

# Oxidative Stress in the Molecular Mechanism of Pathogenesis at Different Diseased States of Organism in Clinics and Experiment

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**Abstract:** According to modern images and results of our observations the oxidative stress (OS) is a non-specific though certain component of pathogenesis at numerous diseased states of organism having in the basis the thoroughness of pathogenic disturbances of phospholipids (PL) metabolism and processes of their free radical oxidation (FRO), which takes place in the membrane formations of as the whole cell, as well as the mitochondrial and microsomal fractions (MCF and MSF) of the white rat brain, liver mitochondria, lung shadows, at the same time erythrocyte and lymphocyte shadows at brain acute edema, ischemia, reperfusion and desympathization, infarction of myocardium, tuberculosis of lungs, diabetes, Familial Mediterranean Fever (FMF), intoxications under halothane anaesthesia (HA) and with micotoxin zearalenon. The regularities observed promote to understand from the point of view of modern approaches the molecular mechanisms of initiation, development and generalization of factors for OS formation under pathologic conditions. It is more obvious at zearalenon intoxication with intensification of lipids FRO processes and failures in PL-PL ratio phenomena. The lymphocytes membranes of the white rats spleen subjected OS induced by zearalenon intoxication permit us to conclude that the general immune status of the organism decreases. It is generally peculiar to the states under conditions of generalized intoxication. The observed increase of phospholipase A<sub>2</sub> activity induces the release of high concentrations of lysophosphatidylcholines (LPC) and non-etherified fatty acids (NEFA) of polyenic range with prevail of arachidonic acid as a pathogenic factor, namely, at modelling brain acute edema by tetraethylolovo to white rats. Formation of the above mentioned disturbances to some extent depends on hydrophobic properties of toxins, particularly, zearalenon. The latter gives certain tropism to dopamine- $\beta$ -monooxygenase (DBM), and ability to stimulate functional activity of the enzyme. Striking haemolytic properties of phospholipase A<sub>2</sub> induced by existence of LPC and NEFA high concentrations, and products of their peroxidation, promote elimination of separate protein fractions of erythrocyte membranes (EM) responsible for OS formation and decrease of erythrocytes resistance to peroxide hemolysis.

Increase of DBM activity under the effect of relatively moderate doses of zearalenon (1-15  $\mu$ g/ml) is accompanied with extra intensification of catecholamine synthesizing function of the organism with lethal result.

Data of publications represented testify exceptional efficiency of sodium thiosulfate (STS) as a powerful synergist for endogenous factors of antioxidant effect, particularly  $\alpha$ -tocopherol ( $\alpha$ -T), which is the main component for the system of cell antiradical defence. Detoxifying effect of STS can be demonstrated indeed on the example of zearalenon intoxication during the first two hours with the reduction of metabolism disturbances of PL and products of its peroxidation [1, 2]. Comparative evaluation of molecular mechanisms of STS normalizing effect as a supplier for hydrogen and sulphur ions, as well as an effective synergist for  $\alpha$ -T on the level of various formations of the live cell in compare with the effects of  $\alpha$ -T and ubiquinone [3-11], allowed to make a special accent on the role of STS in interaction with energy-dependent enzymatic systems of cell antiradical defence, as well as accumulation and transformation of energy on the level of mitochondrial membranes [12, 13]. The results obtained by us confirm a number of clinical experimental observations, which demonstrate treatment and prophylactic role of STS at different pathologic states of the organism [14-25]. STS protectory role at toxic injuries of the organism is higher at its preliminary introduction to the organism before modelling of the studied diseased states, especially at zearalenon and halothane (H) intoxication (in the last case before HA). These data serve a sound affirmation for protectory function of STS, detailed revelation of molecular properties of pathogenesis of the studied intoxication to which a part of our clinical and experimental studies at present is devoted.

**Keywords:** Phospholipids, free radical oxidation, antioxidants, neutral and acidic phospholipids, brain edema.

## EVALUATION OF THE ROLE OF ARACHIDONIC ACID IN BRAIN EDEMA PATHOGENESIS AND IN FORMATION OF ORGANISM COMPENSATORY AND ADAPTATION FUNCTIONS

Studying of the processes dependent on the development of swelling, the brain edema (BE) is one of the current trends in modern theoretical and applied medicine [9].

BE is characterized by a large number of morphological, functional [26] and metabolic disorders taking place in various biological formations of central nervous system (CNS), namely on the level of nerve cells membranes [27]. It is shown that ischemia induced by oxygen radicals mediated [28, 29] and water balance affected [30] processes, plays also an important role in the initiation, development and generalization of BE processes, as it takes place in the pathogenesis of a number of CNS injuries [31, 32].

Being the carriers of high concentrations of different lipids, mainly of PL [33-37], the polyunesterified fatty acids [6, 19] play an important barrier function in neuromembranes [38-41].

The functional activity of PL, as regulators of microenvironment for membrane proteins [41], receptor proteins [19, 20, 42-45], and numerous membrane-bound lipid-depending enzymes [91-94], is in a close dependence on the qualitative contents of their fatty acids (FA) spectrum and on percent ratio of saturated and unsaturated representatives of these

compounds. Proceeding from the above mentioned the harmful impact of the high concentrations of free forms of arachidonic acid (C<sub>20:4</sub>) has to be indicated. They are able to initiate BE processes and make difficult it's processing [1, 3, 23, 49-55].

Taking into account the literature data concerning the role of C<sub>20:4</sub> in the disturbances of blood-brain barrier (BBB) [56, 57] and in the BE formation [9, 58-62, 28], as well as during formation of OS accompanied by activation of neuronal degeneration processes and apoptosis [12, 63, 64], we have got interested in the mechanism of formation of the mentioned disturbances. By some indications [59, 61] the most probable mechanism of C<sub>20:4</sub> participation in the mentioned disturbances are the cyclooxygenase and lipoxygenase pathways. Moreover, together with endothelia xantinoxygenase pathway it can participate in the formation of superoxide anion-radicals [61]. Thus, it becomes obvious the ability of C<sub>20:4</sub> to be incorporated into the reactions of formation of superoxide radicals, in the processes of development of BE and BBB disturbances. It is also possible that the mentioned changes lead to the formation of demyelination processes in the brain. The presence of transferrin [65] and activation of phosphatidylinositols (PI) signaling cycle [66] play an essential role in myelination, and myelin requires cooperative interaction with axon.

Resuming the above mentioned, we consider the described changes of C<sub>20:4</sub> occur only at its free form, while the etherified C<sub>20:4</sub> in the contents of PL molecules is so-called "biological neutralized form". The compensatory-protective function of organism provided particularly by limiting the intensity of incorporation of C<sub>20:4</sub> into the reactions of FRO, lead to the formation of high concentrations for lipid peroxides (hydroperoxides, mono-, di- and trienic conjugates). The latter products possess membranotoxic and membranolytic effect and induce brain

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disorders of different character, such as ischemia, hypoxia [17], BBB dysfunction [67, 68], epilepsy [63], BE [62, 69-72], schizophrenia [73], etc. [74].

So, the main purpose of this part of the present study was to investigate the dynamics for the early and late quantitative changes of  $C_{20:4}$  in white rat brain membrane-bound PL during BE. For elucidating the abovementioned data there appeared a problem to clarify the regularities in the changes of FA composition (especially the qualitative spectrum of the mentioned compounds) in white rat brain PL under the conditions of experimental BE.

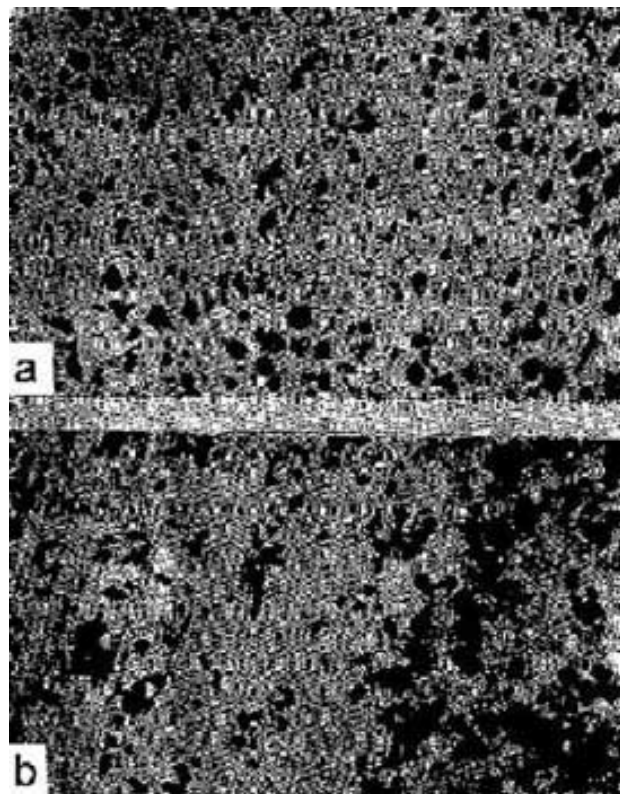
Studying of the dynamics of quantitative shifts for peculiarities of  $C_{20:4}$  in brain tissue during BE is of a great interest. As a fine biochemical mechanism it is possibly underlying in the pathogenesis of BE formations.

The early and late time points in experimental BE at 37 mongrel white male rats weighing 180-200g were induced by intra-abdominal injections of 0,5 ml of 2% tetraethyltin solution (10 mg/kg of body weight) [9]. The same number of animals was used in control experiments.

PL obtained from brain tissue acetone powder were extracted by J. Folch *et al.* [75] in K. Karageuzyan's modification [116].

Methyl esters of PL FA were obtained by W.Stoffel method [76], their qualitative and quantitative determination was made on HPLS (Chrom-5, with flame ionizing detector, CzSSR). FA identification was made by comparing chromatograms with those of standard mixtures and unsaturated FA with  $C_{14-22}$ .

The results demonstrate that BE (induced by tetraethyltin) is characterized by large histomorphological changes and metabolic disorders in brain, resulting in softening of white matter Fig. (1 a, b). There is no need of representing figure for the late period of BE as they are almost similar.



**Fig. (1).** a. Control. Parietal Lobe cortex. Colloidin. Hematoxilin. Incr, 100x. b. Experimental animal. Parietal Lobe cortex. Edematous loosening of the white substance at early period. Colloidin. Hematoxilin-eozin. Incr. 290x.

The content of FA (saturated and unsaturated) in brain PL (Table 1) represents a decrease of saturated  $C_{16:0}$ ,  $C_{18:0}$  and approximately 2-fold increase of the  $C_{20:4}$  level, as well as a slight decrease of  $C_{16:1}$ . Due to these changes the effect of so-called "biological neutralization" in high concentrations of newly etherified more "aggressive"  $C_{20:4}$  is realized probably to be one of the mechanisms of the general compensatory adaptation under extreme conditions of its existence.

As it follows from the Tables 1 and 2, the comparative study of qualitative and quantitative contents of FA permits us to conclude, that at some extent the above mentioned decrease of the quantity of saturated FA, and  $C_{16:1}$  in the structure of those compounds promotes the intensive involvement of  $C_{20:4}$  into the PL structure, the quantity of which was increased 4 fold.

Thus, the changes mainly in PL metabolism and in FA content were examined. The high degree of etherification of  $C_{20:4}$  in brain PL in the BE model is a confirmation of the activation of the enzyme system that catalyses reacylation, hence resulting the synthesis of new PL.

According to our preliminary results the increased content of the etherified  $C_{20:4}$  was observed at BE mainly in the contents of phosphatidylserines (PS) and phosphatidylethanolamines (PE) in MCF.

The further study of the fact represents an essential interest as a mechanism strictly limiting the concentrations of non-etherified  $C_{20:4}$ , as well as an indication of PL active role in respiratory function of brain mitochondria.

So, we assume, that the selective saturation of PL molecules of brain tissue at BE by the major quantity of  $C_{20:4}$  both in the early period (Table 1) and especially in the late period (Table 2) can really prevent the involving of the free  $C_{20:4}$  into metabolic mechanisms of the BE pathogenesis.

The change in PL by the increase of  $C_{20:4}$  indicates the formation of a so-called "pathologic" variety with altered metabolism. We suppose the selective increase in PL of brain in BE by  $C_{20:4}$  residues (and not by other mono- and polyenic FA) to be of importance in defense mechanisms. It is not excluded that  $C_{20:4}$  also participates to some extent in the biosynthesis of physiologically active substances, e.g. prostaglandins.

The brightly expressed substitutions in PL structure by the increased quantity of polyenic  $C_{20:4}$  lead to the sensible deviations of the qualitative and quantitative standard contents of tissue PL.

Our data are in agreement with the results of Chan *et al.* [49, 50, 58, 61, 69]. The results demonstrate the absence of BE development at the injection of  $C_{20:4}$ , as well as at the influence of cold-injure factor at preliminary or simultaneous administration of superoxide dismutase (SOD) in liposomes. At SOD free form (not in liposomes) injection there appears a pronounced development of BE. Thus, the leading pathogenic role of  $C_{20:4}$  is out of doubt at BE. On the other hand, according to our results among all the polyenic FA studied, the  $C_{20:4}$  is the only one, the quantity of which increases at experimental BE approximately 5 fold.

So, the etherification of  $C_{20:4}$  distinguished by us as bio- a "biological neutralization" excludes its participation in the pathogenic mechanism of BE.

#### PHOSPHOLIPID POOL, LIPID PEROXIDATION, AND SUPEROXIDE DISMUTASE ACTIVITY UNDER VARIOUS TYPES OF OXIDATIVE STRESS OF THE BRAIN AND THE EFFECT OF LOW-ENERGY INFRARED LASER IRRADIATION

Increased FRO of lipids in ischemic and reperfusion lesions and during vascular edema of the brain contributes to the development of a certain metabolic disorder considered as one of the types of OS [77]. Free-radical attack by very reactive superoxide  $O_2^-$ ,  $OH^\bullet$ , and other reactive oxygen species is associated with intensification of lipid peroxidation (LP), which influences the spectrum and level of membrane PL and conformational transitions in membrane proteins resulting in direct chemical modification of membrane-bound proteins including enzymes and receptors *via* changes in their lipid microenvironment [78]. The guanosine triphosphate (GTP)-binding proteins are one of the critical targets reacting to ischemic damage to the brain [21]. Apart from active oxygen species, during lowering and prolonged deficiency of cerebral circulation,  $NO^\bullet$  actively generated by neurons, endothelium, and leukocytes in cerebral ischemia also damages the brain [20]. Our previous studies indicate that He-Ne laser irradiation (LI) corrects normal phylogenetically stabilized PL-PL interactions in cerebral ischemia [79, 80] and that LI suppresses free-radical oxidation in the lipid phase of biomembranes and photo reactivation of SOD [81]; so, contents of various types of PL in the brain were measured at certain types of OS as well as during low-energy infrared (IR) LI. Together with brain PL, the parameters characterizing the LP intensity and SOD activity were determined.

The study has been carried out on 270 mature male mongrel white rats (with 180-200 g weight) divided into several groups according to experimental pathology. Cerebral ischemia was induced by occlusion of

**Table 1. Fatty Acids Contents in White Rats Brain Phospholipids (% of the sum of FA) in Norm and on the 3rd Day (early time point) of BE Induced by Tetraethyltin**

Fatty acids	Control	BE
Myristic (C <sub>14:0</sub> )	Insignificant	Insignificant
Palmitic (C <sub>16:0</sub> )	28.91 ± 0.28	20.12 ± 0.29 <sup>x</sup>
Palmitoleic (C <sub>16:1</sub> )	1.81 ± 0.03	1.56 ± 0.02 <sup>x</sup>
Margarinic (C <sub>17:0</sub> )	Insignificant	Insignificant
Stearic (C <sub>18:0</sub> )	30.97 ± 0.51	25.93 ± 0.19 <sup>x</sup>
Oleic (C <sub>18:1</sub> )	29.91 ± 0.29	32.19 ± 0.20 <sup>x</sup>
Nonadecylic (C <sub>19:0</sub> )	Insignificant	Insignificant
Arachinic (C <sub>20:0</sub> )	Insignificant	Insignificant
Gadoleinic (C <sub>20:1</sub> )	5.69 ± 0.59	4.31 ± 0.39 <sup>xx</sup>
Arachidonic (C <sub>20:4</sub> )	3.77 ± 0.03	6.98 ± 0.21 <sup>x</sup>
Undecanic (C <sub>21:0</sub> )	Insignificant	Insignificant
The sum of saturated FA (C <sub>14:0</sub> )+(C <sub>16:0</sub> )+(C <sub>17:0</sub> )+(C <sub>18:0</sub> )+(C <sub>19:0</sub> )+(C <sub>20:0</sub> ) <sup>+</sup>	59.88 ± 0.69	46.05 ± 0.61 <sup>x</sup>
The sum of monoenic FA (C <sub>16:1</sub> )+(C <sub>18:1</sub> )+(C <sub>20:1</sub> )+(C <sub>21:0</sub> )	37.41 ± 0.59	38.00 ± 0.51 <sup>xxx</sup>
The sum of polyenic FA (C <sub>20:4</sub> )	3.74 ± 0.03	7.59 ± 0.21 <sup>x</sup>
The sum of mono- and polyenic FA	41.12 ± 0.81	45.73 ± 0.59 <sup>x</sup>

x-P<0,001, xx-P<0,01, xxx-P>0,1.

both carotid arteries for 20 min in animals narcotised with intraperitoneal injection of nembutal (3 mg/kg body weight). The reperfusion period was 20 min (restoration of circulation in carotid arteries); the control group included sham-operated rats. Acute BE was determined by water accumulation in the brain tissue and by microstructural lesions [82]; it was induced by single intraperitoneal injection of 10 mg tetraethyl tin (as 0.2Vo solution) per kg body weight [26]. Animals were decapitated under slight ether anaesthesia; the brain was excised, cleaned from blood vessels and membranes, and washed with cooled physiological saline in the cold as rapidly as possible. Acetone powder of the brain was prepared as described [74] and its constant weight indicates that the maximal extraction of PL was achieved [75] with various volume ratios of the chloroform-methanol mixture (1:1, 1:2, 2:1). PL were fractionated by one-dimensional ascending chromatography on silica gel [139] and the spots of tri-, di- and monophosphoinositides (TPI, DPI, and MPI, respectively), sphingomyelins (SPM), phosphatidylcholines (PC), PS, PE, and cardiolipins (CL) were detected with iodine vapour using marker PL (Sigma, USA). Amount of PL was expressed in micrograms of mineralized lipid phosphorus per gram dry residue (in figures, the amount of PL is expressed in percentage). Phosphorus was determined by the method of Fiske and Subbarow [83]. Subcellular fractions of the brain [84] were isolated in medium containing 0.25 M sucrose and 0.01 M Tris-HCl by differential centrifugation (protein was also assayed) [85]. Colour of the products of the malonic dialdehyde (MDA) reaction with thiobarbituric acid and of the hydroperoxide (HP) reaction with ammonium thiocyanate was proportional to the concentration of the compounds and was determined at 535 [86] and 480 nm [87], respectively. Activity of SOD was assayed by inhibition of O<sub>2</sub> generation in a model system containing phenazine methosulfate, nicotineamide adenine dinucleotide reduced form, and nitrotriazolium blue [88]. NEFA were determined by gas-liquid chromatography using a gas chromatograph by Pay-UNICAM (UK). The brain was irradiated with a laser by a non-invasive method through the crown bone with a low-energy IR (YAG:Nd<sup>3+</sup>) laser. The wavelength was 1064 nm, spot diameter 1.52 cm, pulse power 100-150 mJ, total energy 66 J, and LI mode pulses at 2 Hz frequency and 10 sec exposure. LI of the brain was performed before and after experimental ischemia and reperfusion; in case of acute BE the animals were irradiated 3 times:

2, 24, and 48 h after the injection with tetraethyltin. The data were evaluated using the parametric Student's test.

The data indicate that in ischemia, reperfusion, and acute BE the spectrum and levels of PL are significantly altered predominantly in case of inositol-containing PL. Lowered levels of TPI and DPI in the brain characterize these disorders, whereas the content of MPI is not changed in acute edema and is increased in ischemia and reperfusion. These experimental lesions are associated with relatively stable levels of PS and PE and are characterized by increased content of PC in the brain during ischemia development and especially after acute BE; in reperfusion, PC content is lowered. Typically, in these types of OS the SPM content is lowered in the brain but the amount of CL is not changed Fig. (2). Changes in the PL spectrum of the brain in case of acute edema are associated with activated peroxidation in the crude homogenate and especially in the subcellular fractions including the enzymatic nicotineamide adenine dinucleotide phosphate-dependent (NADPH) lipid peroxidation system. Interestingly, changes in the level of HP and MDA are more pronounced in the MCF of brain: somewhat lower changes are detected in the microsomes and crude homogenate Fig. (3). Activation of LP in acute BE is associated with simultaneous development of dramatic changes in FA composition of PL, i.e., lowered amount of palmitic (C<sub>16:0</sub>) and stearic (C<sub>18:0</sub>) acids and significant increase in unsaturated NEFA (predominantly arachidonic acid C<sub>20:4</sub>) which is 5-fold higher versus the initial level Fig. (4). On the other hand, the intracerebral injection of C<sub>20:4</sub> inhibits the membrane ATPases [49], thus promoting the accumulation of sodium ions and water in the cells increasing permeability of the BBB, and causing acute BE. The effects of transcranial low-energy IR LI on brain PL is of special interest in the control group of animals and in experimental OS. The data of Fig. (5) indicate that LI of the control brain slightly influences the PL pool. The levels of TPI and DPI were not significantly altered but the content of MPI is lowered. The amount of SPM and CL is stable but the PC cycle components are elevated. Analysis of the effects of LI on the PL pool of the brain in experimental OS Fig. (6) revealed important changes in inositol-containing PL. In ischemia and reperfusion the LI does not lower the content of TPI, whereas in acute BE it has a beneficial influence on TPI changes. The lowered level of DPI is apparently photo corrected but the MPI content is lowered in ischemia with LI; MPI level is not

Table 2. Fatty Acids Contents in White Rats Brain Phospholipids (% of the sum of FA) in Norm and on the 7th Day (late time point) of BE Induced by Tetraethyltin

Fatty acids	Control	BE
Myristic (C <sub>14:0</sub> )	Insignificant	Insignificant
Palmitic (C <sub>16:0</sub> )	27.91 ± 0.31	22.63 ± 0.37 <sup>x</sup>
Palmitoleic (C <sub>16:1</sub> )	1.67 ± 0.03	1.33 ± 0.02 <sup>x</sup>
Margarinic (C <sub>17:0</sub> )	Insignificant	Insignificant
Stearic (C <sub>18:0</sub> )	28.93 ± 0.47	25.91 ± 0.19 <sup>x</sup>
Oleic (C <sub>18:1</sub> )	31.97 ± 0.19	32.97 ± 0.15 <sup>xx</sup>
Nonadecylic (C <sub>19:0</sub> )	Insignificant	Insignificant
Arachinic (C <sub>20:0</sub> )	Insignificant	Insignificant
Gadoleinic (C <sub>20:1</sub> )	4.77 ± 0.57	3.91 ± 0.14 <sup>xxx</sup>
Arachidonic (C <sub>20:4</sub> )	2.73 ± 0.1	10.92 ± 0.29 <sup>x</sup>
Undecanic (C <sub>21:0</sub> )	Insignificant	Insignificant
The sum of saturated FA (C <sub>14:0</sub> )+(C <sub>16:0</sub> )+(C <sub>17:0</sub> )+(C <sub>18:0</sub> )+(C <sub>19:0</sub> )+(C <sub>20:0</sub> )+(C <sub>21:0</sub> )	58.51 ± 0.69	49.87 ± 0.71 <sup>x</sup>
The sum of monoenic FA (C <sub>16:1</sub> )+(C <sub>18:1</sub> )+(C <sub>20:1</sub> )+(C <sub>21:0</sub> )	38.41 ± 0.81	38.21 ± 0.79 <sup>xxx</sup>
The sum of polyenic FA (C <sub>20:4</sub> )	2.73 ± 0.01	10.92 ± 0.29 <sup>x</sup>
The sum of mono- and polyenic FA	41.14 ± 0.83	49.13 ± 0.73 <sup>x</sup>

x-P<0,001, xx-P<0,01, xxx-P>0,1.

changed in reperfusion and acute BE. Among the components of the PC cycle, the level of PC increases in ischemia and is further increasing in case of LI, whereas the amount of PS is lowered and the content of PH slightly increases.

In reperfusion the PC levels are relatively stable but LI promotes its increase in the brain though it does not influence the levels of PE, and decreases the amount of PS. In acute BE, on the contrary, LI inhibits increase in PC, elevates the level of PE, and has no effect on PS in the brain. In all cases of OS in the brain the SPM level is lowered. In ischemia

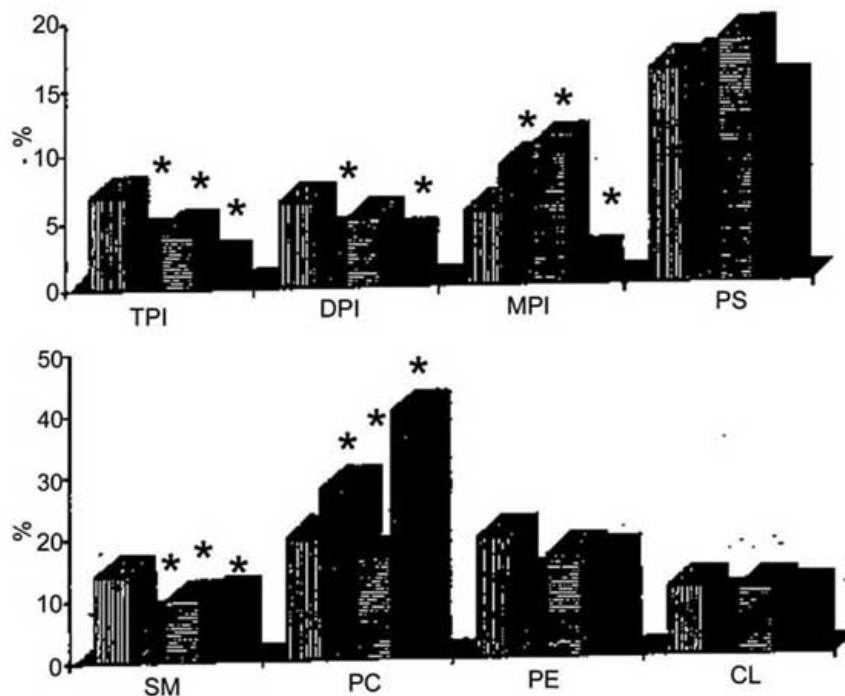
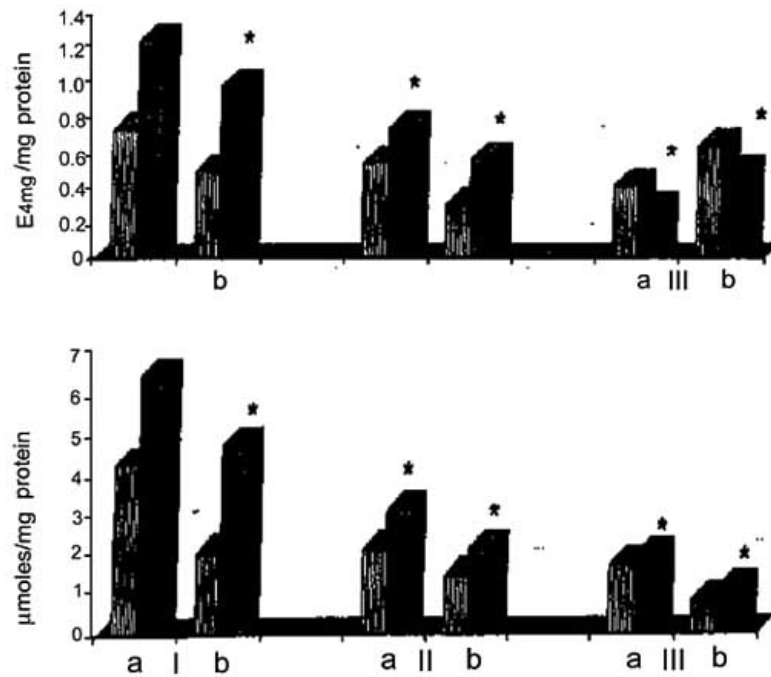


Fig. (2). Changes in acidic (I) and neutral (II) phospholipids in the brain of white rats under various types of oxidative stress: 1) control; 2) ischemia; 3) reperfusion; 4) acute edema. Asterisks indicate significant differences versus control (p < 0.01).

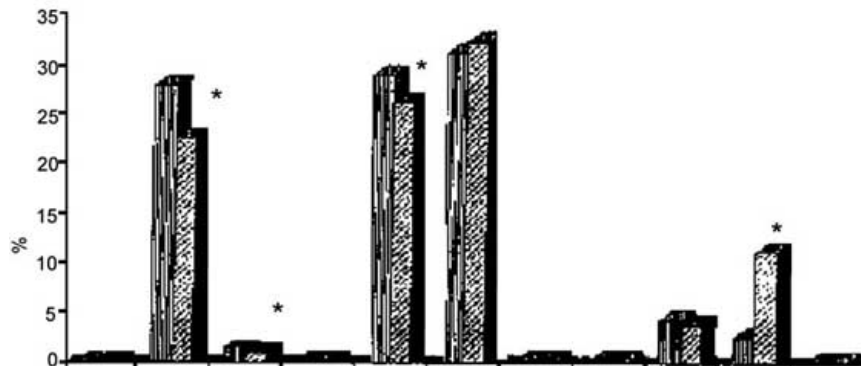


**Fig. (3).** Contents of hydroperoxides (HP) and malonic dialdehyde (MDA) in the brain of white rats in nonenzymic (a) and enzymatic (b) systems of lipid peroxidation in control and acute edema of the brain (AEB): I) mitochondrial fraction; II) microsomal fraction; III) crude brain homogenate. Asterisks indicate significant differences versus control ( $p < 0.01$ ).

the SPM content is not changed, and it is essentially normalized by LI increasing in acute BE. Under LI the level of CL increases in reperfusion and especially in ischemia and decreases in acute BE Fig. (6).

Thus, the data indicate that in experimental OS variable photo induced changes in the contents of individual PL in the brain occur: thus, SOD activity in the brain was studied under these conditions. In cerebral ischemia SOD is slightly activated but the enzyme activity remains stable in reperfusion and decreases in acute BE. Considering such variable changes in SOD activity the data of Fig. (7) indicate that LI has no effect on enzyme activity in reperfusion and promotes its maximal increase in acute BE (characterized by initially lowered SOD activity) versus that in case of ischemia, i.e., when the enzyme activity is already elevated. It should be noted that in experimental cerebral lesions the IR laser promotes the lowering of the increased contents of HP and MDA in the brain in all types of OS Fig. (8). This is especially pronounced in case of the enzymatic LP system.

Thus, the data indicate that transcranial low-energy IR LI corrects the PL pool lesions in the brain in various pathologies (ischemia, reperfusion, acute edema), which are characterized by OS, suppresses lipid peroxidation, and enhances the activity of SOD, one of the main enzymes of the antiradical defense of the cell.



**Fig. (4).** Fatty acid composition of phospholipids (% of total) in the brain of control animals and in acute edema of the brain (AEB). Asterisks indicate significant differences versus control ( $p < 0.05$ ).

#### ROLE OF PHOSPHOLIPIDS METABOLISM DISORDERS IN PATHOGENESIS OF HALOTHANE-INDUCED INTOXICATION

The formation of narcotic effect under HA influence [89] is accompanied with special reactivity of the organism on the level of sarcoplasmic reticulum of cardiomyocytes [90-92], liver MCF (LMCF) and liver MSF (LMSF) at the state of inflammation of different loading [93-96], EM [97] and other biological formations, affect the ones peculiar to intoxication with carbon tetrachloride and ethanol [98-101].

In the basis (as a main pathogenic factor) the extra activation of intensity of FRO intoxication with H is characterized by more wide spectrum of effects in compare with the ones of many other compounds of similar influence expressed in particular in the loss of stability and resistance of EM to peroxide hemolysis [97].

Thus, we have determined the goals of this investigation, which represent a study of peculiarities for disturbances of qualitative composition and quantitative contents of certain categories of PL in outer and inner membranes of white rats hepatocytes under HA influence, as well as for differences normalizing the effect of STS on this background [7, 8, 102-105]. At present it is of no doubt that in the basis of molecular mechanisms of the STS wholesome effect at diffuse inflammations and hepatocyrrosis are of pronounced antioxidant properties [106, 107]

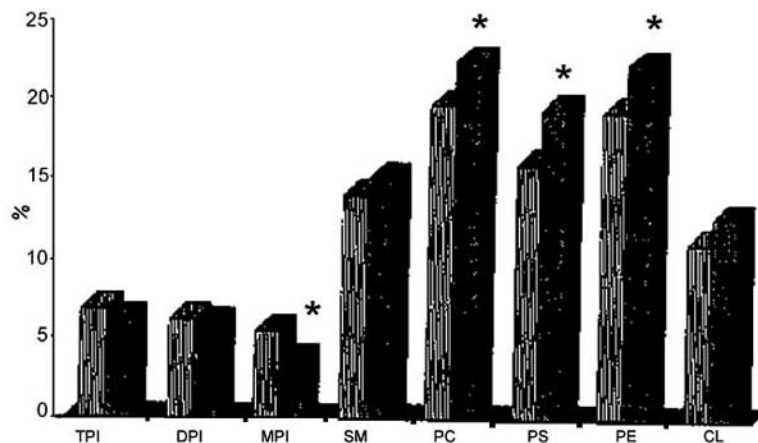


Fig. (5). Effect of infrared laser on phospholipids content in the brain of control white rats. Asterisks indicate significant differences versus control ( $p < 0.05$ ).

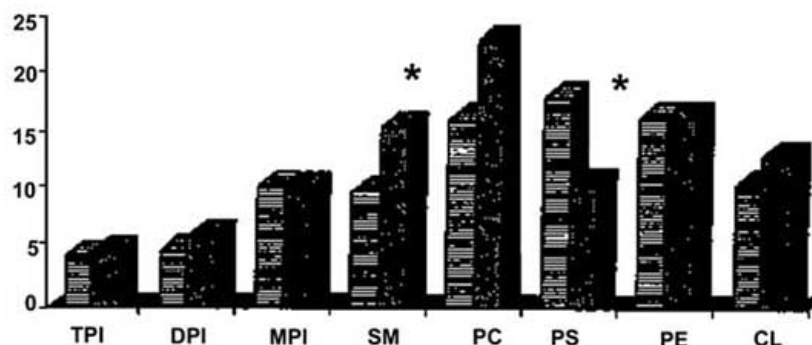


Fig. (6). Effect of infrared laser on phospholipids contents in the brain of white rats in ischemia (I), reperfusion (II), and acute edema of the brain (III): 1) no irradiation; 2) laser irradiation. Asterisks indicate significant differences versus control ( $p < 0.01$ ).

similar to membrane protectory effect of  $\alpha$ -T and glutathione [108]. It is accompanied with parallel increase of glutamate oxalacetate transaminase and glutamate pyruvate transaminase activity [109]. These examples are bright verifications for the opinion existing on the paramount of phylogenetically programmed constancy for membrane P1 qualitative-quantitative ratio [110, 111], the slightest deviations of which may lead to the development of membranotoxic and membranolytic effects, so promoting the start for ill state.

The experiments were carried out on 60 mongrel male rats weighing 180-200 g, and fed on ordinary vivarium support. They were divided into four groups (by 15 animals in each group) as follows: 1) control, 2) subjected to 30 min influence of HA, the concentration of which in the oxygen inspired was about 1-1,5 vol%, 3) the same as in the point 2, only in 30 min after intra-abdominal administration of STS 35% solution, 4) the same as in the point 3, only 0,15 ml of STS of the same concentration was used. The animals were decapitated in 60 min after the last manipulation under ether anaesthesia.

The liver slices isolated on cold after the detailed cutting into the small slices were homogenized in the medium containing 0,25 M of saccharose and 0,01 M Tris-HCl buffer with pH=7,4. The suspension was centrifuged

to obtain subcellular formation, such as: nuclei (at 850 x g during 15 min), LCMF (at 11000 x g during 20 min in centrifuge K-24, GDR), LMSF (at 105000 x g during 60 min in centrifuge VAC-601, GDR) [2]. The LCMF fraction obtained was washed with the mixture of saccharose with Tris-HCl buffer and subjected trice centrifugation: first 600 x g for 10 min, then twice 8500 x g for 10 min. Having removed the supernatant fluid, the precipitant was added 25 ml of cooled distilled water (osmotic shock), then it was put into the refrigerator for 24 h to separate the mitochondrial membranes. After the completion of the mentioned period of time there were isolated the extra and intra mitochondrial membranes in the gradient of saccharose density: 34, 44, and 52% solutions prepared on Tris-HCl buffer. The gradient of saccharose density was obtained by gradual forming of layer of the prepared solutions in descending concentration (52, 44, 34%) in volume 8-10 ml (the first two solutions), and 6-8 ml (the third solution), which was added to the 5-7 ml of mitochondrial suspension (prepared beforehand) in the centrifuge test tubes. Centrifugation was made by bucket-rotor at 45000-x g during 60 min. After that the fraction of mitochondrial extra membranes appeared to be between the layers of saccharose solution concentrations of 34 and 44%. The fraction of hepatocytes inner mitochondrial membrane (HIMM) was situated in the gradient between the layers of saccharose solution concentrations 44 and

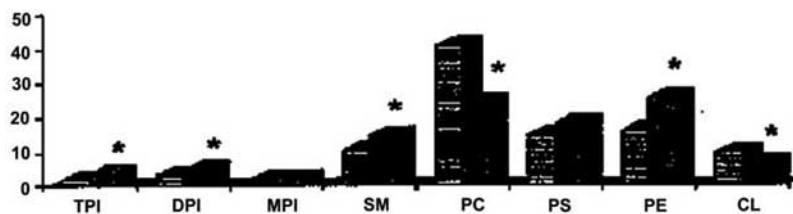
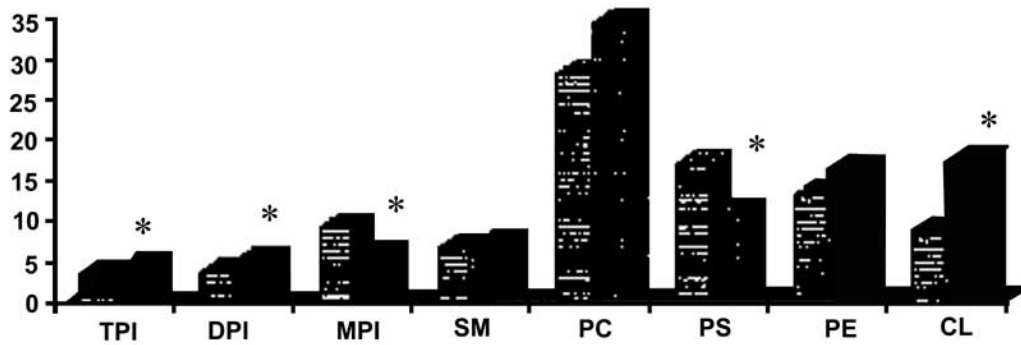


Fig. (7). Effect of infrared laser irradiation on superoxide dismutase activity in the brain of white rats in various types of oxidative stress: 1) control; 2) ischemia; 3) ischemia and laser irradiation; 4) reperfusion; 5) reperfusion and laser irradiation; 6) acute edema; 7) acute edema and laser irradiation. Circles indicate differences between control and experimental pathology ( $p < 0.01$ ) and asterisks indicate differences between experimental pathology and laser irradiation ( $p < 0.01$ ).



**Fig. (8).** Effect of infrared laser contents of hydroperoxides (HP) and malonic dialdehyde (MDA) in the brain of white rats in non-enzymatic (I) and enzymatic (II) lipid peroxidation systems in various types of oxidative stress: 1) control; 2) ischemia; 3) ischemia and laser irradiation; 4) reperfusion; 5) reperfusion and laser irradiation: 6) acute edema; 7) acute edema and laser irradiation. Circles indicate differences between control and experimental pathology ( $p < 0.01$ ) and asterisks indicate differences between experimental pathology and laser irradiation ( $p < 0.01$ ).

52%. After washing with cooled distilled water the isolated (with syringe) fractions of mitochondrial membranes were precipitated by the above-

mentioned centrifugation [112]. Their degree of purity was controlled by electron microscope analysis.

**Table 3.** Dynamics of Quantitative Changes of Phospholipids ( $\mu\text{g}$  lipid phosphorus/mg dry fraction) in Intact Mitochondria (1), their Extra (2) and Intra (3) Membranes in Control (a) and at 30 min Halothane Anesthesia (b)

INDICES	1		2		3	
	A	B	A	B	A	B
MPI	1,29±0,09	2,51±0,08 <sup>x</sup>	1,26±0,06	0,80±0,02 <sup>x</sup>	1,19±0,06	2,08±0,20 <sup>x</sup>
LPC	1,19±0,03	2,79±0,04 <sup>x</sup>	1,49±0,09	2,56±0,09 <sup>x</sup>	0,91±0,02	2,08±0,13 <sup>x</sup>
SPM	2,18±0,08	2,89±0,07 <sup>x</sup>	2,23±0,01	1,03±0,09 <sup>x</sup>	1,23±0,05	2,41±0,21 <sup>x</sup>
PC	4,98±0,11	3,13±0,12 <sup>x</sup>	3,73±0,15	1,39±0,09 <sup>x</sup>	4,99±0,09	4,09±0,14 <sup>x</sup>
PS	1,83±0,12	0,78±0,08 <sup>x</sup>	1,99±0,06	0,96±0,05 <sup>x</sup>	1,31±0,04	3,93±0,15 <sup>x</sup>
PE	2,22±0,06	4,91±0,07 <sup>x</sup>	1,11±0,08	2,46±0,04 <sup>x</sup>	2,11±0,13	3,69±0,19 <sup>x</sup>
CL	2,73±0,13	3,99±0,12 <sup>x</sup>	1,61±0,10	1,07±0,09 <sup>x</sup>	2,44±0,13	4,41±0,25 <sup>x</sup>
TPL	16,42±0,22	21,00±0,25 <sup>x</sup>	13,42±0,23	10,27±0,2 <sup>x</sup>	14,18±0,27	22,69±0,28 <sup>x</sup>
TNPL	10,57±0,12	13,72±0,14 <sup>x</sup>	8,56±0,12	7,44±0,17 <sup>x</sup>	9,24±0,14	12,27±0,23 <sup>x</sup>
TAPL	5,25±0,11	7,28±0,09 <sup>x</sup>	4,86±0,13	2,83±0,14 <sup>x</sup>	4,94±10,15	10,42±0,17 <sup>x</sup>
C TNPL /TAPL	1,80±0,03	1,88±0,04 <sup>x</sup>	1,76±0,03	2,60±0,05 <sup>x</sup>	1,87±0,03	1,18±0,05 <sup>x</sup>

n=15; x -  $P < 0,001$ .

**Table 4.** Dynamics of Quantitative Changes of Phospholipids ( $\mu\text{g}$  lipid phosphorus/mg dry fraction) in Intact Mitochondria (1) of White Rats Hepatocytes, their Extra (2) and Intra (3) Membranes in Control (a) and at 30 min Halothane Anaesthesia Released in 30 min after Preliminary Administration of Sodium Thiosulphate (b)

INDICES	1		2		3	
	A	B	A	B	A	B
MPI	1,29±0,09	1,31±0,09 <sup>xxx</sup>	1,26±0,06	1,25±0,04 <sup>xxx</sup>	1,19±0,06	1,18±0,08 <sup>x</sup>
LPC	1,19±0,05	1,26±0,07 <sup>x</sup>	1,49±0,09	1,51±0,09 <sup>xxx</sup>	0,91±0,02	0,89±0,04 <sup>x</sup>
SPM	2,17±0,08	2,23±0,08 <sup>x</sup>	2,23±0,08	2,20±0,08 <sup>xxx</sup>	1,23±0,05	1,22±0,07 <sup>x</sup>
PC	4,98±0,11	4,81±0,13 <sup>x</sup>	3,73±0,15	3,68±0,22 <sup>x</sup>	4,99±0,08	4,92±0,13 <sup>x</sup>
PS	3,78±0,12	3,69±0,14 <sup>xx</sup>	1,99±0,06	1,97±0,07 <sup>x</sup>	1,31±0,04	1,29±0,08 <sup>x</sup>
PE	2,22±0,06	2,25±0,09 <sup>xx</sup>	1,11±0,03	1,09±0,09 <sup>xx</sup>	2,11±0,13	2,08±0,15 <sup>x</sup>
CL	2,73±0,13	2,71±0,16 <sup>xxx</sup>	1,61±0,10	1,58±0,11 <sup>x</sup>	2,44±0,13	2,39±0,12 <sup>x</sup>
TPL	18,36±0,32	18,96±0,41 <sup>xx</sup>	13,42±0,34	13,28±0,29 <sup>x</sup>	14,18±0,90	13,97±0,99 <sup>x</sup>
TNPL	10,56±0,12	10,55±0,14 <sup>xxx</sup>	8,56±0,33	8,48±0,38 <sup>x</sup>	9,24±0,14	9,11±0,15 <sup>xxx</sup>
TAPL	7,80±0,11	7,71±0,12 <sup>x</sup>	4,86±0,12	4,80±0,13 <sup>x</sup>	4,94±0,12	4,86±0,13 <sup>x</sup>
C TNPL /TAPL	1,35±0,03	1,37±0,03 <sup>x</sup>	1,57±0,02	1,77±0,03 <sup>x</sup>	1,87±0,03	1,87±0,03

n=25; x -  $P < 0,001$ ; xx -  $P < 0,01$ ; xxx -  $P > 0,5$ ; without marks data are unverified.

**Table 5. Dynamics of Quantitative Changes of the Products of Lipids Free Radical Oxidation (in nm of malonic dialdehyde/mg protein) in White Rats Hepatocytes, Intact Mitochondria (1), their Extra (2) and Intra (3) Membranes at Control (a) and on the Background of 30 min Halothane Anaesthesia (b)**

INDICES	1		2		3	
	A	B	A	B	A	B
<b>Non-enzymatic peroxidation</b>	11,81±1,13	20,35±1,03 <sup>x</sup>	8,46±0,97	17,58±1,13 <sup>x</sup>	6,12±0,97	8,99±0,75 <sup>x</sup>
<b>Enzymatic peroxidation</b>	9,88±1,03	15,89±1,30 <sup>x</sup>	5,92±0,85	9,98±0,71 <sup>x</sup>	5,99±1,80	7,28±0,98 <sup>x</sup>

n=25; x - P&lt;0,001; data are without marks are unverified.

**Table 6. Dynamics of Quantitative Changes of the Products of Lipids Free Radical Oxidation (in nm of malonic dialdehyde/mg protein) in White Rats Hepatocytes, Intact Mitochondria (1), their Extra (2) and Intra (3) Membranes at Control (a) and on the Background of 30 min Halothane Anaesthesia (b) Released in 30 min after Preliminary Administration of Sodium Thiosulphate**

INDICES	1		2		3	
	A	B	A	B	A	B
<b>Non-enzymatic peroxidation</b>	12,00±1,13	12,04±1,01	8,46±0,97	8,39±0,88 <sup>x</sup>	6,12±0,97	6,66±0,91 <sup>x</sup>
<b>Enzymatic peroxidation</b>	9,88±1,03	10,12±1,14 <sup>x</sup>	5,92±0,81	6,22±0,81 <sup>x</sup>	5,99±1,30	6,76±1,19 <sup>x</sup>

n=25; x - P&lt;0,001; data without marks are unverified.

Fractionation of individual PL was made by one-dimensional descending chromatography in thin layer type of silicagel (KSK) using system of solvents: chloroform-methanol-ammonia in volumes 65:35:5. The identification of PL spots was carried out by the substances of "Sigma" (USA). The mineralization of lipid phosphorus was done in the medium of 72% chloral acid with further counting of the quantity of phosphorus in µg for 1 mg of dry masses of the corresponding fraction [13].

The activity of lipids peroxidation was demonstrated by the output of MDA, which together with thiobarbituric acid results in colour staining. Its intensity has been registered spectrophotometrically (SF-4A) at wavelength 535 nm [66]; the quantity of peroxides was counted for 1 mg of the whole protein of the fraction [67].

Study of STS effects was made by the scheme and concentrations mentioned above.

The results given in Table 3 testify HA toxic effect on cell biological systems expressed particularly in essential disturbances of PL all categories studied in hepatocytes external mitochondrial membrane (HEMM) and HIBM.

Shifts in the levels of PC, PE and PS are observed more clearly. At HA their interrelations are expressed by statistically verified decrease of PC quantity accompanied simultaneously by the developing contents of PE. It can be explained by obvious activation of PC demethylation processes, which are worthy to be studied in future. At the same time on the background of the observed decrease of PS level in IMC and HEMM it is noticed a pronounced increase of PS contents too, as well as MPI and CL in HIBM. On our opinion this fact can be interpreted by the important role of the mentioned PL in the stimulation of MCF respiration function. The latter is especially necessary under HA toxic effect, when a certain part of PC being deacylated transforms into LPC. At their high concentrations there takes place an unavoidable development of membranotoxic and membranolytic effects. Thus, the above-mentioned increase of the level of acidic PL (APL) and total APL (TAPL), which is an original expression of compensatory-adaptation activity of the organism in a whole, can be interpreted as a reaction directed to the general support of cell respiratory function. Thus, the increase of TAPL contents in HIBM appeared to be more demonstrative in compare with the level of monodirected changes of the contents of as separate representatives of neutral PL (NPL), as well as

**Table 7. Changes in the Contents of Certain Categories of Phospholipids (µg lipid phosphorus/0,5 g dry residue of the studied material) in General Brain Homogenate (1), Mitochondrial (2), and Microsomal (3) Fractions of White Rat Brain in Control (4) and at Halothane Anaesthesia (5)**

INDICES	1		2		3	
	4	5	4	5	4	5
<b>LPC</b>	326,6±3,02	504,4±3,11	28,8±2,81	120,8±4,03	42,7±1,12	60,82±1,23
<b>SPM</b>	129,0±2,01	72,9±1,09 <sup>x</sup>	93,3±2,16	52,0±1,99	49,3±2,00	55,21±2,23
<b>PS</b>	105,8±2,00	122,0±2,32	47,1±3,11	84,1±3,03	26,5±1,13	22,82±1,63
<b>MPI</b>	246,8±3,07	191,5±2,22	142,0±3,09	29,0±2,02	95,0±2,03	74,80±2,91
<b>PC</b>	607,7±6,01	450,0±4,13	421,0±4,03	262,7±4,40	239,8±2,01	204,21±2,01
<b>PE</b>	553,8±3,00	493,0±3,01	320,0±2,23	301,4±1,73	152,0±2,00	140,02±1,97
<b>CL</b>	106,8±0,32	108,2±0,91	31,1±0,40	37,0±0,51	-	-
<b>TPL</b>	2076,5±6,02	1942,0±4,00	1083,3±6,04	887,0±5,00	605,3±2,04	557,88±3,04
<b>TNPL</b>	1617,1±6,09	1520,3±6,05	863,1±4,02	736,9±3,07	483,8±1,1	460,25±1,32
<b>TAPL</b>	459,4±3,69	421,7±4,00	220,2±4,05	150,1±4,16	121,5±1,16	97,62±1,22
<b>C TNPL /TAPL</b>	3,5	3,6	3,9	4,9	4,0	4,7

x-P&lt; 0,001; data without marks are unverified.

of total NPL (TNPL). Namely, it is testified by noticeable decrease of coefficient TNPL/TAPL (C TNPL/TAPL) defined at HIMM in the limits of 1,18 at HA influence, and 1,87 of control. Study of peculiarities of STS effect is a logic development of the investigations fulfilled.

As it is clear from Table 4, the saturation of organism with STS before HA influence is effective in reference to maintenance of peculiar to norm qualitative and quantitative contents of all the categories of PL in HEMM and HIMM.

The results obtained allow us to suppose that in the basis of protectory role of STS (as a metabolically rather active compound containing sulphur) are at least two main factors. The first one is an antioxidant property of STS promoting destructive influence of the substance on lipids peroxidation products formed, and the second one is the ability of STS to level the start of free radical chain reactions characterized by high level of peroxidation. In this aspect the statistic unverified shifts are of special interest. We define them in the studied problems of PL metabolism in IM, especially in HEMM and HIMM. It is reflected correspondingly in TPL, TNPL, TAPL, and C TNPL/TAPL.

The results of investigations given in Tables 5, testify at all the intensification of lipids FRO reactions in all the studied by us biological objects of hepatocytes under HA and levelling role of STS. According to data obtained HA influence is characterized by a strong increase of lipids peroxidation products level in HEMM, and HIMM in non-enzymatic, and enzymatic systems. Nevertheless, as at study of the dynamics of PL quantitative changes, as well as in this case STS normalizing effect appears more demonstratively at its preliminary administration to the organism, when HA anaesthesia is released on the background of certain conceptions of exogenous administration of the antioxidant. As it is demonstrated in Table 6, in this variety of the experiment HA is not accompanied by any noticeable shifts in the quantity of formed lipid peroxides in compare with control. In all the cases the mentioned shifts are statistically unverified. The above indicated data pave a way for the further detailed study of molecular mechanisms of HA toxic influence on organism of patients to be subjected to surgical treatment, as well as for the revelation of properties of STS wholesome effect.

#### HALOTHANE ANAESTHESIA IN MOLECULAR MECHANISM OF OXIDATIVE STRESS PATHOGENESIS

The enlarged use of different narcotic remedies in surgery leads at present to an increasing interest for the discovery and detailed study of peculiarities of molecular mechanisms origin, the development and generalisation of intracellular metabolism disturbances under their influence. On this point of view it is of a special interest the harmful effect of H on the PL metabolism and on the intensity of lipids FRO reaction at different level of cell structures organisation, which to some extent promotes the physiological level of cell activity.

Having close relation to the structural organisation and functional-metabolic peculiarities of the surfaces for cell and subcellular formations division, the state of separate PL fractions in normally functioning biological systems are characterised by status of phylogenetically strongly programmed constancy [110, 111] supplying in a whole the physiological level of the cell vital activity. In this respect the main issue for membrane PL is based on their maintaining the necessary level of either liquidity, fluidity of biological membranes formed due to the complex variety of PL-PL ratio, formation of numerous complexes creating unique character for lipid surrounding of membrane proteins, necessary level of hydrophobicity for the given biological system as an element of regulation of its functional activity. It concerns more certainly to the membrane-bound, lipid-dependent enzymes catalysing reactions of transmembrane transfer of substances, transduction of outer signal [112, 113], as well as the constant maintaining of physiological level for ligand - receptor interrelations, and normal stereotype of cell functioning in a whole [114, 115].

Thus, in the present part of work we have studied specific metabolic changes of PL [75] in general brain homogenate (GBH), MCF, and MSF of animals brain being under HA. It refers to the qualitative and quantitative changes of TPL, TNPL, TAPL, C TNPL/TAPL, separate fractions of NPL, such as LPC, SPM, PC, PE, as well as, APL - MPI, DPI, TPI, PS, phosphatidic acids, CL. PL extraction from the studied materials [75] has been made after their preliminary dehydration by acetone into acetone powder [116] with further fractionation by one-dimensional ascending thin layer chromatography in silicagel ("Merck", Germany) in the solvents system: chloroform-methanol-concentrated ammonia (65:35:5). PL quantity was expressed in  $\mu\text{g}$  of mineralised lipid phosphorus 0,5 g dry residue.

Simultaneously there were investigated in details the peculiarities of changes of lipids FRO reactions intensity, results of which promoted principally new determination of mechanisms for PL metabolism products participation as pathogenic factors depending on the nature of H toxic effects.

Investigations were carried out on white female rats weighing 180-200 g fed by general ration in vivarium experimental HA was caused by putting the animals into the special glass camera enriched with oxygen and H vapour (1,0-1,5 v%). Isolation of brain MCF (BMCF) and brain MSF (BMSF) was made in medium containing 0,25 M saccharose solution and 0,01 M, Tris-HCl buffer solution using method of differential centrifugation and simultaneous determination of general protein concentration.

Measurement of intensity of the developing staining of MDA in reaction with thiobarbituric acid and hydroperoxides with ammonium sulphocyanide of adequate concentrations of the mentioned compounds were made on spectrophotometer SP-26 at wave length 535 nm and 480 nm, respectively.

According to the results given in the Table 7, the experimental HA at white rats is characterized by statistically verified changes of PL-PL ratio in GBH, BMCF, and BMSF dependent on qualitative and quantitative changes of the contents of individual representatives of PL.

The most acknowledged one is the decrease of PC quantity induced, perhaps, by the increase of phospholipase A<sub>2</sub> activity, which is testified by simultaneous output of high concentrations of LPC and NEFA of polyenic range, actively involved in FRO reactions, and promoting the formation of certain quantity of lipid peroxides which possess membranotoxic effect.

The latter is considered at present as one of the main consistent of CNS complex pathogenic diseases (up to acute BE) [104, 113]. On the other hand the decrease of PC was observed with parallel increase of PS in a result of probable carboxylation of PE. The formation of the latter from PC is possible as a result of activation of demethylation processes of PC.

The probability of the supposed mechanism of PS formation is apparent on our point of view, as under H toxic effect it obtains a real compensatory-adaptation character involving PS as stimulators for inhibited respiratory function of brain BMCF.

It is of no less interest the lowering of SPM in GBH and BMCF, which is interpreted by us as a free response to the reactivity of myelin substance of brain tissue to H toxic effect.

In affirmation to the abovementioned ones it attracts our attention to the fact of quantitative increase of CL in MCF. The formers are considered the main representatives of APL participating in the catalyzing reactions of the cell respiratory function, especially at its inhibition.

At last, the importance of the mentioned PL (including MPI) under HA, probably, is of more wide significance, namely MPI and PC cycles are of the primary significance in transduction of outer signal into the cell, thus in supplying its activity in a the cell membrane [117], which, perhaps, undergoes certain shifts under the effect of the studied anaesthetic remedy.

Interfractional changes of PL in GBH and intracellular brain formations under H effect defined by us, having various appearances, elucidate the understanding and interpretation of the obtained C TNPL/TAPL. It is interesting to note that in spite of the developing shifts of TAPL to the incrementation, their level in TPL is statistically verified for the decrease only in BMSF, especially in BMCF, but not in GBH, hence being less informative in the last case [58, 118]. Simultaneously the process of peroxide formation as in GBH, as well as in brain BMCF and BMSF according to table 8 is noticeably activated, especially in non-enzymatic (ascorbate-dependent) system of lipids peroxidation.

It must be noted that comparatively high level of HP output is an objective index of high rate of peroxide formation process, which at a known extent is a verification of unique development and generalization of disease process. We must note also that the described regularity in the dynamics of quantitative changes of HP and MDA in more expressed forms were observed in brain tissue BMCF and BMSF, and to some extent in GBH. Activation of processes of radical formation (which is compulsory, but it is not a specific index for pathogenic complex of different states of disease in the organism, including the states initiated at toxic effects of anaesthetic compounds) is accompanied both by PL deacylation with the release of NEFA involved in FRO reactions, and by sensitive changes of intermolecular fatty acid contents of PL, causing cardinal changes of their physical, chemical, functional and metabolic

**Table 8.** Changes in the Contents of Hydroperoxides (e<sub>480</sub>/mg protein) and Malonic Dialdehyde (nm/mg protein) of General Brain Homogenate, Mitochondrial and Microsomal Fractions in White Rat Brain in Non-enzymatic (1) and Enzymatic (2) Systems of Lipid Peroxidation in Control (C) and Halothane Anaesthesia

INDICES	Control (C)		Halothane anaesthesia			
	1	2	1	% Changes from C	2	% Changes from C
<b>MITOCHONDRIAL FRACTION</b>						
HP	0,75±0,03	0,51±0,03	1,25±0,05 <sup>x</sup>	67,0	0,99±0,05 <sup>x</sup>	94,0
MDA	4,21±0,09	2,02±0,07	6,42±0,21 <sup>x</sup>	52,5	4,67±0,06 <sup>x</sup>	131,0
<b>MICROSOMAL FRACTION</b>						
HP	0,55±0,04	0,31±0,03	0,73±0,03 <sup>x</sup>	33,0	0,58±0,02 <sup>x</sup>	90,0
MDA	2,07±0,07	1,42±0,08	3,04±0,17 <sup>x</sup>	47,0	2,07±0,11 <sup>x</sup>	46,0
<b>GENERAL BRAIN HOMOGENATE</b>						
HP	0,42±0,06	0,29±0,05	0,65±0,03 <sup>x</sup>	55,0	0,49±0,04 <sup>x</sup>	68,0
MDA	1,66±0,02	0,81±0,07	1,86±0,02 <sup>x</sup>	12,0	1,04±0,02 <sup>xx</sup>	29,1

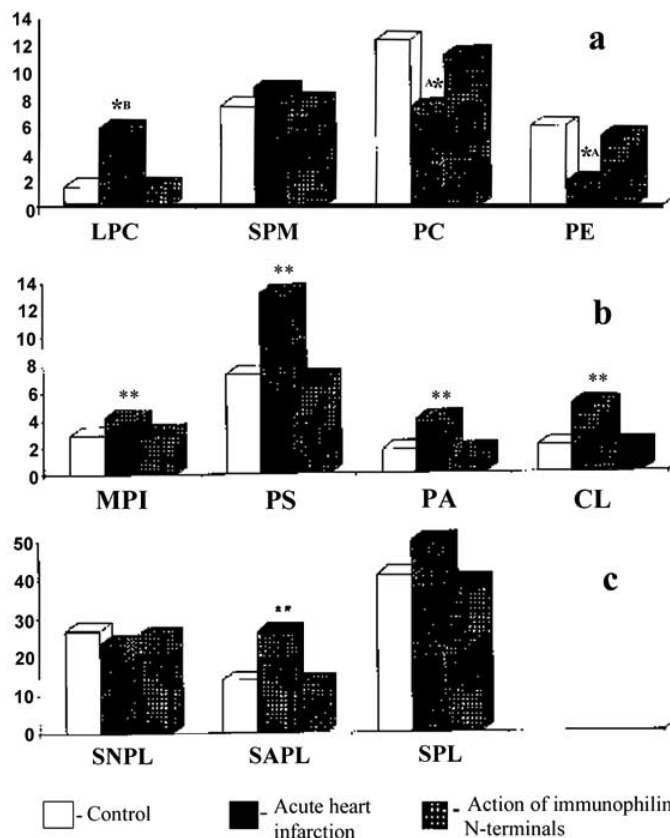
x-P,0,001; xx-P<0,002; data without marks are unverified.

peculiarities, which will become the subject of our further special investigation.

**ROLE OF SYNTHETIC N-TERMINALS OF BRAIN IMMUNOPHILIN IN NORMALIZATION OF LYSPHOSPHATIDYLCHOLINES METABOLISM IN BLOOD LYMPHOCYTE MEMBRANES OF RATS WITH EXPERIMENTAL ACUTE HEART INFARCTION**

Membranes of blood erythrocytes and lymphocytes of the rats with modulated acute heart infarction were characterized with the significant changes in phylogenetically stabilized PL-PL interrelations. It is well known that abnormalities mentioned play an important negative role in the

development of pathogenic mechanisms, which condition inactivation of the membrane-bound enzymatic systems, catalyzing the reactions transport through membranes, as well as transduction of the external signals into the cell. During the incubation of the indicated membranes for 1,5 h in the presence of water-soluble synthetic peptide fragments corresponding to the residues 1-9 and 1-15 of the N-terminus of immunophilin (IPh) the effect on the PL composition and intensity of free radical peroxidation processes have been examined. It was shown that significant and dose-dependent decreases in the content of PC, and simultaneous increase of LPC, PE and PS concentrations were normalized at the end of incubation period. Similar changes were registered in the quantity of lipid peroxidation products. The results are discussed in terms of the possible effects of IPh on modulating PL turnover in blood



**Fig. (9).** Effect of 1, 5 and 10 µm of synthetic 1-9 and 1-15 N-terminals of immunophilin (average data) on quantitative changes of individual fractions of NPL (a), APL (b) and SNPL, SAPL, SPL (c) (in µg of lipid phosphorus/g wet material) in rat blood EM in control, acute heart infarction and after 1.5 h incubation in presence of the N-terminals immunophilin.

erythrocyte and lymphocyte membranes, significantly changed under condition of the experimental acute heart infarction.

**Table 9.** Effect of 1, 5 and 10  $\mu\text{m}$  of Synthetic 1-9 and 1-15 N-Terminals of Immunophilin (average data) on Coefficient SNPL/SAPL in Erythrocyte and Lymphocyte Membranes in Control, Acute Heart Infarction and after 1,5 h Incubation of the Latter in the Presence of N-Terminals Used

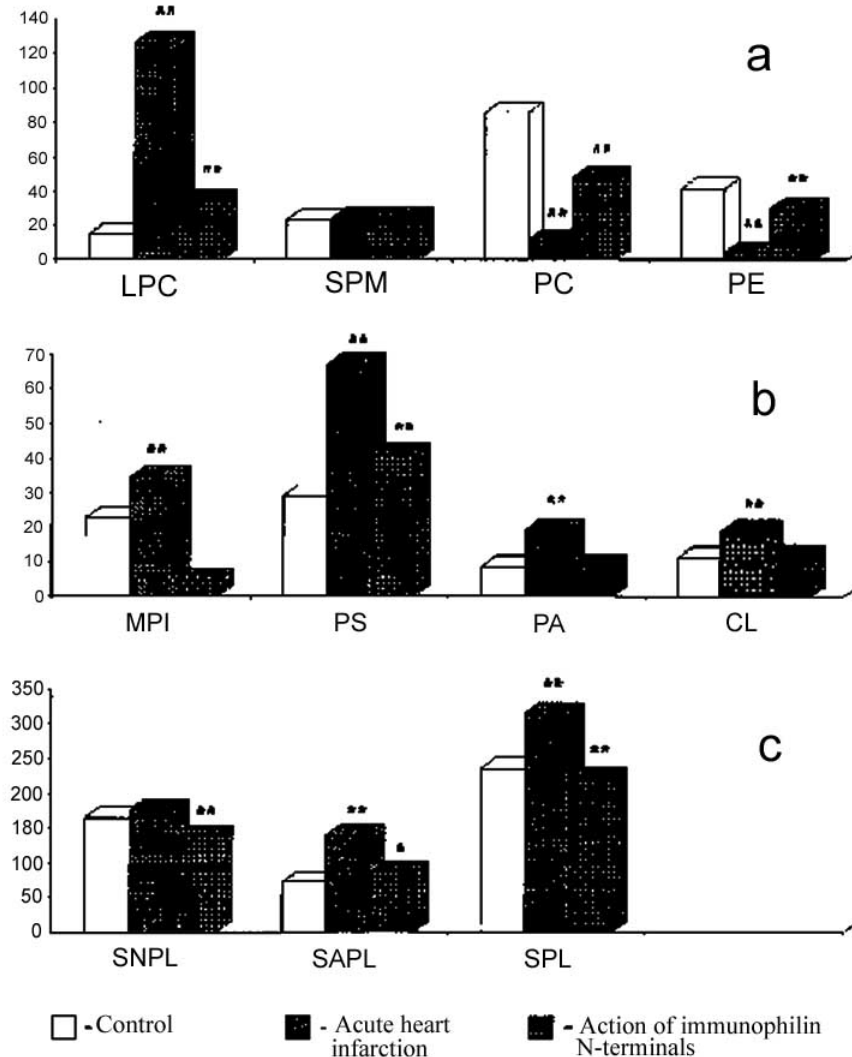
INDICES	CONTROL	HEART ACUTE INFARCTION	EFFECTS OF N-TERMINALS
EM	1,93 $\pm$ 0,01	0,89 $\pm$ 0,01	1,87 $\pm$ 0,01
LM	2,30 $\pm$ 0,01	1,26 $\pm$ 0,01	1,58 $\pm$ 0,01

Iph are proteins, which bind immunosuppressive drugs such as FK506, rapamycin and cyclosporin A [119, 120]. They are present in all cell types, in which they have been sought, and involvement in a number of immunological, endocrinological and chaperone-mediated pathways have been suggested [16, 121, 122]. Multifunctional role of IPh is consistent with sequence of hydrophobic cluster analysis, which indicate that FK506 and calmodulin binding and peptidyl-prolyl *cis-trans* isomerase activity, believed to be associated with protein folding, are all found within the IPh molecule [123]. The FK506 binding site and isomerise activity domains are located at the N-terminus [119]. The domains appear to be close to a nucleotide binding domain but remote from the calmodulin binding consensus sequence located in the C-terminal part of the molecule.

In 1992 a receptor of immunosuppressor FK506 (FK 506 BP) possessing peptidyl-prolyl *cis-trans* isomerase activity in the bovine hypothalamus was discovered [124]. The primary structure was determined by mass-spectral analysis and Edman degradation method [125]. Taking into account the existence of IPh in the neurosecretory parts of hypothalamus A.Galoyan suggests that multiple forms of PPIase and particularly IPh are synthesized by the hypothalamic neurosecretory nuclei and play a role in the protein folding as well as can take part in the neuroendocrine regulation. Recently in his laboratory the existence of IPh in the nucleus Supraopticus and nucleus Paraventricularis was demonstrated. Authors demonstrated the influence of IPh fragment (1-15) on the energetic metabolism. It was interesting to study the effect of this fragment on metabolism of PL. The 1-15 fragment was synthesized by solid-phase synthesis using Fmoc amino acid derivatives in A.Galoyan's Moscow laboratory.

They succeeded to prepare peptides with sequences corresponding to amino acids 1-9 and 1-15 of the N-terminus of the bovine hypothalamic IPh.

This present paper describes the effect of peptide fragments 1-9 and 1-15 on the metabolism of PL in rat brain and blood corpuscles [126-127]. It has been shown recently IPh to modulate phosphorylation of growth-associated protein-43 in brain [128] *via* its interaction with calcineurin therapy regulating neuronal regeneration. We have investigated the changes in PL composition of rat brain slices incubated in the presence of the N-terminal peptides of IPh to investigate the role of membrane lipid turnover in the action of IPh. We report marked changes in particular PL



**Fig. (10).** Effect of 1, 5 and 10  $\mu\text{m}$  of synthetic 1-9 and 1-15 N-terminals of immunophilin (average data) on quantitative changes of individual fractions of NPL (a), APL (b) and SNPL, SAPL, SPL (c) (in  $\mu\text{g}$  of lipid phosphorus/g dried residue of LM) in rat blood LM in control, acute heart infarction and after 1.5 h incubation in presence of the N-terminals immunophilin.

classes isolated from the tissue, which are related to the concentration of peptide in the incubation medium.

## EXPERIMENTAL PROCEDURES

Preparations of EM and lymphocyte membranes (LM) were obtained from corresponding blood elements of the mongrel male rats (180-200g). They were fixed on a special bench for small animals and were divided into two groups (control and experimental) by 12 animals in series of investigation. Blood samples in volume 2 ml were taken under light ether anaesthesia from angulus venosus (confluence of vena cava superior and vena subclavia) and stabilized by mixing it with 3,8% sol. of sodium citrate in correlation 9:1. Erythrocytes were sedimented by 10 min centrifugation at 2000 g, and then they were stored in refrigerator at 4°C for 24 h in the hypotonic solution of sodium chloride to obtain the EM by the osmotic shock. EM (or ghosts) was isolated by high-speed centrifugation at 10000 g for a 20 min. The sedimented EM was used for PL assay [74, 75]. Blood lymphocytes were isolated by centrifugation in ficol-400-verografin gradient density [22] and used in quantity 10<sup>7</sup> ml for preincubation at 37°C in 0,01 M solution of Tris-HCl buffer (pH 7,4) in the presence of medium 199 (1:4) and mitogen concanavaline A (6 µg/ml). LM were centrifuged and used for PL determination.

## RESULTS AND DISCUSSION

As one can see in Fig. (9) the 1,5 h incubation of control EM in the presence of 1,0-5,0-10,0 µm of 1-9 and 1-15 synthetic N-terminal peptides of IPh is accompanied with significant changes of qualitative and quantitative composition of membrane-bound PL of EM. The presence of N-terminal peptide fragments in the incubation medium in the mentioned concentrations induced noticeably significant and dose-dependent changes in the pattern of all PL categories.

The magnitude of the change in the amount of each PL class was 1-9 fragment equivalent concentrations. According to the data obtained decreasing of PC concentration, which is more demonstrative in LM, is accompanied with the simultaneous increasing of LPC quantity. The level of the latter in LM was also more pronounced. The changes mentioned were characterized by the parallel decrease of PE level. The metabolic perturbances mentioned in the dynamics of NPL affect the SNPL and are characterized by the opposite changes in the APL and their SAPL, as well as influence the rate of C SNPL/SAPL (Table 9). The obtained data indicate increasing of PS quantity, which may be explained by the activation in the studied membranes of the PS-decarboxylase, which catalyses the transformation of PE into PS. That is the reason for registration always the decrease of PE concentration. At the same time it is not excluded that deacylation of the main quantity of PC (certain part of PC) is involved in demethylation processes. Due to the latter the PC is partly transformed into PE, which is actively included into the abovementioned processes of PE carboxylation leading to the formation of high quantities of PS.

As it was mentioned already these changes have more incomparable intensive development in LM than in EM. The results of our investigations have demonstrated that the synthetic N-terminals of IPh used lead to the normalization of PL-PL interrelation in EM and LM. According to the data reflected in the Fig. (9) and Fig. (10) IPh fragments 1-9 and 1-15 in doses used (average data) lead to the loosening of qualitative and quantitative abnormalities of all classes of PL especially in EM. More demonstrative changes were registered when 1-15 synthetic N-terminal of IPh was used. In this case all disorders in PL metabolism of EM become normalized, and the differences between control and experimental samples were found statistically reliable. At the same time, under the same mentioned conditions the tendency to the correction of PL-PL interrelation in LM has been registered too.

However, it concerns to numerous individual representatives of NPL and APL except PC, PE, FS and LPC. The level of these categories of PL was in the definite limits, which thoroughly differed from the control indices. Similarly on the background of normalized qualitative and quantitative composition of main count of LM-bound PL the quantity of PC and PE was decreased while the level of LPC and PS was simultaneously increased. At the same time as one can observe on the table, the rate of PL-PL in EM does not demonstrate the statistically reliable differences in compare with control data. On the other hand it is interesting that in LM even under the action of N-terminals studied the coefficient of SNPL/SAPL continues to remain lower than in control.

This phenomenon is conditioned by high concentrations of APL in compare with NPL in the whole sum of all PL, and confirms the results of

our previous investigations [4, 14, 104, 117], which have demonstrated the role of PL deacylation and reacylation processes at initiation, development and generalization of immunological reactions in different links of the whole system, particularly in LM. According to the recent scientific information [129-136] LPC play an important role in the regulation of cell activity, and especially in conditioning the whole immune arrangement and immune stabilisation of the organism. At the same time it is known that the concentration of PS in LM of the infarcted rats has been supported by physiologically active synthetic peptides used in the ranges statistically higher of the control indices. These data would be interpreted as biologically very important reactions, when the organism uses high concentrations of PS for additional stimulation of PS-dependent enzymatic systems catalysing energy formatting reactions, which are of high significance in realization of the immune reactions as well.

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## ABBREVIATIONS

α-T	=	alfa tocopherol
APL	=	acidic phospholipids
BBB	=	blood-brain barrier
BE	=	brain edema
BMCF	=	brain mitochondrial fraction
BMSF	=	brain microsomal fraction
CL	=	cardiolipins
CNS	=	central nervous system
C TNPL/ TAPL	=	coefficient TNPL/TAPL
DBM	=	dopamine-β-monooxygenase
EM	=	erythrocyte membranes
FA	=	fatty acids
FMF	=	Familial Mediterranean Fever
FRO	=	free radical oxidation
GBH	=	general brain homogenate
GTP	=	guanosine triphosphate
H	=	halothane
HA	=	halothane anaesthesia
HEMM	=	hepatocytes external mitochondrial membrane
HIMM	=	hepatocytes inner mitochondrial membrane
HP	=	hydroperoxides
IPh	=	immunophilin
IR	=	infrared
KSK	=	type of silicagel
LI	=	laser irradiation
LM	=	lymphocyte membranes
LMCF	=	liver mitochondrial fraction
LMSF	=	liver microsomal fraction
LP	=	lipid peroxidation
LPC	=	lysophosphatidylcholines
MCF	=	mitochondrial fraction
MDA	=	malonic dialdehyde
MPI	=	monophosphoinositides
MSF	=	microsomal fraction
NEFA	=	non-etherified fatty acids
NPL	=	neutral phospholipids
OS	=	oxidative stress
PC	=	phosphatidylcholines
PE	=	phosphatidylethanolamines
PI	=	phosphatidylinositols
PL	=	phospholipids

PS	=	phosphatidylserines
SPM	=	sphingomyelins
SOD	=	superoxide dismutase
SPM	=	sphingomyelins
STS	=	sodium thiosulfate
TAPL	=	total acidic phospholipids
TNPL	=	total neutral phospholipids
TPI, DPI, and MPI	=	tri-, di- and monophosphoinositides

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