TABLE 1. Effect of Fe^{++} and ^3H-Thymidine Incorporation into DNA Fraction and on MDA Accumulation under the Influence of Fe^{++} and Ascorbate

Experimental conditions	^3H-thymidine uptake, % of control	MDA concentration, % of control
Control	100±5,5	100
Fe^{2+} + ascorbate	191±14	211
Fe^{2+} + ascorbate + tocopherol	172±8	104
α-Tocopherol	106±10	100

Legend. Concentrations: Fe^{++} 2 μM, ascorbate 30 μM, α-tocopherol 10^{-4} M.

which the intensity of repair processes is depressed [7]. In our view, therefore, the study of processes of this kind in brain neurons in the postmitotic phase, which is characterized by low activity of reparative enzymes, and the partial oxygen consumption is very high, may therefore be particularly interesting.

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^{31}P-NMR SPECTROSCOPY OF HUMAN LIVER AND BILE

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The study of the composition and physicochemical properties of bile, a product of the external secretory activity of the liver, can be used to evaluate both the synthetic and the excretory function of the liver. In particular, changes in the concentration of one component of native bile — phosphatidylcholine (PCh) — reflect changes in its acid-dependent (dependent on secretion of bile acids) secretion by the hepatocyte [3, 7, 8]. Meanwhile, secretion of inorganic phosphorus (P_i, orthophosphate) and of other inorganic ions is acid-independent (does not depend on secretion of bile acids), and is effected by hepatocytes along the concentration gradient [1]. As a result of this, the P_i concentration in bile is directly linked with its concentration in the hepatocytes and, consequently, it depends on the level of metabolic processes taking place in the liver cells with the participation of orthophosphate.

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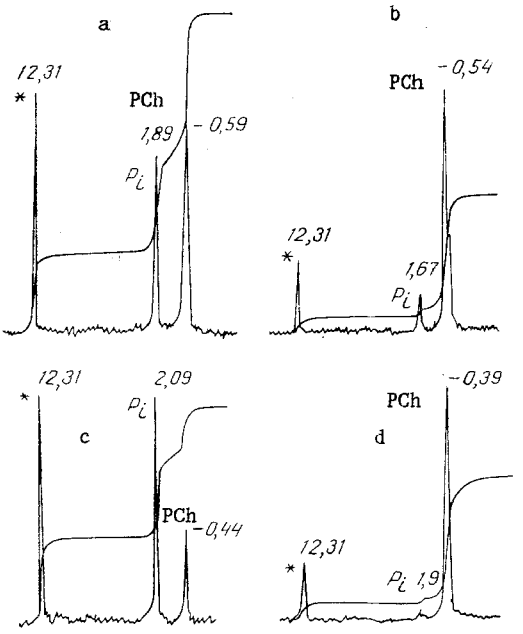

Fig. 1. ^{31}P-NMR spectra of hepatic (a, c) and cystic (b, d) portions of bile from a patient with PBC (c, d) and a clinically healthy man (a, b). PCh) Signal of phosphatidylcholine (lecithin), P_i) signal of inorganic phosphate; *) signal of external standard. Numbers indicate chemical shifts of signals (in ppm).

The high-resolution nuclear magnetic resonance (NMR) method is a noninvasive method for the rapid and simultaneous analysis of concentrations and the dynamic state of the principal metabolites in native biological cells, tissues, and organs and in biological fluids [6].

In the present investigation high-resolution NMR on phosphorus nuclei (^{31}P-NMR) was used to assess the level of phosphate-containing compounds in native bile of patients with primary biliary cirrhosis of the liver (PBC) and in biopsy material from the liver of patients with chronic calculous cholecystitis (CCC).

EXPERIMENTAL METHOD

Bile from 17 patients with PBC at different morphological stages of development of the disease and from 14 clinically healthy men was studied. The bile was obtained by multistage fractional duodenal catheterization. For investigations of the ^{31}P-NMR method 1.5 ml each of cystic (B) and hepatic (C) portions of bile was taken and kept at 4°C for not more than 4 h before recording of the spectra. Liver biopsy material from the patients with CCC was obtained by peripheral liver biopsy during operation.

High-resolution ^{31}P-NMR spectra of specimens of bile and liver tissue were recorded on a WH-360 NMR spectrometer (Bruker, West Germany), with a working frequency of 145.78 MHz at 4°C on phosphorus nuclei without rotation of the ampul with the specimen and without suppression of spin–spin interaction of ^{31}P nuclei with protons. The spectra were obtained by the use of a 60° pulse and a scanning time of 0.82 sec, and with a delay time of 0.35 sec. The noise level was reduced by multiplication by an exponential function with an exponent of 15 Hz. Values of chemical shifts of signals (δ) in the spectra in parts per million (ppm) are given relative to the 85% H_3PO_4 signal. Values of chemical shifts and intensities of signals in the spectra were estimated relative to the signal of an external standard — the disodium salt of ethylenediaminetetraphosphonic acid (δ = 12.31 ppm).

EXPERIMENTAL RESULTS

Typical ^{31}P-NMR spectra of portions B and C of bile from a patient with PBC and a clinically healthy individual are given in Fig. 1. Signals of phosphorus nuclei in the region between −0.25 and −1.05 ppm, observed in the spectra illustrated, are due to the phosphate group of PCh and its lysis products in bile, and those in the region between +1.7 and +2.3 ppm are due to orthophosphate. The integral intensities of the signals in the ^{31}P-NMR spectra are

TABLE 1. Relative Concentration of Lecithin and Orthophosphate in Cystic (B) and Hepatic (C) Portions of Bile from Patients with PBC and Subjects of Control Group (M ± m)

Parameter	Patients with PBC (n = 17)	Control group (n = 14)
Lecithin		
B	1,04±0,22*	7,18±1,01
C	0,45±0,10*	2,07±0,34
Orthophosphate		
B	0,39±0,09	0,61±0,14
C	0,29±0,07*	1,21±0,30

Legend. *p < 0.05.

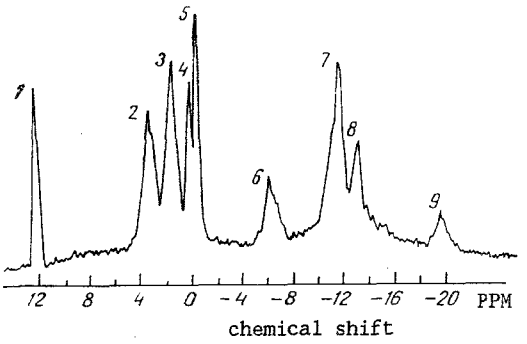

Fig. 2. High resolution ^{31}P-NMR spectrum of the liver of a patient with CCC. 1) Signal of external standard; 2) signal due to phosphomonoesters; 3) signal of orthophosphate; 4) superposition of signals of glycerol-3-phosphorylserine and glycerol-3-phosphorylethanolamine; 5) signal of glycerol-3-phosphorylcholine; 6) superposition of signals of γ-P-group of ATP and β-P-group of ADP; 7) superposition of signals of α-P-groups of ATP and ADP, and also of phosphate groups of NAD and NADP (oxidized and reduced forms); 8) signals of uridine diphosphoglucose; 9) signal of β-P-group of ATP.

proportional to the number of phosphorus nuclei and can be used to determine concentrations of phosphorus-containing compounds in the specimen. The results of measurements of the intensities of the P_i and PCh signals in portions B and C of bile from clinically healthy individuals and from patients with PBC are summarized in Table 1.

As can be seen from Table 1, the observed PCh content in the portion of bile B is greater than in portion C, as a result of the concentration of PCh in the gallbladder on account of the reabsorption of water by the bladder mucosa. The ratio of the PCh concentrations in the portions of bile B and C can evidently be used to characterize the concentrating ability of the human gallbladder in normal and pathological states. The decrease in the concentration of P_i in the portion of bile B in healthy people in comparison with its concentration in portion C may be evidence of reabsorption, probably passively, along the concentration gradient, of part of the P_i by the gallbladder mucosa together with water.

Thus comparison of the PCh and P_i concentrations in the hepatic and cystic bile enables the concentrating and absorption capacity of the human gallbladder to be judged.

Data in the table also indicate that the PCh concentration in portions B and C of bile from patients with PBC is much lower than its concentration in healthy human bile. Since PCh forms mixed micelles in bile with bile acids and cholesterol, the fall of PCh recorded may be linked with a decrease in the secretion of bile acids and cholesterol into the bile, which we found in these same patients.

Reduction of the intensity or complete absence of the P_i signal in the spectrum of bile from patients with PBC may indicate that its secretion from hepatocytes into the lumen of the

intercellular biliary capillaries is reduced. A fall in the transport of P_i, taking place along the concentration gradient, is evidently a result of a decrease in the P_i concentration in the hepatocytes. We know that a marked increase in activity of alkaline phosphatase is observed in patients with PBC, and an increase in alkaline phosphatase activity in the blood is the result of its rapid production in the hepatocytes [5]. Simultaneous enhancement of alkaline phosphatase activity and a fall in the concentration of P_i formed under the influence of this enzyme is evidence of active utilization of P_i in metabolic processes taking place in the liver of patients with PBC. Possibly P_i is used for phospholipid biosynthesis as a compensatory mechanism for binding the excess of bile acids in the hepatocytes.

By the ^{31}P-NMR method it is possible to assess the level of phosphate-containing compounds directly in liver tissue [2]. However, removal of the tissue from the body leads to rapid anaerobiosis and to hydrolysis of phosphate-containing compounds in the cells. Choice of the optimal conditions for keeping samples of liver tissue (nutrient medium, temperature, degree of oxygenation) in the period between removal of the tissue from the body and recording the ^{31}P-NMR spectra was determined in CMK mice at each experimental point five animals were used. It was shown that although the sample of liver tissue after removal is in anaerobiosis, rapid cooling can reduce uncontrollable enzymic processes to a minimum, so that the greater part of the original pools of phosphate-containing metabolites are preserved in the tissue. The best results are obtained by the use of a combination of cooling and oxygenation of the sample: The liver is kept in nutrient medium 199, cooled to 2-4°C, and intensively aerated with oxygen. By using these conditions stable ^{31}P-NMR spectra can be observed with a sufficiently high signal to noise ratio for 2-2.5 h. Human liver biopsy specimens were transported and their spectra recorded under the conditions described above, and the whole process did not take more than 2 h.

The typical ^{31}P-NMR spectrum of liver biopsy material from a patient with CCC is shown in Fig. 2. The signals in the spectrum were identified in accordance with the observations of Burt et al. [4]. The main contribution to the spectrum is made by phosphomonoesters (phosphorylated sugars, phosphoryl choline), P_i, adenine nucleotides, and uridine diphosphoglucose.

These investigations demonstrate the possibility of rapid comparative assessment of the concentrations of the main phosphate-containing compounds in human bile and liver biopsy material, for use in the clinical diagnosis of diseases of the liver and biliary tract.

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