EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Bile Congestion on Lipid Peroxidation and Structural Composition of the Bile *In Vitro*

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Oxidative processes in the bile caused by congestion lead to bile restructuring consisting in decreased phospholipid content paralleled by increased content of their peroxidation products and shifts in relative composition of components toward the predominance of more hydrophobic ones, which is more pronounced in calculous bile during incubation.

Key Words: *lipid peroxidation; cholecystitis; cholelithiasis, bile*

Bile nucleation takes place at 37°C; the terms of this process are different in patients with calculous and acalculous cholecystitis. For the bile from patients with acalculous cholecystitis the term is 32 days, for cholelithiasis it is 5±2 days. All bile components are changed, which is apparently caused by lipid peroxidation (LPO) determining the restructuring of physicochemical properties of the bile leading to cholelithiasis. The type of and relationships between these changes were studied under conditions of experimental cholestasis.

MATERIALS AND METHODS

Closed tubes with bile were incubated for 19 days at 37°C, and its components were tested on days 1, 5, 10, 15, and 19. Bile specimens from 16 patients were examined: from 7 patients with cholelithiasis and 9 patients without calculi in simulated *in vitro* cholestasis demonstrating different effects of LPO on bile components. For separating bile components according to polar characteristics, they were extracted with a chloroform-methanol mixture by the method of Volch.

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The levels of malonic dialdehyde (MDA) and hydrophobic components of chloroform extract of bile lipids were measured by spectrophotometry at a wavelength of 450 nm: phospholipids (P) [3], cholesterol [1], and bilirubin chloroform fraction. Extinction of water-methanol component of the extract was measured at a wavelength of 440 nm (E_{440}) corresponding to the content of bilirubin polar fraction.

The content of MDA in chloroform extract was determined by thiobarbituric acid test in acid water-free medium.

In order to level the concentration differences in individual bile specimens, relative coefficients were calculated: MDA/P reflecting the content of MDA per unit phospholipids, $(E_{450}/E_{440})\times 100$ — the ratio of chloroform to water-methanol bilirubin fractions, cholesterol-lecithin index (C/L), and the ratio of measured components.

RESULTS

Bile with the mean MDA/P relative values 2.5-3.1 nmol/mg was studied. According to our preliminary data, these values correspond to normal LPO values and values in patients with remission of calculous and acalculous cholecystitis.

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Starting from the first days of incubation, phospholipid content decreased in all samples, the decrease depending on the initial level of this component and its oxidation, bile concentration, and effects of other components on LPO (Table 1).

The relative cholesterol content showed a tendency to increase during incubation; this tendency was more obvious in patients with cholelithiasis. The C/L index (mg/mg) regarded by many researchers as an objective parameter reflecting sedimentation capacity of bile dispersions (Table 1) significantly increased by day 5 in this group. Later, the differences in the values caused by notable individual changes in bile specimens made these shifts statistically insignificant.

Study of chloroform extracts of bile after its incubation showed regularities in restructuring of water-soluble (E_{440}) and hydrophobic fractions of bilirubin. Chloroform extract pigment showed a tendency to increase in the course of bile incubation: by day 5 in patients with acalculous cholecystitis, after which its level was more or less stable, and during the entire period of incubation in patients with cholelithiasis. Water-methanol fraction of the extract showed a tendency to a decrease in extinction (Table 2).

Table 2 shows the differences in the time course of $(E_{450}/E_{440})\times100$ in patients with acalculous and calculous cholecystitis. On day 5 of incubation the parameter increased 11 times in the acalculous cholecystitis group and 1.7 times in the calculous cholecystitis group. Its initial value was 3.7 times higher in cholelithiasis patients than in those with acalculous cholecystitis. After 10-day incubation, the parameter tended to decrease in patients with acalculous cholecystitis, whereas in cholelithiasis patients it was still increasing. Therefore, during *in vitro* incubation of the bile, when additional bile inflow is ruled out, bilirubin migrates from aqueous fraction to the hydrophobic portion of the extract. In acalculous bile this transfer is more expressed at the early stages of incubation, whereas in calculous bile it is pronounced throughout the entire incubation period. The different time course of $(E_{450}/E_{440})\times100$ changes in these groups can be explained by different concentrations of

TABLE 1. Time Course of Phospholipid Content and C/L Index during Incubation (*M±m*)

Parameter	Day of incubation				
	1	5	10	15	19
Acalculous cholecystitis					
Phospholipids,mg.	3.3±1.4	3.1±1.0	1.7±0.8	0.9±0.3	0.6±0.2
%	100.0	75.5±7.4*	46.8±4.7***	27.5±4.7***	16.5±3.9***
C/L index	0.25±0.03	0.55±0.14	0.99±0.20*	1.24±0.10**	1.90±0.40*
Cholelithiasis					
Phospholipids,mg	38.0±12.7	25.7±8.6	13.±6.6	7.8±4.4*	5.5±3.3*
%	100.0	67.9±4.3***	27.9±17.9***	13.4±5.0***	9.0±3.7***
C/L index	0.15±0.02	0.22±0.02*	1.96±1.19	5.59±3.20	11.80±6.90

Note. Here and in Table 2: *$p<0.05$, **$p<0.001$, ***$p<0.0001$ *vs.* day 1 of incubation.

TABLE 2. Time Course of Changes in Water-Soluble and Hydrophobic Fractions of Bilirubin and $(E_{450}/E_{440})\times100$ during Incubation

Parameter	Day of incubation				
	1	5	10	15	19
Acalculous cholecystitis					
E_{440}, U. light absorb.	2.3±1.5	1.5±1.1	0.8±0.6	0.8±0.6	0.8±0.6
Bilirubin, mg/ml	0.007±0.003	0.056±0.034	0.047±0.034	0.057±0.047	0.053±0.044
$(E_{450}/E_{440})\times100$	2.9±0.7	31.9±6.9*	38.6±2.9***	35.8±5.7**	30.3±6.7*
Cholelithiasis					
E_{440}, U. light absorb.	13.3±4.9	9.4±3.6	8.5±2.7	7.3±2.3	7.4±2.3
Bilirubin, mg/ml	0.12±0.04	0.25±0.10	0.51±0.18	0.63±0.23	0.74±0.28*
$(E_{450}/E_{440})\times100$	11.7±3.4	17.7±3.1	31.1±4.4*	42.1±9.1*	47.1±11.2*

the bile, differences in its physicochemical structure, and LPO effects on bile components in two groups.

Together with proteins, bilirubin can alter the borderline of cholesterol solubilization as a substance 3.5×10^3 times less soluble in water than cholesterol. In the presence of decreasing levels of solubilizers, an increase in the free bilirubin concentration leads to metastability of colloid corpuscles. Analysis of centers of cholesterol bile calculi shows that in the majority of cases their matrix consists of macromolecular mucin and bilirubin complex [2,5,6].

The decrease in phospholipids is paralleled by increase in lipid peroxides reacting with thiobarbituric acid (TBA); this increase is reflected by the MDA/P value. This increase is different in patients with chronic cholecystitis in the presence of cholelithiasis and with acalculous cholecystitis. Increased content of TBA-reactive products during incubation in the acalculous cholecystitis group is smooth, with exponential increase toward the end of incubation, whereas in cholelithiasis the exponent manifests itself earlier (Fig. 1).

A trend to MDA/P increase by day 5 was more expressed in the acalculous group, but by day 10 the values showed a tendency to increase in the cholelithiasis group, increasing still more by days 15-19 of incubation.

To rule out the effects of concentrations, the absolute values (MDA, phospholipids, cholesterol, and bilirubin content) were converted into percent (Fig. 2).

MDA increased similarly in both groups, the differences being observed only at early stages. The differences in the composition of hydrophobic components of the bile in the two groups consist in a

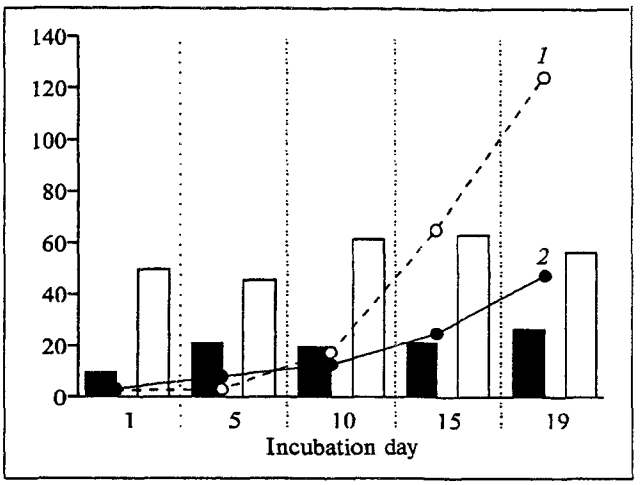

Fig. 1. Time course of changes in malonic dialdehyde level in acaculous cholecystitis (dark bars) and cholelithiasis (light bars) during incubation, nmol/ml. Malonic dialdehyde content per phospholipid unit in cholelithiasis (*1*) and acalculous cholecystitis (*2*), nmol/ml.

nonuniform increase of bilirubin, which is an important component of heterogeneous nucleation.

Although bilirubin transfer from hydrophilic into hydrophobic phase of the bile is more expressed at the first stages of incubation in acalculous cholecystitis, specific contribution of hydrophobic components to the total volume during incubation was greater in cholelithiasis. Due to a higher concentration of the bile, chloroform fraction of bilirubin can increase for a long time in these patients. The time course of this increase is conditioned by mutual ratio of all components which better than individual values reflects the nature of structural changes in the hydro-

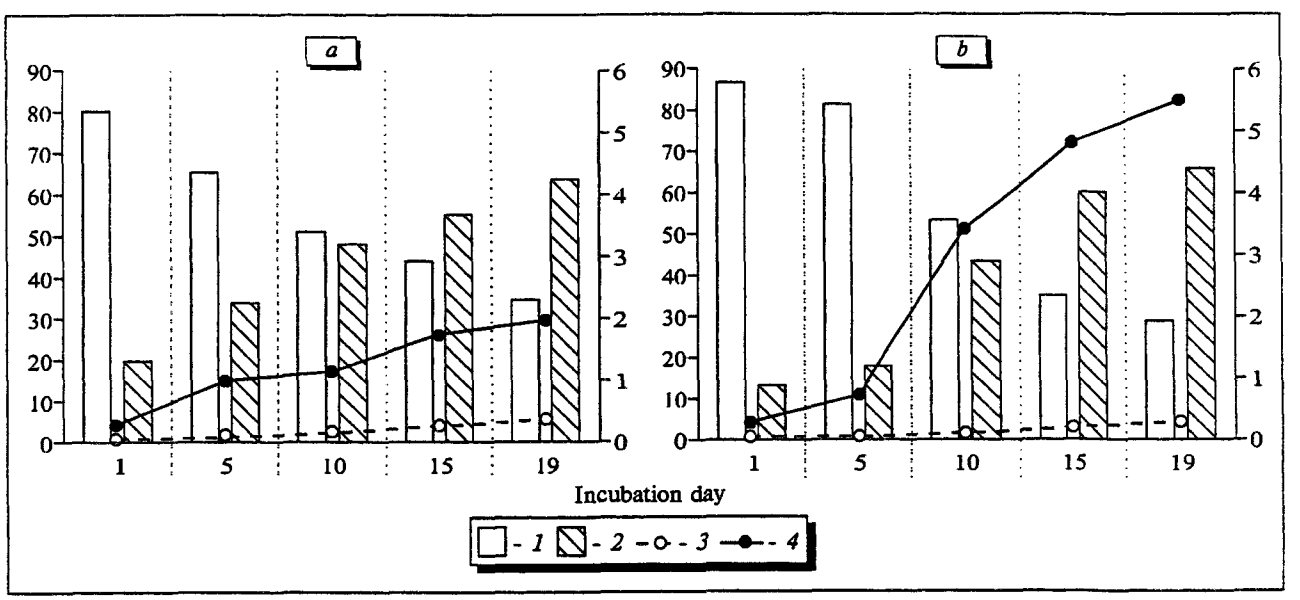

Fig. 2. Time course of changes (in %) of ratios of biochemical components of the bile in acalculous (*a*) and calculous (*b*) cholecystitis. *1*) phospholipids; *2*) cholesterol; *3*) malonic dialdehyde; *4*) bilirubin. Ordinate: concentration, %; left: for 1, 2; right: for *3*, *4*.

phobic phase of the bile, occurring during congestion and, like the concentration factor, can be regarded as a pathogenetic factor of calculi formation under conditions of active absorption of water and water-soluble component in a blocked gallbladder.

Therefore, restructuring of bile components resulting from oxidative processes caused by congestion consists in decreased phospholipid content paralleled by an increased LPO products, increase of C/L index, and increase of bilirubin content in the hydrophobic phase of the bile (chloroform extract) parallel with its decrease in hydrophilic phase (water-methanol portion of the extract). The changes differed in the two groups by the end of incubation; they were greater in the majority of patients with cholelithiasis.

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