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Changes in Fatty Acid Composition of Thymus Cells, Liver, Blood Plasma, and Muscle Tissue in Mice with Solid Ehrlich Carcinoma

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Abstract—The fatty acid composition of thymus cells, liver, blood plasma, muscle tissue, and tumor focus has been studied in mice with solid Ehrlich carcinoma. The tumor growth in the mice was associated with an increase in the total content of monounsaturated fatty acids in all organs and tissues studied and with a decrease in the total amount of polyunsaturated fatty acids in all tissues except blood plasma. The tumor tissue was characterized by increased levels of monounsaturated fatty acids in comparison with their levels in organs and tissues of intact animals. In the thymus of tumor-bearing mice, the contents of myristic and palmitic saturated fatty acids, which are associated with activation of the T-cell immunity, were increased. The most expressed and considerable changes in the fatty acid composition during tumor growth were observed in the muscle tissue of the animals. A possible role of changes in the fatty acid composition in the investigated organs and tissues of tumor-bearing mice in the organism's response to tumor growth is discussed.

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Key words: Ehrlich carcinoma, fatty acids, thymus, liver, blood plasma, muscle tissue

Carcinogenesis is a very severe pathology significant as the second leading cause of death in many countries of the world. Studies on mechanisms of the formation and progression of tumors are very important for development of approaches for prevention and treatment. The influence of fatty acids on tumor growth can be studied as one such approach. Fatty acids are known to be an extremely important component of immune reactions of humans and animals under conditions of both health and pathology. They determine the state and functions of cell membranes, are precursors of bioactive messengers, and are involved in regulation of gene expression [1, 2]. Studies on the role of long-chained polyunsaturated fatty acids (PUFAs) of the omega-3 and omega-6 families are especially interesting because derivatives of these fatty acids (eicosanoids) are involved in various pathological processes, including inflammation and carcinogenesis [3]. Antitumor effects of exogenous PUFAs added into the culture medium of tumor cells and to the diet of tumor-bearing animals and humans have been shown in some works [4-7]. However, there is virtually no literature data on the fatty acid composition of tumor-free organs and tissues in tumor-bearing animals. Studies on the fatty acid composition of organs and tissues of mammals inoculated with tumor cells are important for understanding of mechanisms of the organism's response to tumor growth.

The purpose of the present work was to study the fatty acid composition of the thymus, liver, blood plasma, muscle tissue, and the tumor focus in mice with the solid form of Ehrlich carcinoma.

MATERIALS AND METHODS

Procedures on animals. Experiments were performed on two-month-old male mice of the BALB/c strain with body weight of 23-25 g. The animals were maintained on a standard diet with water and food *ad libitum*. Ehrlich ascites carcinoma cells grown *in vivo* and washed in sterile saline (0.9% NaCl) were inoculated into the femoral muscle of mice at the concentration of $2.5 \cdot 10^6$ cells per 100 µl of saline. Tumor growth was evaluated starting from sixth

Abbreviations: MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

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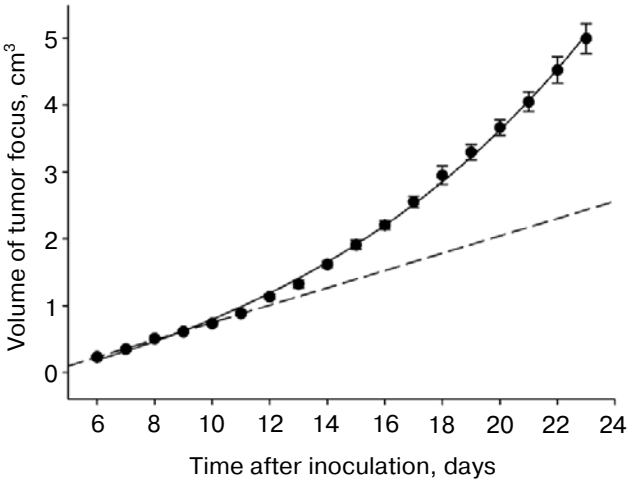

Fig. 1. Growth of solid Ehrlich carcinoma in mice ($n = 15$).

day after the inoculation by measuring the length (a), width (b), and thickness (c) of the tumor focus. The tumor focus volume was calculated using the formula:

$$V = \pi(a \cdot b \cdot c)/6.$$

Preparation of specimens. The animals were decapitated on the ninth day after the inoculation of tumor cells. Blood, thymus, liver, and muscle tissue from the tumor-free leg, as well as the tumor focus itself were isolated. Similar tissues from intact animals were used as the control. The tissue specimens were washed in ice-cold phosphate-saline buffer (pH 7.4), homogenized in the same buffer, and samples were taken to analyze the fatty acid composition. Blood (~0.5 ml) was taken with addition of 20 µl of 10% EDTA as an anticoagulant. Blood plasma was prepared by centrifugation of the blood at 600g.

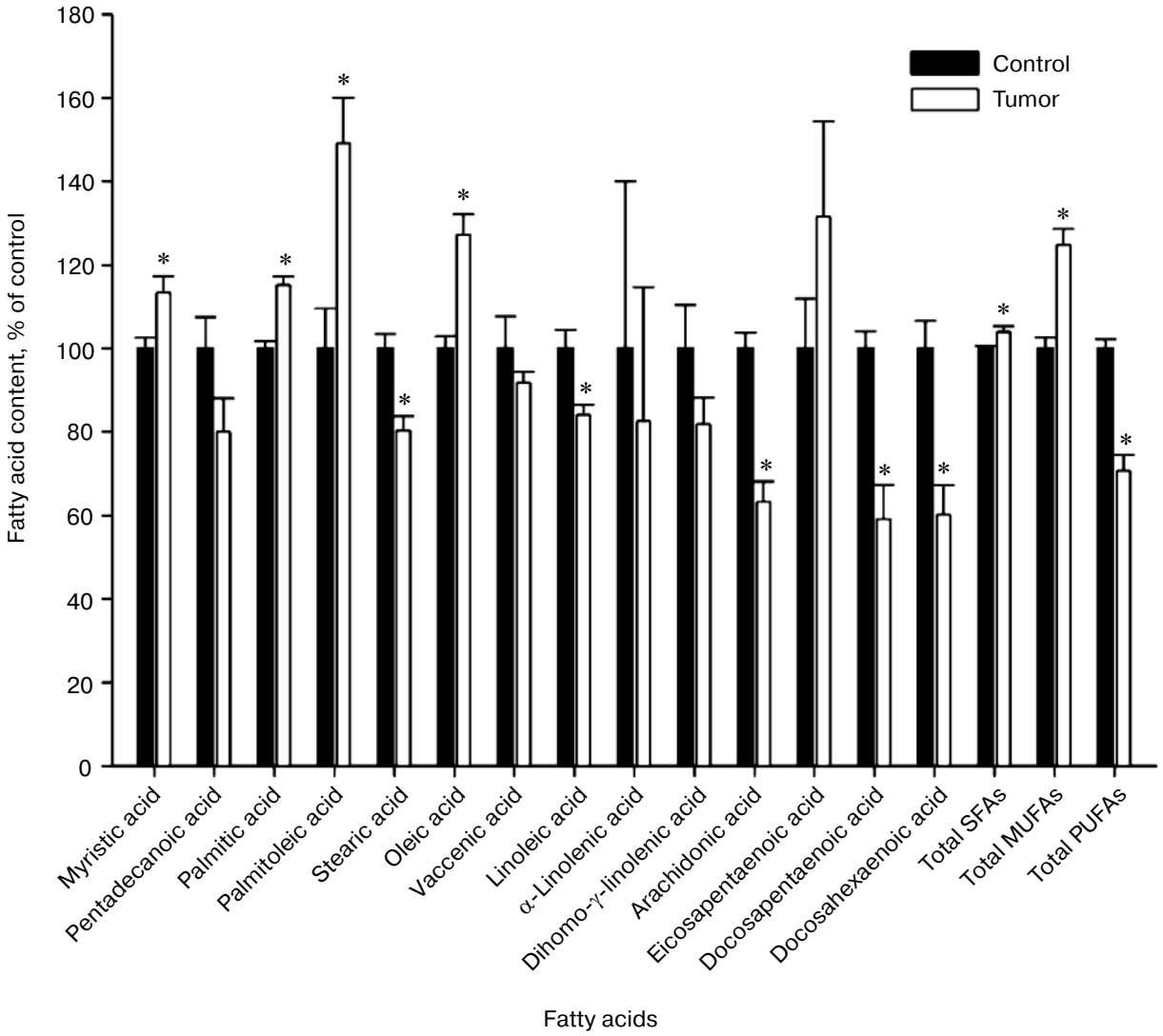

Fig. 2. Changes in fatty acid composition of thymus tissue of tumor-bearing mice. Here and further: * $p < 0.05$ as compared to the level of the corresponding fatty acid in intact animals (control), $n = 7$.

Determination of fatty acid composition. Samples of plasma and tissue homogenates (~150 μl) were stabilized by adding 0.5% antioxidant ionol (2,6-di-tret-4-methylphenol). To prepare derivates, 100 μl of a specimen was placed into a standard tube to be treated under pressure, supplemented with 100 μl of the internal standard dissolved in methanol (margaric acid, 320 μg/ml), and dried in a rotation-vacuum concentrator (Savant Instruments, USA) at room temperature for 30-60 min. Saponification and methylation were performed in a thermally controlled block (Lab Line, USA) [8]. Methyl esters of fatty acids extracted into the hexane phase were determined by gas chromatography using a GC 3900 analytical gas chromatograph (Varian, USA) supplied with a flame-ionization detector (the detector temperature was 260°C). Methyl esters of fatty acids were separated using a quartz capillary column (15 m × 0.25 mm × 0.3 μm) with SUPELCOWAX-10 stationary phase (Supelco, USA). The samples were introduced in the following regimen: 2 μl without division of the flow of the gas-carrier (helium), after 12-30 sec flow division was started depending on the concentration of the studied substances. The temperature program of the analysis was from 90°C (0.5 min) to 240°C (5 min) with the rate of 6°C/min. The data were analyzed using the Multichrom-1.5x program (Ampersand, Russia). Concentrations of individual fatty acids in the samples were determined using the internal standard (with corresponding calibration coefficients previously calculated from chromatograms of fatty acid mixture with margaric acid $C_{17:0}$). In every sample of plasma or tissue homogenate, relative contents of individual fatty acids were calculated.

Statistical processing of data. The growth of solid Ehrlich carcinoma was studied in 15 animals. Fatty acid composition of organs and tissues was determined in two groups of mice (intact mice and tumor-bearing mice),

Fatty acid composition of thymus, liver, blood plasma, and muscle tissue of intact mice and of the tumor focus of tumor-bearing mice ($n = 7$)

Fatty acids	Content of individual fatty acids, %				
	thymus	liver	blood plasma	muscle tissue	tumor focus
Myristic ($C_{14:0}$)	2.53 ± 0.07	0.22 ± 0.03	0.60 ± 0.07	1.51 ± 0.08	1.99 ± 0.06
Pentadecanoic ($C_{15:0}$)	0.229 ± 0.017	0.079 ± 0.007	0.155 ± 0.017	0.196 ± 0.017	0.137 ± 0.009
Palmitic ($C_{16:0}$)	28.7 ± 0.5	24.6 ± 0.4	26.8 ± 0.9	24.3 ± 0.6	20.0 ± 0.7
Palmitoleic ($C_{16:1;\ n-7}$)	3.90 ± 0.37	1.63 ± 0.34	1.83 ± 0.34	3.65 ± 0.50	4.27 ± 0.35
Stearic ($C_{18:0}$)	14.3 ± 0.5	11.4 ± 0.7	9.7 ± 0.6	10.5 ± 0.4	14.1 ± 0.8
Oleic ($C_{18:1;\ n-9}$)	17.8 ± 0.5	14.7 ± 1.6	16.0 ± 1.4	14.4 ± 1.1	24.1 ± 0.5*
Vaccenic ($C_{18:1;\ n-7}$)	4.19 ± 0.32	3.10 ± 0.59	2.64 ± 0.65	3.08 ± 0.09	4.55 ± 0.25
Linoleic ($C_{18:2;\ n-6}$)	9.6 ± 0.4	13.0 ± 0.5	18.9 ± 1.1	15.9 ± 0.5	14.6 ± 0.9
α-Linolenic ($C_{18:3;\ n-3}$)	0.029 ± 0.012	0.042 ± 0.005	0.068 ± 0.011	0.152 ± 0.015	0.090 ± 0.014
Dihomo-γ-linolenic ($C_{20:3;\ n-6}$)	1.05 ± 0.11	2.26 ± 0.31	2.06 ± 0.32	1.03 ± 0.06	1.63 ± 0.12
Arachidonic ($C_{20:4;\ n-6}$)	14.0 ± 0.5	17.7 ± 0.9	15.8 ± 1.4	11.8 ± 0.5	9.7 ± 0.7
Eicosapentaenoic ($C_{20:5;\ n-3}$)	0.060 ± 0.007	0.062 ± 0.007	0.105 ± 0.010	0.132 ± 0.009	0.073 ± 0.007
Docosapentaenoic ($C_{22:5;\ n-3}$)	0.55 ± 0.02	0.32 ± 0.02	0.19 ± 0.02	1.52 ± 0.07	0.48 ± 0.03
Docosahexaenoic ($C_{22:6;\ n-3}$)	3.1 ± 0.2	10.7 ± 1.2	5.1 ± 0.7	11.8 ± 0.4	4.3 ± 0.4
Total SFAs	45.7 ± 0.3	36.3 ± 0.6	37.3 ± 1.0	36.5 ± 0.6	36.2 ± 0.5
Total MUFAs	25.9 ± 0.7	19.5 ± 2.5	20.5 ± 2.2	21.1 ± 1.2	32.9 ± 0.5*
Total PUFAs	28.4 ± 0.6	44.2 ± 2.3	42.2 ± 2.6	42.4 ± 1.0	30.9 ± 0.5

* Differences are significant with respect to tissues of intact animals ($p < 0.01$).

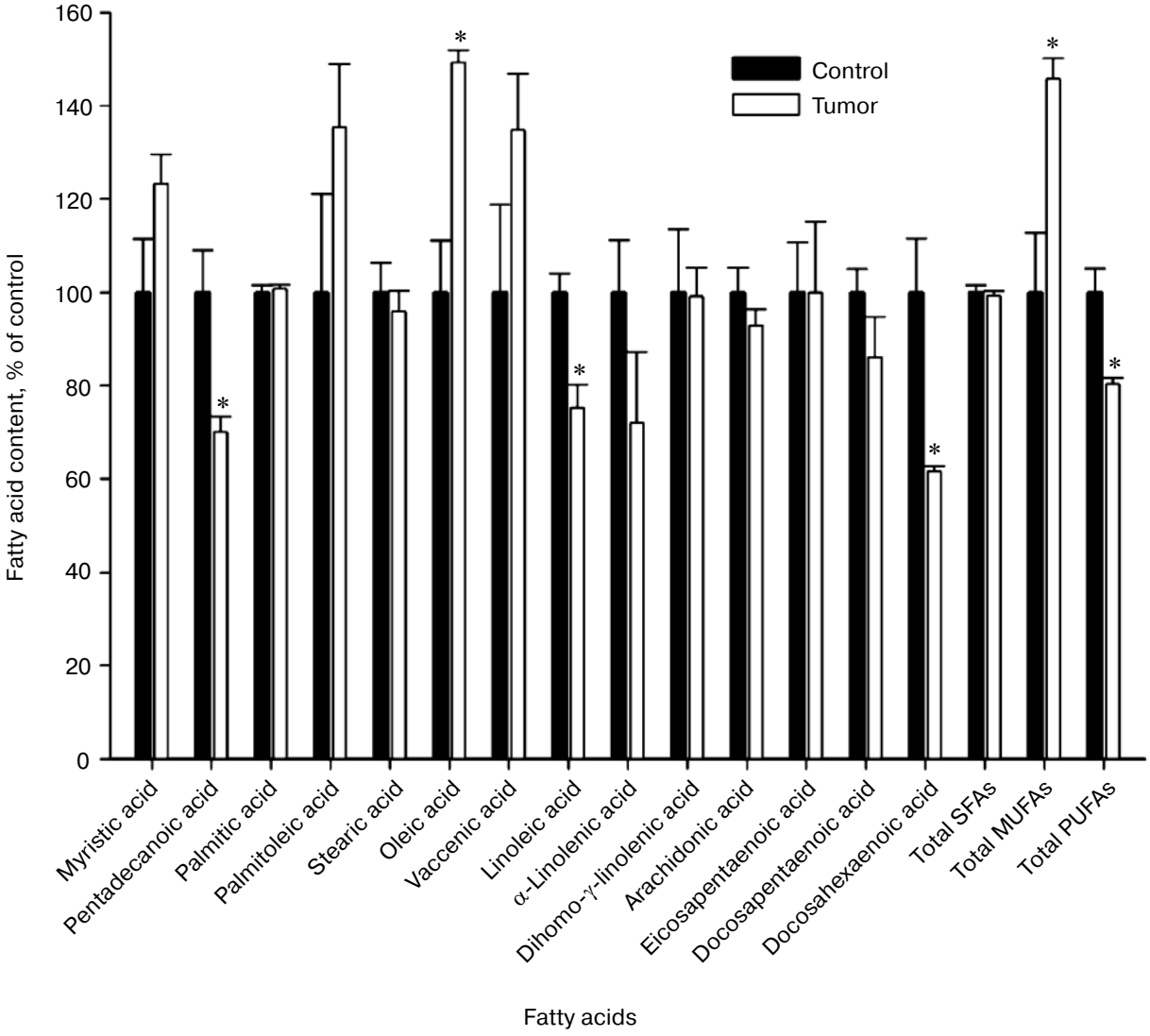

Fig. 3. Changes in fatty acid composition of the liver of tumor-bearing mice.

with seven animals in each group. All data are presented as the mean value ± standard error. Because of normal distribution of all data (by the Kolmogorov–Smirnov test), they were analyzed using Student's *t*-test. Differences were considered to be significant at $p < 0.05$.

RESULTS

The tumor focus growth in mice with solid Ehrlich carcinoma was described by an exponential dependence within a period of 23 days (Fig. 1). However, the tumor growth during the sixth-eleventh days could also be approximated by a linear dependence, and the mean rate of the tumor growth was 0.13 cm^3/day. It seems that from the twelfth day after the inoculation the organism undergoes irreversible changes associated with the tumor growth. The observed tumor growth corresponded to the modern theory of immunosurveillance and immunoediting as interrelations between the growing tumor and the immune system. According to this theory, in the initial stages of tumor growth there is no pronounced immune depression, but immune reactions are activated in response to tumor cells [9, 10]. In the late stages of tumor growth the immune cells lose their competence to the tumor cells, which began to actively grow and spread that finally results in the death of the animal. In our experiments, the animals began to die from the 24th day after tumor cell inoculation, and deaths were recorded during the subsequent three weeks [11]. Based on the observed tumor growth, the fatty acid composition of different tissues of the tumor-bearing animals was analyzed on the ninth day after the inoculation, when no pronounced immunosuppression seemed to occur.

Contents of individual fatty acids (in percent) in the organ and tissue specimens from intact animals and from

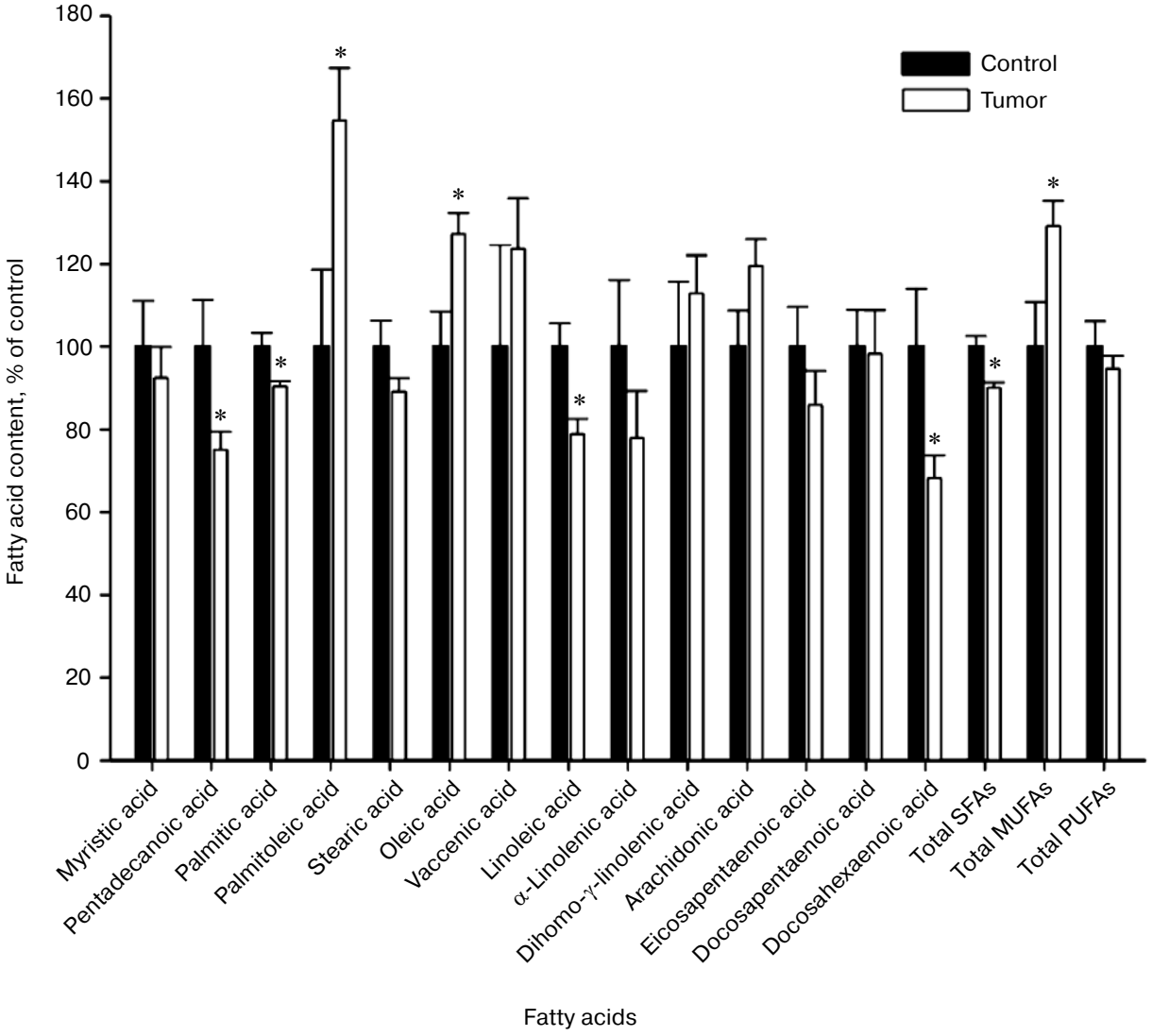

Fig. 4. Changes in fatty acid composition of the blood plasma of tumor-bearing mice.

the tumor focus of tumor-bearing mice are presented in the table. The contents of monounsaturated fatty acids (MUFAs) in the tumor tissue were higher than in the tissues of intact animals.

The presence of the tumor in the mouse's organism resulted in changes in fatty acid contents in the thymus as compared to the intact animals. Contents of saturated myristic and palmitic acids and also of monounsaturated palmitoleic and oleic acids were increased. Tumor growth was accompanied by a decrease in contents of a saturated stearic and of polyunsaturated linoleic, arachidonic, docosapentaenoic, and docosahexaenoic acids. As a result, in the thymus of the tumor-bearing animals the total content of PUFAs was decreased and the contents of saturated fatty acids (SFAs) and MUFAs were increased (Fig. 2).

In the liver of the tumor-bearing mice, the contents of pentadecanoic, linoleic, and docosahexaenoic acids were decreased along with a significant increase in the content of oleic acid. The total content of MUFAs was increased, whereas the total content of PUFAs was decreased (Fig. 3).

In the blood plasma of the tumor-bearing mice, the contents of pentadecanoic, palmitic, linoleic, and docosahexaenoic acids, as well as the total content of SFAs were decreased. The contents of palmitoleic and oleic acids and of the total MUFAs were increased (Fig. 4).

The most pronounced changes in the fatty acid composition in the tumor-bearing mice were observed in the muscle tissue taken from the tumor-free thigh. In this sample contents of palmitic, palmitoleic, oleic, and α-linolenic acids and of the total MUFAs were increased. The contents of pentadecanoic, stearic, dihomo-γ-linolenic, arachidonic, eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids and of the total PUFAs were decreased (Fig. 5).

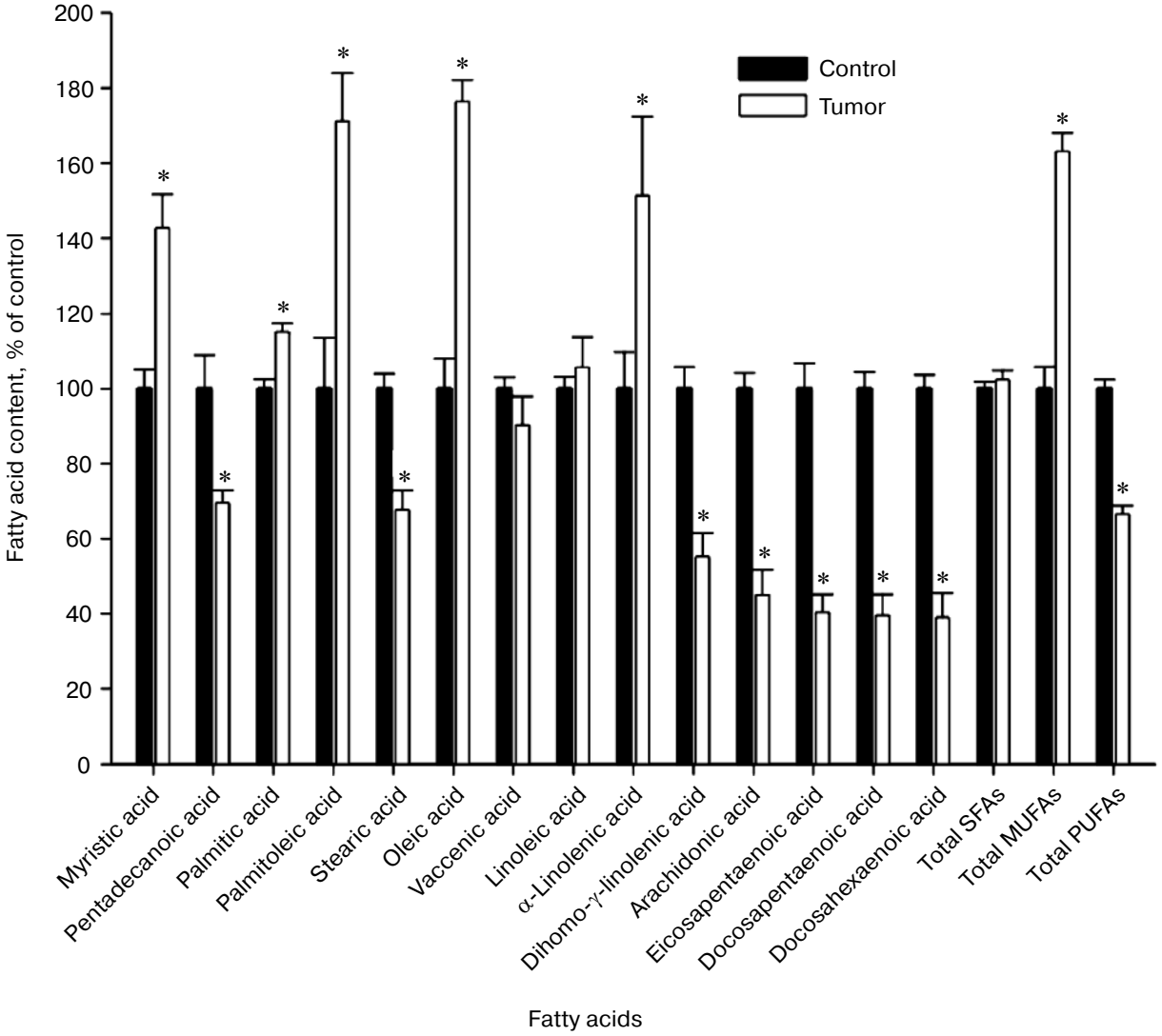

Fig. 5. Changes in fatty acid composition of the muscle tissue of tumor-bearing mice.

DISCUSSION

The expression of stearoyl-CoA desaturase, which is a key enzyme of MUFA synthesis, was found to be increased and correlated with the MUFA content in some genetically and chemically induced tumors [12-14]. Ehrlich ascites carcinoma cells are known to contain an enzyme Δ9 desaturase located on the endoplasmic reticulum [15]. Our findings indicated a significantly increased level of MUFAs not only in the tumor tissue (table), but also in all tissues of tumor-bearing mice (the increase was 25-63% with respect to the corresponding control) (Figs. 2-5). The high level of MUFAs in the tumor cells seems to be necessary for increasing the membrane fluidity to provide the stimulation of cell metabolism and increase in the rate of cell division characteristic for tumor cells [14, 16]. Inhibition of stearoyl-CoA desaturase 1 resulted in the apoptotic type death of tumor cells [17-19], which suggests an important role of MUFAs in tumor growth.

On the inoculation of mice with the tumor cells and the subsequent tumor growth, the immune system response manifested itself by significant changes in the fatty acid composition of the thymus cells (Fig. 2) because the cell membrane could rapidly respond to changes in the environment by changes in their structural composition and microdomain organization [20]. Formation in the plasma membrane of individual microdomains is the most important element of immune cell activation. The activation of T-cells is associated with plasma membrane condensation and formation of ordered structures – lipid rafts, which include saturated acyl groups of myristic and palmitic acids [21, 22]. The increases in the contents of myristic and palmitic acids (Fig. 2) seem to be due to activation of thymocytes in response to appearance in the organism of foreign antigens.

The decrease in the PUFA content in the thymus during tumor growth (Fig. 2) may be associated with generation and release from the thymocytes of PUFA metabolites involved in the antitumor defense of the organism. PUFA metabolites are known to influence proliferation, differentiation, and apoptosis of tumor cells through multiple signaling mechanisms by both autocrine and paracrine pathways [3].

The fatty acid composition of the liver was characterized by a decreased content of pentadecanoic acid, but its role in the growth of tumors is unknown. The oleic acid content in the liver of the tumor-bearing animals was increased (Fig. 3). Because stearoyl-CoA desaturase 1 is present in liver cells [23], the increased level of this acid seems to be due to activation of this enzyme. Liver is the central organ of lipogenesis responsible for provision with lipids of other organs and tissues of the organism. The increased levels of oleic acid in the liver (Fig. 3) and blood plasma (Fig. 4), which performs the transport function, seem to indicate an activation of synthesis of oleic acid for needs of other tissues.

Significant changes in the fatty acid composition in some ways similar to those observed in the thymus were found in the tumor-free muscle tissue of the animals (Fig. 5). This seems to be associated with the functioning of skeletal muscles as the main organ regulating energy homeostasis and glucose oxidation in the organism. Fatty acids, which contribute significantly to the regulation of muscle cell metabolism, can rapidly induce the expression of key genes involved in their metabolism. They influence the expression of the major metabolic regulators, which control the switching of the preferential glycolytic metabolism into predominant lipid oxidation [24].

Changes in the oleic acid contents in different tissues of the tumor-bearing animals are especially interesting because oleic acid plays an important role in the activation of secretion of matrix metalloproteinase-2 and -9, which are directly related with tumor progression including angiogenesis, tumor cell proliferation, and invasion across the basal membranes and intra-tissue matrix [25, 26]. Oleic acid is thought to mediate different signal transduction pathways on cell responses to different tumor types [27].

Along with an increase in the MUFA content in all tissues studied (except blood plasma), the tumor growth was accompanied by a decrease in the total content of omega-3 and omega-6 PUFAs. But mechanisms of pro- and antitumor action of these acids are still unclear, and further studies are needed.

Thus, the findings indicate the active involvement of fatty acids in the responses of the studied organs and tissues to the presence of a tumor in the organism. Data on changes in fatty acid composition in the tumor-free organs and tissues of tumor-bearing animals can be important for development of approaches for correcting the fatty acid composition of organs and tissues as a component of complex antitumor therapy.

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