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State of Liver Mitochondrial Respiratory Chain in Rats with Experimental Toxic Hepatitis

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Abstract—Polarographical determination of oxygen concentration has shown that in rats with experimental hepatitis induced by combined ethanol and CCl_4 administration for 4 weeks, the functioning of the hepatocyte mitochondrial respiratory chain is impaired. Development of liver pathology was accompanied by adipose dystrophy, fibrosis, and an increase of triglycerides and lipid peroxidation products in the liver tissue. The endogenous respiration rate in hepatocytes isolated from the pathologically altered liver was 34% higher than in the control. Cell respiration was not stimulated by the addition of the substrates malate and pyruvate with digitonine. An uncoupler of oxidation and phosphorylation, 2,4-dinitrophenol, increased the hepatocyte oxygen consumption rate by 37%, while addition of the inhibitor of the I complex, rotenone, decreased cell respiration in pathologically altered hepatocytes by 27%. The states 3 (V_3) and 4 (V_4) of mitochondrial respiration with malate + glutamate as substrates were found to be higher by 70% and 56%, respectively, as compared with the control level. When using malate + glutamate or succinate as substrates, V_3 and Vd (dinitrophenol respiration) in the toxic hepatitis hepatocyte mitochondria did not differ from the control, which indicates no uncoupling occurred of the oxidation and phosphorylation processes. Cytochrome *c* oxidase activity was elevated (+80%) as compared with the control. Administration of the hypolipidemic agent symvastatin simultaneously with ethanol and CCl_4 resulted in a reduction of the degree of liver adipose dystrophy, prevented activation of lipid peroxidation, and decreased the hepatocyte endogenous respiration rate. Addition of malate + pyruvate, dinitrophenol or rotenone produced oxygen consumption changes similar to those in the control. However, in mitochondria isolated from the pathologically altered liver, symvastatin induced an uncoupling effect on the respiratory chain in the presence of the substrates malate + glutamate, but did not change the cytochrome *c* oxidase activity. We suggest that functioning of the NCCR complex in the hepatocyte mitochondria of animals with experimental toxic hepatitis is impaired, which leads to an intensive superoxide anion production at the level of this complex. Under these conditions, the defect of the NADH-coenzyme Q-oxidoreductase is compensated by functioning of other complexes of the respiratory chain (SCCR, coenzyme Q-cytochrome *c*-reductase, cytochrome c oxidase, and ATP-synthase activities).

Key words: hepatitis, hepatocytes, mitochondria, respiratory chain, lipid peroxidation, symvastatin.

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Abbreviations: MTT—3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyltetrazolium bromide, LPO—lipid peroxidation, NCCR-NADH-coenzyme Q-oxidoreductase, SSCR—syccinate-coenzyme Q-oxidoreductase.

INTRODUCTION

Liver diseases are characterized by one of the highest mortalities in the general population. The most severe of these diseases are hepatitis of various etiologies and alcoholic liver disease, two pathologies often complicated by liver cirrhosis. Recently, an increasing incidence of mixed hepatitises and liver cirrhoses has been recorded (Bosch et al., 1994; Lieber, 1994; Andreeva et al., 1996).

Liver pathologies, especially those associated with ischemic disturbances, are accompanied by essential alterations in the functional state of the mitochondrial respiratory chain (Krähenbühl and Reichen, 1992). The basis of the respiratory chain is an assembly of electron carriers inserted in the internal membrane of these organelles. The carriers are arranged into 4 complexes and include NADH-coenzyme Q-oxidoreductase, succinate-coenzyme Q-oxidoreductase, coenzyme Q-cytochrome *c*-oxidoreductase, and cytochrome *c* oxidase. Synthesis of ATP is provided by ATP-synthase localized in the internal mitochondrial membrane in close proximity to the electron transport chain (Cross, 1994; Boyer, 1997). Data on the state of the mitochondrial respiratory complexes during liver pathology are rather

controversial. Some authors believe that electron transport by the first and second respiratory chain complexes are disturbed in liver pathology (Krähenbühl et al., 1989; Nozu et al., 1992), whereas others consider SCCR to be the least vulnerable complex, which is not impaired, even in liver cirrhosis (Cederbaum et al., 1974; Bottnus et al., 1982; Yang et al., 2004). Controversy about the data reported by various authors and the importance of this problem stimulate the performance of additional studies of the hepatocyte mitochondrial respiratory chain during liver pathology.

Most medications used in the therapy of various diseases are hepatotoxic. This is due to the liver's detoxicational function responsible for metabolism of xenobiotics entering the human organism (Sherlock, Dooley, 1999). Many toxins, including CCl_4 and alcohol, as well as medicinal drugs, activate the processes of formation of free radicals and thereby enhance liver lesion (Britton and Bacon, 1994). Earlier we used a model of complex CCl_4 and ethanol toxic liver damage and demonstrated the use of the hypolipidemic drug symvastatin simultaneously with toxins to decrease the degree of liver adipose dystrophy and blood cholesterol level (Okovityi et al., 2004). Symvastatin is an inhibitor of 3-hydroxy-3-methyl glutaryl coenzyme A reductase; this is an enzyme participating in cholesterol formation in liver, which also has an antioxidant action (Lyakishev, 2001). Free radical oxidation is enhanced in pathology and can be one of the mechanisms of damage of the hepatocyte mitochondrial respiratory chain. From this point of view it would be interesting verify the possibility of correction by this agent, which is characterized by hypolipidemic antioxidant activities, of lesions of the hepatocyte mitochondrial electron transport chain during the development of toxic hepatitis.

The goal of our work was to perform studies in isolated hepatocytes and mitochondria of: (1) the state of hepatocyte mitochondrial respiratory chain in rats with experimental toxic hepatitis, and (2) possible correction by symvastatin of disturbances in the activity of the hepatocyte mitochondrial electron-transport chain.

MATERIALS AND METHODS

Experimental animals and modeling of toxic hepatitis. The study was carried out on 36 Wistar male rats weighing 180–200 g at the beginning of experiment. The animals were kept under standard conditions of light regime and of food ration. All animals were divided into 4 groups. The 1st group was the control (intact rats). Rats of the 2nd and 3rd groups were used for modeling experimental toxic hepatitis (Strubelt et al., 1978; Vengerovskii, 2000); for this, 50% CCl_4 in vaseline oil at a dose of 0.2 ml/kg was administered into the stomach twice a week for one month. These animals were given 5% ethanol in their drinking water. Rats of the 3rd group also were administered a water suspension of symvastatin at a dose of 1 mg/kg intragastrically. The effect of the drug itself (the control for the drug only) was assayed in the 4th group of animals that were administered symvastatin by the same scheme as the rats with liver pathology. Two days after the last CCl_4 administration the animals were sacrificed under thiopental anesthesia.

Isolation of hepatocytes from normal and pathologically altered rat liver. Isolated rat hepatocytes were obtained by the modified Seglen's method (Seglen, 1976). Liver was perfused in situ for 5 min via the portal vein with a buffer (pH 7.4) containing 8.3 g/l NaCl, 0.5 g/l KCl, 2.4 g/l HEPES, 2 g/l glucose, and 5.5 ml/l of 1 M NaOH and heated to 37°C. Then liver was removed, placed into a sterile Petri dish, and minced into pieces of about 1 mm^3. After washing with Eagle's medium supplemented with 0.1% glucose, 0.01 M HEPES, 1% fetal calf serum, and 40 μg/ml gentamicin, the pieces were placed into 0.5% collagenase IV (Sigma, USA) in Eagle's medium with 0.1 M HEPES (pH 7.6). After 5 min of incubation in the enzyme solution at 37°C with constant shaking, supernatant was collected and the procedure was repeated two more times while increasing its duration to 20 min and adding fresh enzyme. The pooled hepatocyte suspension was filtered through a sterile nylon filter and the cells were washed three times with Eagle's medium and were sedimented by centrifugation at 20 g. Viability of hepatocytes was estimated by staining with trypan blue, acridine orange, and ethidium bromide as well as using the MTT test (Mossman, 1983). Hepatocyte viability varied from 70% to 90% in various experiments. The number of isolated hepatocytes in the suspension was determined by counting cells in Goryaev's chamber.

Isolation of mitochondria from hepatocytes. Mitochondria were isolated by the method of differentiated centrifugation (Jonson and Lardy, 1969) with small modifications. The liver pieces were homogenized with a hand homogenizer at 0°C in medium 1 containing (mM): 250 sucrose (without Ca^{2+}), 3 Tris, and 0.5 EGTA (pH 7.3). The homogenate was centrifuged for 5 min at 700 g and 0°C. The obtained supernatant was centrifuged for 10 min at 7000 g and 0°C in medium 2 containing 250 mM sucrose (without Ca^{2+}), 3 mM Tris, at pH 7.3. The obtained mitochondrial pellet was washed twice in medium 2 and mitochondria were sedimented by centrifugation at 7000 g. The protein concentration was determined the method of Bradford (1976).

Determination of hepatocyte dry mass. The dry mass of hepatocytes was determined by the method of interference microscopy (Barer, 1953; Barer and Joseph, 1954) using an MBIN-4 microscope with objective 40×0.65. The principle of the method consists of measuring the ray pathway difference introduced by the studied object. From the pathway difference and the area of the object, the total content (mass) of dry substances in the object can be found by the formula:

$$M = \frac{\delta S}{100\alpha}, \quad \delta = \frac{(l_1 - l_2)\lambda}{180};$$

where M—mass of the cell (g), δ—difference of the ray pathway (cm), S—area of the cell (cm^2), α—specific increment of the object refractive index (for glycerol, it amounts to 0.00095 $sm^3\ g^{-1}$), l_1—the first angle of analyzer (grad), l_2—the second angle of analyzer (grad), λ—the light wavelength (nm).

The cell area S was determined in a VideoTest image analyzer by using the Razmer program. In each preparation, 100 cells were measured.

Preparation of histological sections and smears of isolated hepatocytes. Histological sections were prepared by the standard procedure (Roskin, 1957). Rat liver pieces were fixed in 10% formalin and embedded in paraffin blocks, from which 5 μm thick sections were cut on a microtome. The sections were stained with hematoxylin-eosin or Van Gieson picrofuchsin (Pearce, 1962).

Preparations for interferometry were made on standard object glasses as smears of isolated hepatocytes by the method described earlier (Kudryavtseva et al., 1970). For this purpose, the minced liver pieces were placed for 15 min into a mixture of equal volumes of 0.067 M K,Na-phosphate buffer, pH 8.0, and 5% sucrose. Then the pieces were transferred to 0.067 M K,Na-phosphate buffer, pH 7.4, and kept there for 8 min; after that, cell smears from the liver pieces were prepared and fixed with methanol.

Evaluation of the state of mitochondrial respiratory chain. The mitochondrial respiratory chain was studied by the polarographic method (Chance and Williams, 1956; Estabrook, 1967). The functional states of NADH-coenzyme Q-oxidoreductase (I complex), SCCR and coenzyme Q-cytochrome *c*-oxidoreductase (III complex) were evaluated with special substrates and inhibitors of these complexes by using the following parameters for the work on isolated mitochondria:

• V_4 (a high content in the incubation medium of the following substrates—5 mM glutamate and 5 mM malate (substrates of the I complex) or 5 mM succinate (substrate of the II complex) in the absence of ADP;

• V_3 (the same conditions as at the V_4 determination, but in the presence of 200 μM ADP, the factor limiting the reaction rate being the respiratory chain itself);

• Vd (the same conditions as during the V_4 determination, but in the presence of the 30 μM 2,4-dinitrophenol, an uncoupler of oxidation and phosphorylation).

As well, the respiratory control representing the V_3/V_4 ratio was estimated.

When studying the functional state of the mitochondrial respiratory chain complexes in isolated hepatocytes, other parameters were used:

• endogenous respiration V_0 (the rate of oxygen consumption by cells on endogenous substrates);

• respiration on exogenous substrates (5 mM glutamate and 5 mM malate or 5 mM succinate with digitonine);

• respiration upon the action on cells of 1 μM rotenone (an inhibitor of the I complex) and 30 μM 2,4-dinitrophenol.

Determination of cytochrome *c* oxidase. A mitochondrial suspension (1 mg protein/ml) was introduced into the incubation medium containing 5 mM ascorbic acid (a donor of electrons), 0.2 mM Na_2EDTA, as well as 10 μM cytochrome *c* for elimination of the reaction rate limitation due to its deficit. Then 120 μM tetramethylparaphenylenediamine (an electron carrier, with ascorbic acid, to the cytochrome system) was added to the reaction mixture, with measurement of the oxygen consumption rate (Estabrook, 1967). Based on the obtained data, the cytochome *c* oxidase activity was calculated by the method of Lineweaver-Burk.

Determination of LPO parameters. The intensity of lipid peroxidation was studied by measuring the concentration of diene conjugates and Schiff bases in liver homogenate (Bidlack and Dyel, 1959; Stalnaya, 1977).

Determination of triglyceride level. The triglyceride level in the rat liver was measured by the method of Gottfried and Rosenberg (1973).

Statistical treatment of the obtained data. During statistical treatment of data, the means and the mean errors were calculated. Statistical significance of the means was evaluated by using Student's *t* criterion. The differences were considered statistically significant at the confidence probability of 0.95. The calculations were performed using Statistica 6.0 program.

RESULTS

Histological characteristics of the liver in experimental toxic hepatitis. The combined toxic action of alcohol and CCl_4 leads to the appearance in rat liver of signs of inflammation, adipose degeneration, and fibrosis. The organ's lobular structure is disturbed. Liver parenchyma is infiltrated with connective tissue strands (Fig. 1a). Vascular walls are thickened and fibrosed (Fig. 2a). In the liver parenchyma, especially near vessels, activated Kupffer's cells and leucocytic infiltrates are seen (Fig. 2a). The damaged liver parenchyma is regenerating, which is confirmed by hepatocyte mitoses often being present that are practically absent under normal conditions (Fig. 2b).

In the liver parenchyma of the rats that simultaneously with toxic action were given the hypolipidemic agent symvastatin, the adipose dystrophy was pronounced to a significantly lesser degree; however, symvastatin use did not affect the degree of parenchyma fibrosis and the activity of inflammatory processes (Fig. 1b).

Morphology of isolated hepatocytes of normal and pathologically altered liver. Isolated hepatocytes of the normal rat liver had a regular rounded shape with centrally located nucleus, clearly outlined plasma membrane, and a large amount of cytoplasm. Isolated hepatocytes of the pathologically altered liver had sig-

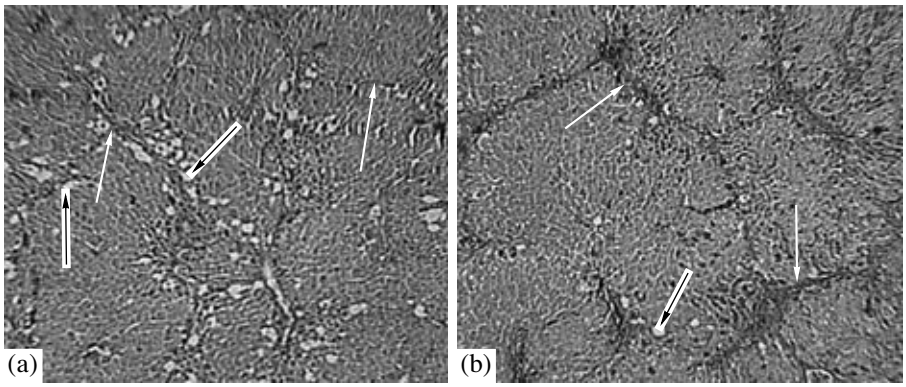

Fig. 1. Histological sections of liver sections in experimental toxic hepatitis.
(a)—hepatitis, (b)—the same under conditions of use of symvastatin. Van Gieson staining. *Black arrows*—lipid droplets, *white arrows*—connective tissue bands. Objective 6.3 × 0.12.

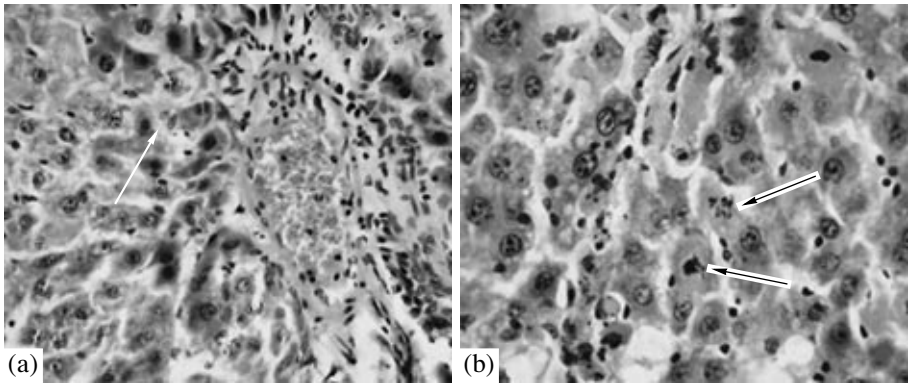

Fig. 2. Histological sections of liver sections in experimental toxic hepatitis.
Hematoxylin-eosin staining. Objective 25 × 0.5 (a), 40 × 0.65 (b). *Black arrows*—mitoses, *white arrows*—Kupffer's cells.

nificantly more diverse sizes; very large cells with large polyploid nuclei were revealed. The cytoplasm often contained vacuoles, the plasma membrane had numerous outgrowths. The percent of intact hepatocytes after their isolation, both from normal and from pathologically changed liver, varied in different experiments from 70% to 90%.

Determination of triglycerides in pathologically altered rat liver. Development of experimental toxic hepatitis in rats was accompanied by an increase of triglycerides (Fig. 3). Use of symvastatin produced a statistically significant decrease of the triglyceride level (Fig. 3). The symvastatin administration induced a statistically significant decrease of the triglyceride level in liver of rats of the 3rd group as compared with group 2 ($p < 0.02$) and in group 4 as compared with group 1 ($p < 0.05$).

Measurement of the liver cell dry mass. In the rats treated with CCl_4 and ethanol, significant hepatocyte hypertrophy developed, which was indicated by an almost two-fold increase of the mean dry mass of these cells (Table 1). Under the use of symvastatin, this hepatocyte hypertrophy was practically absent.

State of the mitochondrial respiratory chain in isolated hepatocytes. Results of measurements of the oxygen consumption rate by isolated hepatocytes of the 1st (control), 2nd (toxic hepatitis), and 3rd (toxic hepatitis + symvastatin) rat groups are presented in Table 2.

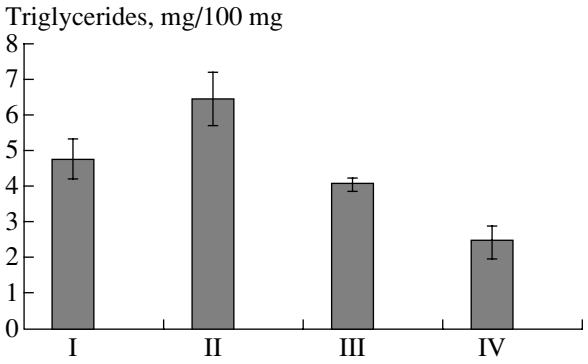

Fig. 3. Content of triglycerides (M ± SEM) in the liver of control rats (I), in rats with toxic hepatitis (II) with the use of symvastatin in a background of development of pathology (III) and without it (IV).

Table 1. Results of measurement of hepatocyte dry mass

	Control	Hepatitis	Hepatitis + + symvastatin
Dry mass, pg	1422 ± 34	2271 ± 160*	1743 ± 170**
Number of rats	5	6	5

Note: * $p < 0.01$ relative to control, ** $p < 0.05$ relative to the group of hepatitis, $M \pm m$.

It was observed that the hepatocyte endogenous respiration rate in the 2nd group was higher by 34% than in control.

Addition of substrates malate and pyruvate with digitonine did not stimulate cell respiration. The 2,4-Dinitrophenol increased the hepatocyte respiration by 37% as compared with the cell endogenous respiration. The presence of rotenone in the isolated hepatocyte suspension of the 2nd group animals decreased the oxygen consumption rate by 27% as compared with respiration on malate and private. This decrease was twice lower than in the control group (–59%).

The hepatocyte endogenous respiration rate in rats of the 3rd group was lower by 30% than in the control and lower by 48% than the hepatocyte endogenous respiration in the 2nd group animals (Table 2).

Unlike the case for the hepatocytes of rats of the 2nd group, addition of malate and pyruvate with digitonine to the hepatocyte suspension of the 3rd group animals increased the cell respiration by 56% relative to endogenous respiration. Addition of 2,4-dinitrophenol into the hepatocyte suspension of the 3rd group increased respiration by 44%. The relative respiration in this case practically did not differ from the response to 2,4-dinitrophenol in animals of the 2nd group. Thus, by the degree of coupling of oxidation and phosphorylation, no differences between the hepatocytes of the three rat groups were revealed.

The hepatocyte respiration rate in the 3rd rat group in response to addition of rotenone decreased by 45% as compared with respiration using malate and pyruvate. This response was statistically significantly more pronounced than in the second group, but lower than in the control group. This could indicate a less pronounced lesion of the respiratory chain first site in the presence of symvastatin.

State of the respiratory chain of rat liver isolated mitochondria. The cell respiration (the rate of oxygen consumption by cell) could depend on such unspecific factors as membrane permeability, activity of lysosomes, etc. To rule out effects of these factors on results of evaluation of the isolated hepatocyte respiration, we studied the state of the respiratory chain on isolated mitochondria.

The respiration intensity of mitochondria isolated from the livers of rats of the 2nd and 3rd groups using succinate as a substrate in state 4 (V_4) and respiratory control did not differ from the corresponding control values (Fig. 4). We revealed no differences between the V_3 and Vd parameters using succinate for liver mitochondria of the 2nd and 3rd rat groups, which indicates the absence of uncoupling of oxidation and phosphorylation processes. These data are similar with those obtained for the oxidation and phosphorylation uncoupling in hepatocytes of the 2nd and 3rd group rats. In the 1st and 4th groups the Vd parameter did not differ statistically significantly from V_3, which indicates the complete coupling of oxidation and phosphorylation processes (Fig. 5). The V_3 parameter of the 2nd group mitochondria turned out to be higher by 23% ($p < 0.05$) than in the control. In the 3rd and 4th groups, V_3 did not differ from control (Fig. 5). These data indicate that in experimental hepatitis the functional activity of hepatocyte mitochondrial respiratory chain was elevated.

Study of respiration of mitochondria on glutamate and malate demonstrated and increase of the V_4 rate in the 2nd rat group by 56% ($p < 0.01$) as compared with the control (Fig. 6). The mitochondrial V_4 of the 3rd and 4th groups did not differ from the control. The respiratory control in all groups was at the control level. No differences were revealed between the V_3 and Vd parameters on glutamate and malate in the 2nd group, which indicates the absence of uncoupling of the oxidation and phosphorylation processes (Fig. 7). In the 3rd and 4th groups of animals treated with symvastatin, differences between the mitochondrial V_3 and Vd parameters amounted to 34% ($p < 0.05$) and 27% (statistically non-significantly), respectively. The mitochondrial V_3 parameter value of the 2nd rat group exceeded that of

Table 2. Rate of oxygen consumption by hepatocytes at addition of the respiratory chain substrates (malate + pyruvate with digitonine), inhibitor (rotenone), and uncoupler (2,4-dinitrophnol)

Oxygen consumption rate, nmole O_2 mln cells^{-1} min^{-1}	Control	Hepatitis	Hepatitis + symvastatin
Endogenous respiration V_0	14.0 ± 0.8	18.8 ± 3.2[1]*	9.9 ± 0.3[1*/2**]
Malate (1 mM) + pyruvate (5 mM)	19.4 ± 0.9	18.7 ± 5.2	15.5 ± 1.2
2,4-Dinitrophenol (5 μM)	18.5 ± 0.7	25.2 ± 9.1	14.3 ± 1.0
Rotenone (5 μM)	8.0 ± 0.5	13.7 ± 1.1	8.5 ± 0.7

Note: $M \pm m$, * $p < 0.05$, ** $p < 0.02$, [1] comparison with control, [2] comparison with hepatitis, $n = 5$.

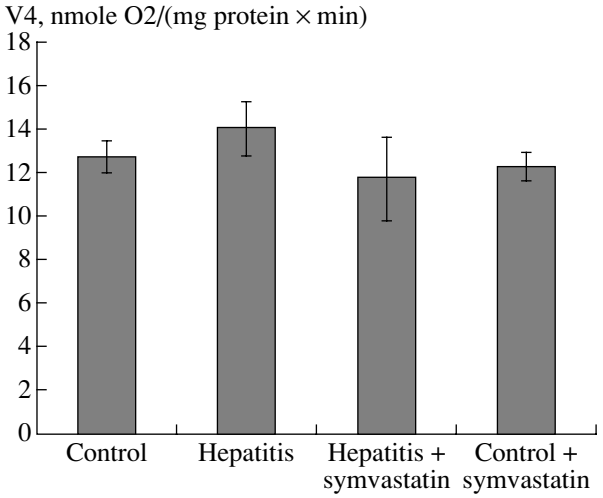

Fig. 4. Rates of oxygen consumption (M ± SEM) by hepatocyte mitochondria in state 4 with succinate as substrate.

Here and in Figs. 5–8: *I*—control rats; *II*—rats with toxic hepatitis; *III*—the same with the use of symvastatin in a background of the development of pathology (*III*) and without it (*IV*).

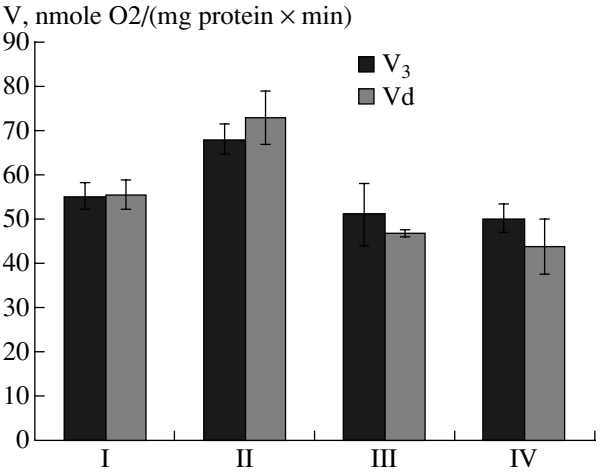

Fig. 5. Rates of oxygen consumption (M ± SEM) by hepatocyte mitochondria in state 3 (V_3) and in the presence of dinitrophenol (Vd) with succinate as the substrate.

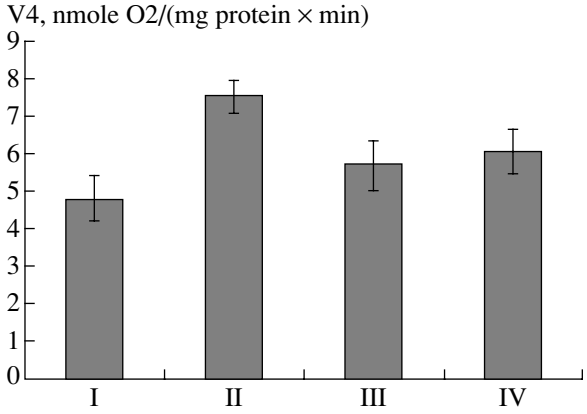

Fig. 6. Rates of oxygen consumption (M ± SEM) by hepatocyte mitochondria in state 4 with malate and glutamate as substrates.

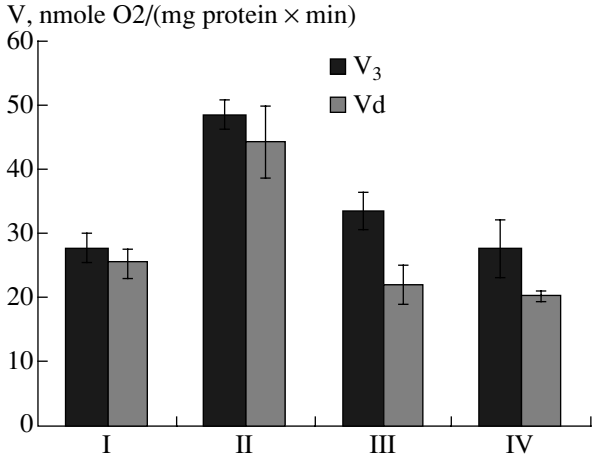

Fig. 7. Rates of oxygen consumption (M ± SEM) by hepatocyte mitochondria in state 3 (V_3) and in the presence of dinitrophenol (Vd) with malate and glutamate as substrates.

the control group by 70% ($p < 0.001$). In the 3rd and 4th groups the V_3 parameter did not differ statistically significantly from the control (Fig. 7).

Activity of cytochrome *c* oxidase. Measurements of the cytochrome *c* activity have shown this activity in rats with experimental hepatitis to be by 80% higher ($p < 0.01$) as compared with the control (Fig. 8). In rats of the 3rd group, the cytochrome *c* activity was the same as in experimental hepatitis. Administration of symvastatin to control animals did not lead to any changes of the cytochrome *c* activity.

Activity of LPO processes in rat liver. The study of LPO showed that the content of Schiff bases and diene conjugates in liver homogenates from rats with experimental hepatitis exceeded that in the control by 145% ($p < 0.05$) and 153% ($p < 0.05$), respectively (Table 3). Administration of symvastatin simultaneously with toxins prevented activation of the rat liver LPO processes. Administration of symvastatin to control animals led to a statistically significant decrease of the LPO parameters (Table 3).

DISCUSSION

The performed study revealed a disorder in the activity of the NCCR site (the 1st complex) of the hepatocyte mitochondrial respiratory chain in rats with toxic hepatitis. This is indicated by the fact that respiration intensity of isolated hepatocytes did not change on addition to the incubation medium of substrates malate

and pyruvate (Table 1). As well, addition of rotenone, an inhibitor of the respiratory chain 1st complex, produced only a slight decrease of isolated hepatocyte respiration (Table 1). Disturbances of the activity of the respiratory chain 1st complex of hepatocyte mitochondria in pathology, specifically in liver cirrhosis, have been reported in several works (Jikko et al., 1984; Krähenbühl et al., 1992, 2000; Yang et al., 2004). The cause of the NCCR damage is suggested to be an increase of the free radical level (Yang et al., 2004).

It is known that CCl_4 and ethanol are powerful activators of the LPO process (Slater, 1966; McCay et al., 1980; Chiarpotto et al., 1981). Our data from the measurement of the concentrations of diene conjugates and Schiff bases confirm an increase of the LPO level in the liver tissue in toxic hepatitis. LPO products have a high reactive activity and are able to interact both with proteins and with lipids by initiating free-radical oxidation of cell membranes, including the internal mitochondrial membrane that contains respiratory chain complexes (Krähenbühl and Reichen, 1992). However, what the reflection of the damage of the mitochondrial respiratory chain is and how this chain is functioning in liver pathology, remains unclear.

We established the endogenous respiration rate of isolated hepatocytes in rats with toxic hepatitis was 34% higher than in the control. However, the elevated oxygen consumption was not necessarily due to oxidative phosphorylation processes, as oxygen can be consumed in processes that are not associated with cytochrome *c* oxidase activity. Results of the study of coupling of oxidation and phosphorylation both on isolated hepatocytes and on isolated mitochondria indicate the absence of uncoupling of these processes. This might possibly be due to the fact that in the first respiratory chain site the electron transfer occurs to oxygen, rather than to iron-sulfur centers of NADH-coenzyme Q-oxidoreductase. The mitochondrial respiratory chain, specifically NADH-coenzyme Q-oxidoreductase and coenzyme Q-cytochrome *c*-oxidoreductase, are able perform the one-electron reduction of oxygen to superoxide anion (Skulachev, 2001). However, in the hepatocytes of normal liver, the intensity of this process is negligible and it does not noticeably affect the respiratory chain activity, as an antioxidant protection system exists in the mitochondria themselves (Agol, 1996). It could be suggested that disturbances of the respiratory chain complex I activity in hepatocyte mitochondria of rats with toxic hepatitis are due to a disorder of electron transport by this complex, which leads to formation of a large amount of superoxide anion. This could explain the increase of oxygen consumption by mitochondria of the pathologically altered hepatocytes. Cytochrome *c* in turn could oxidize the formed superoxide anion. Then the reduced cytochrome c may transfer electrons to cytochrome *c* oxidase (Skulachev, 2001). The increase of the cytochrome *c* oxidase activity, which we revealed in the hepatocyte mitochondria of rats with toxic hepatitis, agrees well with the above suggestion.

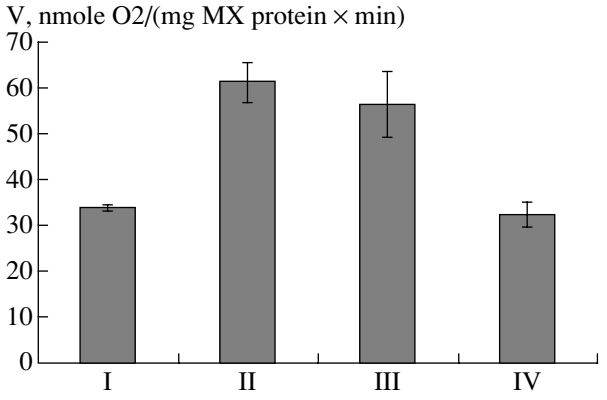

Fig. 8. Changes of cytochrome *c* oxidase activity (M ± SEM) in the liver of rats with toxic hepatitis and with the use of symvastatin.

The data on the measurement of hepatocyte respiratory control in rats with toxic hepatitis also indicated that an increase of cytochrome *c* activity is the mechanism compensating disturbances of the activity of the respiratory chain I complex, which allowed mitochondria to cope with the energy-forming function under conditions of oxygen "leakage" for formation of superoxide anion.

Study of isolated mitochondrial respiration on succinate showed that neither V_4 nor respiratory control of mitochondria in animals with toxic hepatitis differed from the corresponding control values. No differences were also revealed between the V_3 and Vd parameters. These data indicate that the state of the respiratory chain II complex (succinate-coenzyme Q-oxidoreductase) in liver pathology does not differ from the norm. The V_3 parameter, both on malate and on succinate, in hepatocyte mitochondria of rats with toxic hepatitis was higher than that in control animals. We are not aware of the reason for such an increase. This might be due to increased functional activity of ATP-synthase and/or to elevated superoxide anion production. To confirm this suggestion, a study of ATP synthesis and of superoxide anion seems to be necessary.

Our study of the development of toxic liver lesions in the presence of symvastatin showed that this sub-

Table 3. Content of Schiff bases and diene conjugates in rat liver

	Schiff bases, relat. units	Diene conjugates, μmoles/l
Control	172 ± 22	12.5 ± 1.8
Hepatitis	421 ± 49[1]**	31.6 ± 2.1[1]***
Hepatitis + symvastatin	102 ± 15[1]*/[2]***	18.6 ± 2.7[2]**
Control + symvastatin	99 ± 7[1]*	4.5 ± 0.8[1]**

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, [1] comparison with control, [2] comparison with hepatitis, $n = 6$.

stance prevents development of liver adipose dystrophy, as well as leading to a decrease in the level of LPO. This is indicated by the data of histological study and of evaluation of the levels of triglycerides, diene conjugates, and Schiff bases. As well, symvastatin decreased the rate of hepatocyte endogenous respiration in rats with toxic hepatitis. However, in isolated mitochondria, the V_4 parameter with malate and glutamate did not differ statistically significantly from the control. This might possibly be due to the ability of the symvastatin antioxidant properties to reduce the degree of the free radical oxidation-associated damage. Data on the V_3 and Vd parameters indicate that symvastatin uncoupled the respiratory chain (Fig. 7). However, this effect was revealed when measuring these parameters only in the first complex substrates, which suggests that symvastatin somehow affects functioning of the respiratory chain I complex.

The obtained data allow us to propose the hypothesis that in toxic hepatitis the activity of the respiratory chain I complex is disturbed due to leakage of electrons to oxygen and formation of the superoxide anion. As to the revealed changes in the activities of other respiratory chain complexes, they seem to be compensations in order to maintain mitochondrial energy production. In spite of the pronounced hypolipidemic and antioxidant action of symvastatin, it induced the uncoupling effect on the processes of oxidation and phosphorylation in cell respiration, which led to an additional load on the respiratory chain.

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