can be explained by an increase in the total PL content, an increase in the PCH/CM ratio, a decrease in the fraction of free CH, and removal of free CH from the surface monolayer of HDL particles, responsible for their CH-accepting properties. The increase in the fractions of CH esters and of lysophosphatidylcholine in HDL on administration of PCH indicates definite activation of lecithin:CH acyltransferase. Investigation of the fatty acid composition of HDL-PL revealed no marked differences between animals of different groups (Table 4).

The area of the aortas occupied by atherosclerotic lesions in animals receiving PCH was only half of that in the control animals: 14 ± 2.5 and 32 ± 4.3% respectively (Fig. 2).

The results of this investigation thus demonstrate that excess CH can be extracted from biological membranes of animals with experimental atherosclerosis by intravenous injection of positively charged PCH micelles.

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ROLE OF BILIRUBIN AS A NATURAL ANTIOXIDANT IN REGULATION OF THE INTENSITY OF LIPID PEROXIDATION IN ACUTE VIRUS HEPATITIS

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UDC 616.36-002-022-036.11-02:547.937

KEY WORDS: antioxidants; bilirubin; virus hepatitis; lipid peroxidation.

Liver damage in acute virus hepatitis (VH) leads to various metabolic disturbances, among which an important place is occupied by disturbances of lipid metabolism, expressed as high serum levels of phospholipids, triglycerides, cholesterol, and nonesterified fatty acids in the patients. Several workers [7, 8, 13] have found high serum levels of lipid peroxidation (LPO) products in patients, correlating with the severity of the disease. Bilirubin, whose level also is regularly raised in accordance with the severity of VH, exhibits the properties of an antioxidant *in vitro* — an inhibitor of radical reactions [9, 11]. Data showing that bilirubin can perform the function of natural antioxidants *in vivo* (especially, in regulation of the intensity of LPO during VH) are not to be found in the literature.

The aim of this investigation was to study the effect of bilirubin on changes in some LPO parameters, namely levels of diene conjugates and antioxidant activity (AOA) of lipids, in the serum of patients with VH of different degrees of severity.

EXPERIMENTAL METHOD

Altogether 63 patients with VH (A and B) of different degrees of severity were studied at the climax of the disease. The degree of severity of the disease was determined on the basis of the usual clinical and clinical-biochemical parameters. Since preliminary investigations revealed no significant differences in the biochemical parameters of patients with VH A and VH B, patients were placed together in the corresponding groups of severity of course of the disease irrespective of the type of VH. The control group consisted of 35 clinically healthy subjects.

Riga Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 2, pp. 166-168, February, 1985. Original article submitted April 7, 1984.

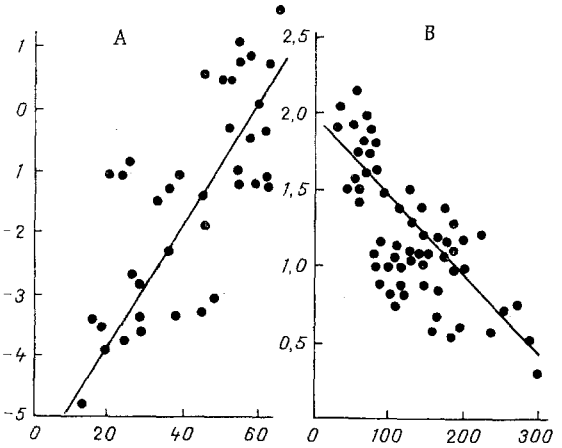

Fig. 1. Dependence of AOA of lipids (A) and degree of oxidation of lipids (B) on serum bilirubin concentration of patients with VH. Abscissa, concentrations of free (A) and total (B) bilirubin (in μmoles/ml serum); ordinate, AOA of lipids (in h/ml/g × 10^{-3}); B) diene conjugates (in E_{233}/mg lipids).

TABLE 1. Changes in Serum Bilirubin Level and Parameters of Lipid Metabolism in Patients with Acute VH (M ± m)

Biochemical parameter	Normal individuals (35)	Patients with VH	
		mild form (29)	moderately severe form (34)
Total bilirubin, μmoles/liter	15±6	86±5[a]	134±7[a, b]
Free bilirubin, μmoles/liter	11±5	25±2	47±3[a, b]
Total lipids, mg/ml	5,7±0,4	7,1±0,7[a]	9,9±0,7[a, b]
Diene conjugates, E_{233}/ml	3,71±0,20	5,04±0,32[a]	5,51±0,30[a, b]
AOA of lipids, h/g/ml × 10^{-3}	—1,28±0,28	—1,70±0,33	—0,96±0,22[c]

Legend. Number of subjects tested given in parentheses; a) $P < 0.05$ compared with healthy individuals, b) $P < 0.05$ compared with patients with mild VH.

Blood serum lipids were extracted by Folch's method [10]. The content of diene conjugates was determined by a spectrophotometric method [12] in the modification in [5]. The AOA level of the lipids was determined on a methyl oleate oxidative model. The results of the investigation were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The serum concentration of diene conjugates, one of the primary products of LPO, in the patients increased in accordance with the severity of the disease: 1.3 times in the mild form and 1.5 times in the moderately severe form of VH (Table 1), in agreement with data obtained by other workers [7, 8]. However, the degree of oxidation of the lipids (the content of diene conjugates calculated per milligram of lipids) in patients with moderately severe VH was 1.5 times lower than in patients with mild VH and 1.3 times lower than in clinically healthy individuals. It can accordingly be postulated that the increased content of LPO products in patients with VH is due, not to intensification of peroxide reactions in lipids, but to elevation of the serum lipid levels themselves.

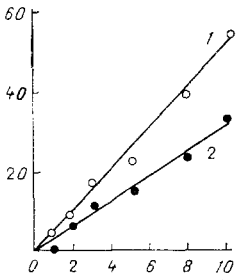

Fig. 2. Dependence of duration of induction period of oxidation of methyl ester of oleic acid at 50°C in presence of bilirubin (1) and ionol (2) on concentration of antioxidants. Abscissa, concentration (in $M \times 10^5$); ordinate, difference between induction period of methyl oleate oxygenation in presence and absence of inhibitors (in h).

AOA of lipids, reflecting the level of natural antioxidants, in patients with moderately severe VH had a tendency to rise above normal, and it was significantly higher than in patients with mild VH (Table 1).

Negative correlation was found between the serum concentrations of total bilirubin and diene conjugates in patients with VH, and positive correlation between the lipid AOA level and lipid-soluble free bilirubin ($r = 0.78$ and $r = +0.56$ respectively; Fig. 1), i.e., the blood level of lipid peroxidation products was lower in patients with a high blood bilirubin level, whereas the level of natural antioxidants was higher than in patients with a low bilirubin level. This is in good agreement with the fact that bilirubin possesses antioxidant properties.

It was interesting to estimate the contribution of bilirubin to the change in AOA of the serum lipids of patients with moderately severe VH compared with those with mild VH. To do this, changes in the induction period of thermal oxidation of the methyl ester of oleic acid were studied in a model system *in vitro* in the presence of bilirubin, compared with one of the most thoroughly studied synthetic antioxidants — ionol (4-methyl-2,6-di-tert-butylphenol). It can be concluded from the results that bilirubin is a much more effective antioxidant than ionol (Fig. 2); its relative antioxidative efficiency (ε) was found to be 1.3 (for ionol, $\varepsilon = 1$).

The calculated difference between the bilirubin content per gram of serum lipids in patients with moderately severe VH and with the mild form was 1.2 ± 0.2 μmole/g (Table 1). The calculated value of the induction period of methyl oleate oxidation for this quantity of bilirubin was 610 ± 20 h (Fig. 2). The difference between the induction period for oxidation of methyl oleate in the presence of 1 g serum lipids in patients with moderately severe and mild forms of VH was 740 ± 120 h. Hence it follows that elevation of the lipid AOA level in patients with moderatley severe VH compared with the mild form of the disease can be attributed virtually completely (by $82 \pm 16\%$) to bilirubin. Incidentally, the value given above estimates the contribution of bilirubin as an individual substance, without consideration of its possible interaction with other substances contained in the lipids, i.e., bilirubin can evidently exhibit the properties of an effective natural antioxidant *in vivo*, in particular, in regulation of the intensity of LPO during VH. In this connection it is interesting to study the intensity of LPO and antioxidant levels in the severe form of VH, when the blood bilirubin level is higher still, and also in patients with jaundice of different genesis.

Natural antioxidants play an important role in the regulation of cell proliferation, and processes connected with more rapid cell multiplication (physiological and reparative regeneration, tumor growth) are accompanied by a high lipid AOA level. Antioxidants affect the rate of proliferation by inhibiting free-radical reactions, in the course of which repressors of cell multiplication are formed [3]. We know that regeneration of hepatocytes, which is more intensive in moderately severe than in mild forms of VH, may arise in the earliest stages of this disease (during the first week) [1, 2, 6]. It can accordingly be postulated that elevation of the bilirubin level in patients with VH with an increase in

the severity of the disease, and the associated rise in AOA of lipids constitute a response aimed at strengthening repair processes in the liver and lowering the levels of toxic products of LPO.

The authors are grateful to Professor E. B. Burlakova and Cand. Chem. Sci. N. G. Khrapova for useful discussion of the results and for technical advice in the course of the work.

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