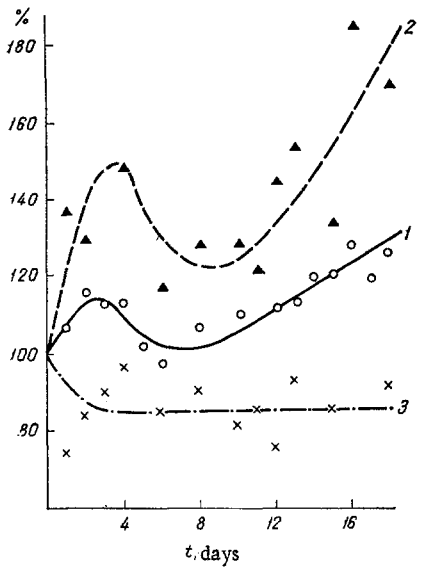


Fig. 1

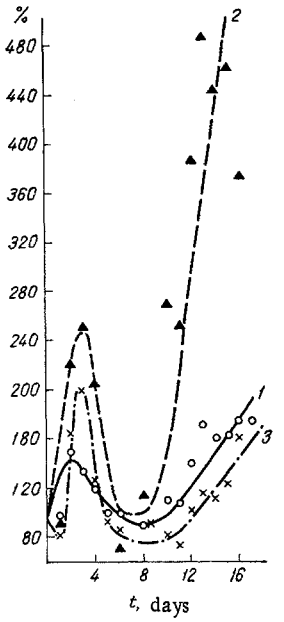


Fig. 2

Fig. 1. Content of total lipids (1), phospholipids (2), and cholesterol (3) in 1 ml packed red cells. Original normal values taken as 100%.

Fig. 2. Content of total lipids (1), phospholipids (2), and total cholesterol (3) in 100 ml plasma (in % of original normal value).

fraction, rich in young red cells, obtained by centrifugation of the cells for 1 h at 3000 rpm had a coefficient equal to half of the lipolytic coefficient of intact red cells.

Complex changes in the content of total lipids and the lipid fractions were observed in the plasma (Fig. 2): the initial period of development of erythromyelosis before the appearance of a visible tumor was characterized by moderate hyperlipemia, whereas in the period of intensive growth of the tumor from the 5th to the 10th day the plasma lipid concentration was the same as in healthy animals. In the terminal stage of the disease the hyperlipemia reappeared and increased.

It will be clear from Fig. 3 that the diene conjugation of 1 mg lipids of the red cells increased from the 1st to the 18th day of development of the erythromyelosis and by the end of the disease it was 2.4-2.8 times higher than initially. Absorption of the plasma lipids in the UV region at $\lambda$ =233 nm changed considerably: it was almost 3 times above its initial normal level on the 1st day and then fell sharply, so that on the 18th day the plasma lipids had a lower value of their diene conjugation (60% of the initial level) than in normal animals.

The increase in the content of total lipids and of the individual fractions was evidently connected with rejuvenation of the cell population and the appearance of large numbers of immature and young erythrocytes and reticulocytes in it [7]. The increase in the diene conjugation is evidence of increased free-radical lipid peroxidation in the erythrocytes of animals with erythroleukemia. A similar effect was observed in organs and cell organelles during the development of several malignant neoplasms [2]. This was evidently due to antioxidant deprivation of the tissue of the tumor-bearing animals [1]. Lipid peroxidation of red cell membranes leads to "leakage" of important structural components of the membranes (cholesterol and arachidic acid) and disturbance of the permeability of the membranes. The test with thiobarbituric acid did not reveal any accumulation of advanced breakdown products of polyunsaturated lipids. Nevertheless, it can be assumed that even slight chronic peroxidation of the membrane lipids leads to a disturbance of the structural and functional integrity of the red cells and to their premature lysis.

Analysis of the red cells by acid resistance showed the appearance of cells of a new immature pathological population with a shortened lifespan in the blood stream. The cells of this new population were

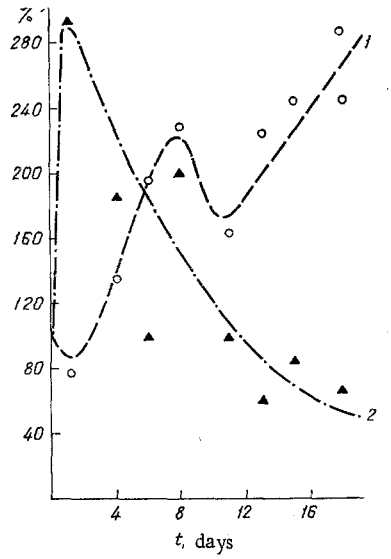


Fig. 3. Absorption in the UV region at $\lambda$ =233 nm of 1 mg lipids of red cells (1) and 1 mg plasma lipids (2). Optical density of lipids of erythrocytes and plasma of intact animals at 233 nm taken as 100%. Asterisk denotes diene conjugation of lipids of "light" fraction of erythrocytes.

possibly richer in arachidic acid than normal red cells (the content of arachidic acid in the youngest red cells in phenylhydrazine anemia has been shown to be higher than in mature cells [7]), and if the organism is deprived of natural antioxidants, lipid peroxidation readily develops in the membranes, as a result of which the arachidic acid disappears from the red cells.

The complex character of changes in the concentration of plasma lipids is due to interaction between two factors: growth of the tumor accompanied by hyperlipidemia [5] and hemolytic anemia, [10]. The extreme increase in the degree of diene conjugation is in accord with this hypothesis of peroxidation of the polyunsaturated fatty acids and also with the decrease in the plasma arachidic acid concentration [4]. The decrease in the diene conjugation to a lower level than in healthy animals probably reflects the rapid destruction of the hydroperoxides formed under the influence of factors of unknown nature [7].

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