It can thus be concluded from these results that LPO at least is one of the causes of the increased negative surface charge on HDL in IHD. This, in turn, weakens interaction of HDL with negatively charged glycosaminoglycans of the vessel wall, i.e., it weakens the acceptor properties of HDL relative to Ch. Meanwhile peroxidation of LDL, if it exists, is not the only cause of the enhanced atherogenic role of this class of LP in IHD.

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END PRODUCTS OF LIPID PEROXIDATION IN ALCOHOL-INDUCED LIVER DISORDERS

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KEY WORDS: lipid peroxidation; alcohol-induced liver damage

Lipid peroxidation (LPO) is a key factor in the development of various pathophysiological processes such as stress, ischemic heart disease, epilepsy, hypoxia, [3, 6, 10]. In particular, intensification of LPO has been demonstrated in patients with liver disease [5, 8]. This is quite understandable if it is recalled that the xenobiotic inactivation sytem of the hepatocytes is closely linked with the LPO enzyme system [1] and that activation of LPO is the initial stage of cytolysis [2].

One of the most widespread and serious forms of liver pathology is that due to alcohol, which is nowadays subdivided into several forms depending on the character of liver damage [4, 7, 9, 11]. However, there are no experimental data on the role of LPO processes in the various forms of alcohol-induced liver damage.

The aim of this investigation was to study the intensity of LPO in patients with different forms of alcohol-induced liver damage.

EXPERIMENTAL METHOD

Altogether 124 patients addicted to alcohol for a long perid of time (from 3 to 20 years) were investigated. All patients had a negative epidemiologic history of acute virus or drug-induced hepatitis, and no HBsAg was present in the blood serum. Clinical and morphological

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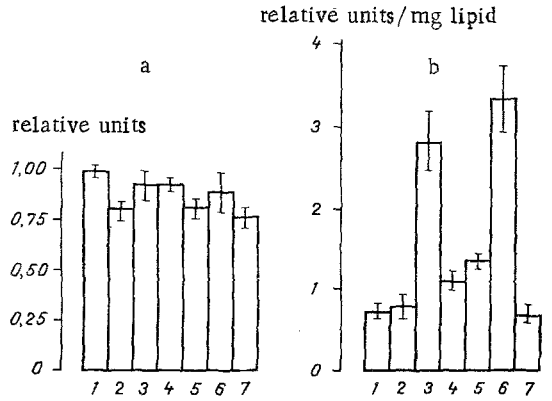

Fig. 1. Concentrations of lipid hydroperoxides with conjugated double bonds (a) and of fluorescent LPO products (b) in plasma from normal subjects (1), patients with alcoholic hepatitis (3), chronic alcoholic hepatitis (4), active alcoholic cirrhosis of the liver without an edema-ascites syndrome (5), active alcoholic cirrhosis of the liver with an edema-ascites syndrome (6), and with inactive alcoholic cirrhosis of the liver (7).

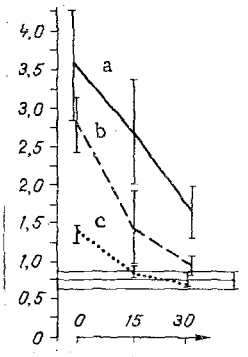

Fig. 2

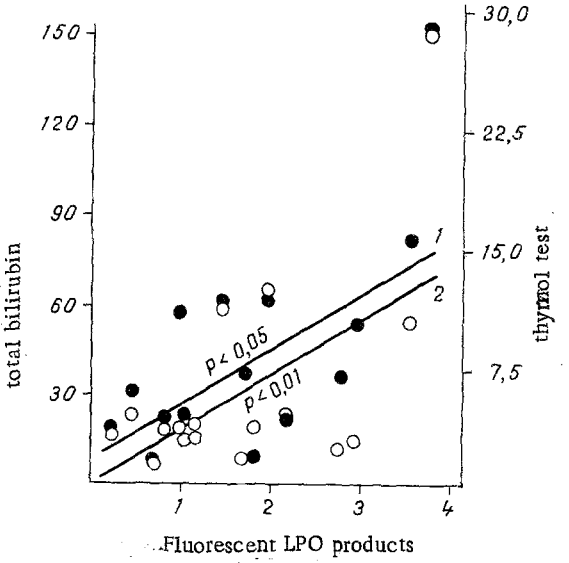

Fig. 3.

Fig. 2. Time course of plasma levels of fluorescent LPO products in patients with alcoholic cirrhosis of the liver with edema-ascites syndrome (a), with acute alcoholic hepatitis (b), and with active alcoholic cirrhosis of the liver without an edema-ascites syndrome (c). Abscissa, time, in days; ordinate, concentration of fluorescent LPO products (in relative units/mg lipid).

Fig. 3. Correlation analysis of plasma levels of fluorescent LPO products and total bilirubin concentration (filled circles, 1) and results of thymol test (empty circles, 2) in patients with active alcoholic cirrhosis of the liver without an edema-ascites syndrome.

forms of alcoholic liver damage were verified by analysis of clinical, biochemical, and morphological tests of liver biopsy material.

Levels of primary and end products of peroxidation were determined in the blood plasma of patients with the following generally accepted clinical and morphological forms of alcohol-induced liver damage: fatty degeneration of the liver, acute and chronic alcoholic hepatitis, active cirrhosis of the liver without and with edema-ascites syndrome, and inactive cirrhosis of the liver. Total lipids isolated from plasma by Folch's method [13]. Concentrations of primary LPO products (hydroperoxides with conjugated double bonds) were recorded spectrophotometrically in methanol solution as the ratio A_{232}/A_{215} [2]. The concentration of fluorescent end products of LPO was determined by measuring fluorescence of methanol solutions of total plasma lipids (λ_{exc} = 350 nm, λ_{flour} = 430 nm) [12].

Optical density of total plasma lipids was measured on a Perkin-Elmer 555 spectrophotometer. Fluorescence spectra were recorded on a Hitachi-850 spectrofluorometer. Statistical analysis of the results and correlation analysis were carried out on a Canon C×I computer (Japan).

EXPERIMENTAL RESULTS

The results of determination of plasma levels of primary LPO products in patients with various forms of alcohol-induced liver damage are given in Fig. 1a. Plasma levels of primary LPO products in patients with different forms of alcohol-induced liver damage were virtually identical with those of the control group. Meanwhile the concentration of fluorescent end products of LPO varied depending on the type of alcohol damage. It will be clear from Fig. 1b that the concentrations of fluorescent LPO products in the plasma of patients with alcoholic fatty degeneration of the liver with fibrosis and patients with moderately active alcoholic hepatitis were virtually indistinguishable from those in the control, whereas plasma levels in patients with inactive cirrhosis of the liver were actually a little depressed. Concentrations of fluorescent LPO products in the plasma of patients with active alcoholic cirrhosis of the liver without an edema-ascites syndrome were significantly higher than in the control. Concentrations of fluorescent LPO products in acute alcoholic hepatitis were three times higher than in the control.

The highest concentration of fluorescent LPO products in the plasma (more than three times higher than the control) was observed in the group of patients with cirrhosis of the liver accompanied by an edema-ascites syndrome.

The results are evidence that plasma levels of end products of LPO are significantly higher in patients with acute alcoholic hepatitis and with active forms of alcoholic cirrhosis of the liver, whereas the hydroperoxide levels are unchanged compared with the control. It is natural to suggest that if the plasma level of fluorescent LPO products in acute alcoholic hepatitis and in active forms of cirrhosis of the liver reflects the state of activity of the pathological processes in the liver, the decrease in the concentration of fluorescent products in the course of treatment ought to correspond to an improvement in other objective clinical parameters.

Figure 2 shows the time course of the plasma levels of fluorescent LPO products in patients and active cirrhosis of the liver without an edema-ascites syndrome, and with their levels during treatment with lipoic, folic, and ascorbic acids together with vitamins B_1 and B_6, and with the preparation "Essentiale." Patients with an edema-ascites syndrome also were treated with diuretics. During treatment of patients with active cirrhosis of the liver without an edema-ascites syndrome the plasma levels of fluorescent LPO products returned to normal by the 15th day, whereas in patients with acute alcoholic hepatitis this occurred on the 30th day. Meanwhile in patients with active cirrhosis with an edema-ascites syndrome, although there was a significant fall in the level of fluorescent LPO products, their concentration still remained 2.5 times higher than in the control even on the 30th day after the beginning of treatment.

A number of biochemical tests are currently used for the diagnosis of liver diseases. Correlation analysis of the concentrations of fluorescent LPO products and the results of the commonly used biochemical tests was therefore interesting. It will be clear from Fig. 3 that the concentrations of fluorescent LPO products correlated significantly with the total plasma bilirubin level and the results of the thymol test in patients with active cirrhosis of the liver without an edema-ascites syndrome. Similar significant correlation also was observed between the concentrations of fluorescent LPO products and the total bilirubin level and results of the thymol test in patients with cirrhosis of the liver, both with an edema-ascites syndrome and with acute alcoholic hepatitis.

It can be concluded from these results that acute alcoholic hepatitis and active alcohol-induced cirrhosis of the liver are accompanied by intensification of LPO. This is manifested as an increase in the concentration of fluorescent end products in the plasma. A higher level of LPO is associated with active cirrhosis with an edema-ascites syndrome and acute alcoholic hepatitis; this is reflected both in the actual concentrations and the time course of normalization of the level of fluorsecent products in these forms of alcohol-induced liver disorder.

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DETECTION OF *DE NOVO* SYNTHESIZED FORM OF MICROSOMAL CYTOCHROME P-448 BY AUTOFLUOROGRAPHY

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The monooxygenase enzyme system, consisting of cytochrome P-450 and NADPH electron transport chain, catalyzes oxidation of hydrophobic foreign compounds (xenobiotics) and thus promotes their elimination from the body. Monooxygenases hydroxylate a wide range of substrates, some of which are genetic inducers of components of the system. More than 300 substances, differing in chemical structure and biological effects, significantly increasing the rate of biotransformation of xenobiotics, and in the substrate specificity of their enzyme system, due to a selective increase in the concentration of certain forms of cytochrome P-450, are now known. For example, during induction by the polycyclic aromatic hydrocarbon (PAH) β-naphthoflavone, five forms of cytochromes possessing different spectral, catalytic, and immunologic properties, and also differing in molecular weight and primary structure, have been isolated from the microsomal fraction of rat liver [7]. After injection of another PAH, namely 3-methylcholanthrene (MCh), only one basic, *de novo* synthesized form of cytochrome (P-448) was isolated from liver microsomes and its characteristics determined sufficiently well. As regards other forms of cytochrome induced by MCh, no data on this problem exist. An increase in the content of certain forms of microsomal cytochromes, which lies at the basis of induction by xenobiotics, may be the result of an increase in the rate of *de novo* protein synthesis or the rate of recursor activation, or a decrease in the rate of degradation. Direct proof of *de novo* syn-

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