Comparative Characteristics of the Mineral and Microelement Composition of Gallstones Extracted from Patients in the Novosibirsk and Omsk Regions

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(Received October 19, 2005; revised December 2, 2005)

Abstract

The mineral and microelement composition of gallstones extracted from patients in the Novosibirsk and Omsk Regions were investigated. It was stated that a common feature of choleolithiasis in the regions under comparison is noticeable predominance of cholesterol gallstones. It was shown that the prevailing element in gallstones is calcium, while differences in content and distribution of microelements in gallstones is determined by regional features. It was established that the main elements of bile are sodium, potassium and phosphorus, which is in good agreement with literature data.

INTRODUCTION

Investigation of biominerals and the process of pathogenic biomineral formation in a human organism is among the most important directions of mineralogical investigations. This direction is urgent in solving the problems connected with the formation of pathogenic biominerals in a human organism, which causes various diseases including cholelithiasis.

The role of microelements, including metals, in cholelithiasis has not yet been investigated completely by present, though it is known that many of these elements play an important part in metabolism and sustain permanency of the internal osmotic stability of tissue liquids. Definite amounts of chemical elements are also included into enzymes, hormones, pigments, protein complexes; they catalyse a large number of exchange processes and sustain normal vital activities of an organism. Along with other factors, the arrival of chemical elements into a human organism is determined by their concentrations in the environment, so any negative environmental processes are accompanied by various disorders of the functions of human organism. Investigation of the pathology connected with the action of microelements is going on for more than 20 years, and some authors made general conclusions about the diseases of biogeochemical nature.

In order to reveal the reasons of cholelithiasis taking into account local factors (both natural and industry-related ones), an important source of information may be the data on phase and microelement composition of gallstones. The distribution of biomineral and organic components over the bulk of concretion (multicomponent as a rule) as well as the character of its inner structure, microelement composition, even the shape – all these parameters depict the features of gallstone-forming medium and its evolution during formation, growth and subsequent transformation of a gallstone [2–4].

The goal of the present investigation is to compare the phase and microelement composition of gallstones extracted operatively from patients living in the Novosibirsk and Omsk Regions.

OBJECTS AND METHODS OF INVESTIGATION

We have studied the collections of 125 gallstones extracted from patients of the Novosibirsk Region, and 120 gallstones extracted from patients of the Omsk Region; gallstones were submitted by surgeons of the Regional Hospitals of Novosibirsk and Omsk. The phase composition was determined by means of diffraction studies, Raman and IR spectroscopy. X-ray analysis was used to determine polymorphous modifications of calcium carbonate and various calcium phosphates. Raman and IR spectroscopy allowed us to record and identify the substances (including the pigment), which could not be determined with the help of X-ray analysis because those substances did not possess crystal structure. Diffraction patterns were recorded with DRON-3M instrument with CuK_α radiation; IR spectra were recorded with Specord 75 IR and Pye Unicam SP3-300. The latter instrument allowed obtaining an intensive spectrum within the region of weak absorption bands without preparing the samples with a large weighed portion. The samples for IR spectroscopic investigation were prepared by pressing tablets with KBr. Raman spectra were recorded with Ramanor UI000 and RFS 100/S involving radiation at 514.5 and 1064 nm, respectively.

Element composition of the samples was determined at the station of energy dispersing X-ray fluorescence element analysis VEPP-3 of the Centre of Synchrotron Radiation of the Institute of Nuclear Physics, SB RAS (Novosibirsk, Russia). Emission spectra of the samples under investigation were excited in the beam of polarized monochromatic radiation with the energy of 25 keV. The samples were prepared by pressing the powder in tablets 30 mg in mass and 6 mm in diameter. The emission spectra were processed with the help of a special programme [5, 6]. The external standard method was used for the quantitative calculation. The detection limits for elements by means of XPA SR corresponded to the range $(2-0.1) \cdot 10^{-4}$ %, error of determination was within the range 2–5 rel. % for all the elements.

The element composition of bile samples was determined by means of atomic emission spectroscopy with inductively coupled plasma (AES-ICP). Measurements were carried out with a Perkin-Elmer Optima 2000 DV ICP-OES. The concentrations of elements were determined after sample mineralization with concentrated nitric acid, followed by decomposition in a microwave furnace. The results were processed with the software of the spectrometer. The detection limits of the elements by means of AES-ICP corresponded to the range $(5-0.1) \cdot 10^{-4}$ mg/kg, the error of determination of all the elements was within the range 1–3 rel %.

RESULTS AND DISCUSSION

Analysis of the phase composition of the collection of gallstones extracted from patients living in the Novosibirsk and Omsk Regions revealed that more than 90 % of the studied objects are composed mainly of cholesterol. Only in the Novosibirsk collection, three stone samples were discovered, which were composed of

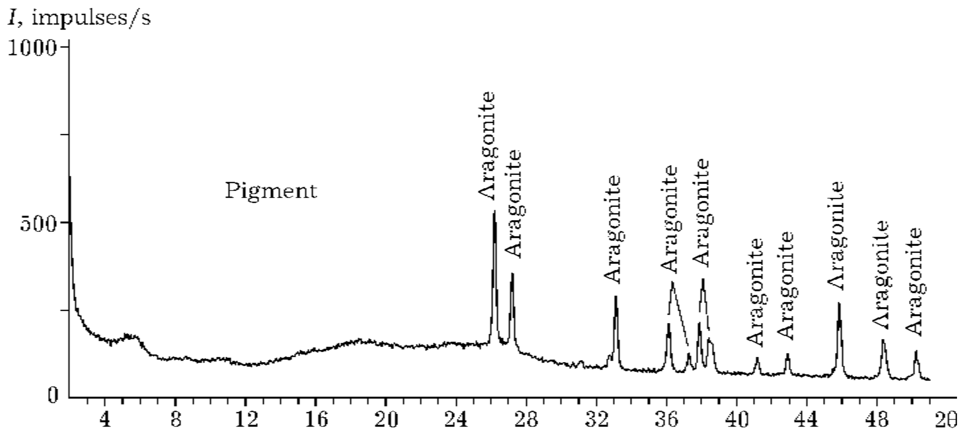

Fig. 1. Roentgenogram of gallstone composed of pigment and aragonite.

the pigment (mainly calcium bilirubinate); two of them contained a substantial amount of aragonite. The pigment was present in the majority of concretions under analysis reaching 20 mass % and more in some samples (Fig. 1). Among other components, the most widespread one was calcium carbonate in the form of three polymorphous modifications (faterite, aragonite, calcite): it was detected in 38 analyzed gallstones of the Novosibirsk collection (30 %), one third of which are carbonate-cholesterol ones, that is, contain comparable amounts of both components (Fig. 2). In general, the amount of $CaCO_3$ varied from insignificant to tens percent in some samples. Only in one patient the gallstones composed of the three modifications of calcium carbonate were revealed by means of X-ray examination.

The occurrence of different polymorphous modifications of $CaCO_3$ in mixed (multicomponent) gallstones from the Novosibirsk collection differs from that in natural systems. Faterite was detected in 87 % of the analyzed carbonate-containing gallstones, aragonite in 55 %, calcite only in 45 %. Somewhat different occurrence of the polymorphous modifications of calcium carbonate was observed in the Omsk collection in which it was present in concretions in insignificant amounts. For example, aragonite was detected in 50 % of carbonate-containing samples, faterite in 33 %, calcite in 17 %.

The results of XPA show that about a half of carbonate-containing stones turned out to be monophase with respect to one or another $CaCO_3$ modification. In all the other samples we observed the presence of two or three polymorphs at the same time in different quantitative relations, as a rule, in association with cholesterol. Both collections of gallstones are characterized by the zonal layered structure with alternation of layers of either different or identical mineral and phase composition, which is connected with the physicochemical properties of the stone formation medium.

In addition to the above-listed main components, we revealed also phosphates (apatite, vitlokite), calcium palmitate and various organic compounds [2, 7] occurring much more rarely and in small amounts. Analysis of the interrelation between $CaCO_3$ polymorphous modifications and the other components of concretions allows us to point out that aragonite formed is more often associated with pigment, whereas it is a rarity in the cholesterol stones. Layer-by-layer analysis of aragonite detected mainly in the cholesterol stones of the Novosibirsk collection showed that it is concentrated in the zones depleted of cholesterol or free from it. For instance, in one

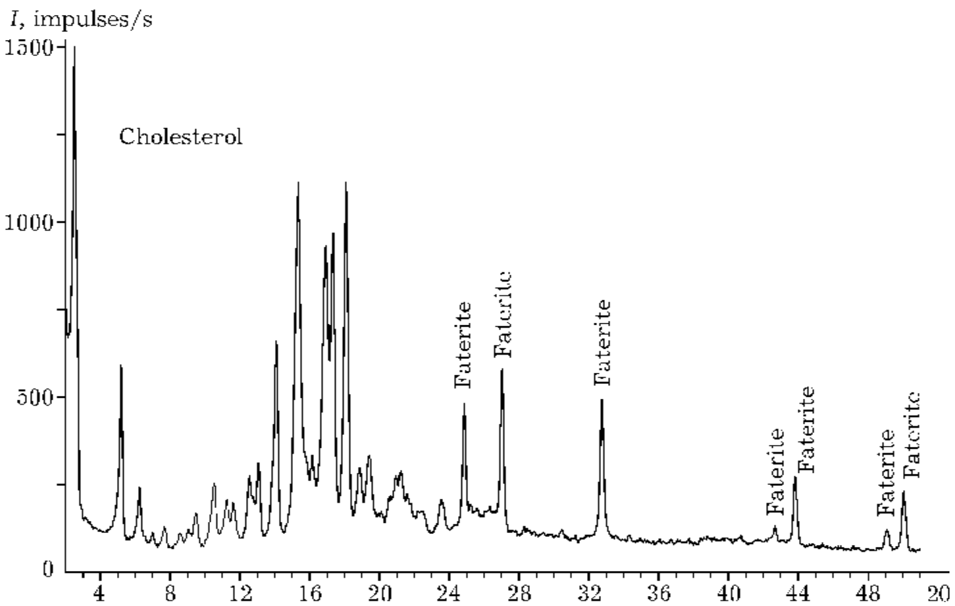

Fig. 2. Roentgenogram of gallstone composed of faterite and cholesterol.

of the carbonate-containing cholesterol stones, aragonite made a thick black outer shell of the stone; no other components were observed in the shell. To the contrary, in another stone aragonite was concentrated in cholesterol-depleted central part, along with the pigment, calcium phosphate and calcite.

When investigating the zonal distribution of mineral phases in gallstones by means of XPA and IR spectroscopy, the most interesting samples were those from the Omsk collection. The nucleus of one of the choleoliths is composed of cholesterol and aragonite. The middle zone contains three minerals – cholesterol, aragonite and faterite. No aragonite was detected in the stone shell; it is composed only of cholesterol and faterite.

So, we may note on the basis of the data obtained that one of the specific features of this stone is a change in the phase composition of the aggregate in consecutive layers from the centre to periphery. This is connected with the transition of calcium carbonate modification from aragonite to faterite and is likely to be due both to changes in stone genesis conditions and to secondary phase transformations characteristic of recrystallization of the deposited phase. Layer-by-layer analysis of another sample showed that all the zones of that stone were identical in composition and represented by cholesterol with the trace amount of aragonite.

In order to identify a wider range of components incorporated into the gallstones of the Omsk collection, the samples were extracted with chloroform (in some cases with ethanol). With the help of extraction separation of the cholesterol component, we succeeded in additional determination of the components of gallstones without a clear crystal structure. The sets of absorption bands for bilirubin occur most frequently (Fig. 3). Isolated instances of the detection of gallstones containing the sodium salt of bile acid and vitlokite were observed. In two cases, analysis of the spectra of insoluble deposits allowed us to assume the presence of vermiculite.

The combined XPA and IR spectroscopic investigation of the samples allowed us to state that the following main components are present in gallstones: cholesterol (92 % of the investigated collection), cholesterol with an additive of the bilirubin component, calcium carbonates of different modifications in cholesterol stones (aragonite, faterite, calcite) with the predominance of aragonite.

More than 36 elements with the concentration above $>10^{-5}$ mass % were identified in gallstones with the help of X-ray fluorescence analysis. The results of the microelement analysis of the samples from Novosibirsk can be represented as the following sequence illustrating the percent relations of microelements in gallstones: Ca>Fe>Cu>Mn> Zn>Cr>Br>Ti>Sr >V>Pb>Se. Calcium concentration varied from insignificant to several tens percent in some samples. Silver (7.68 µg/g) and the highest bis-

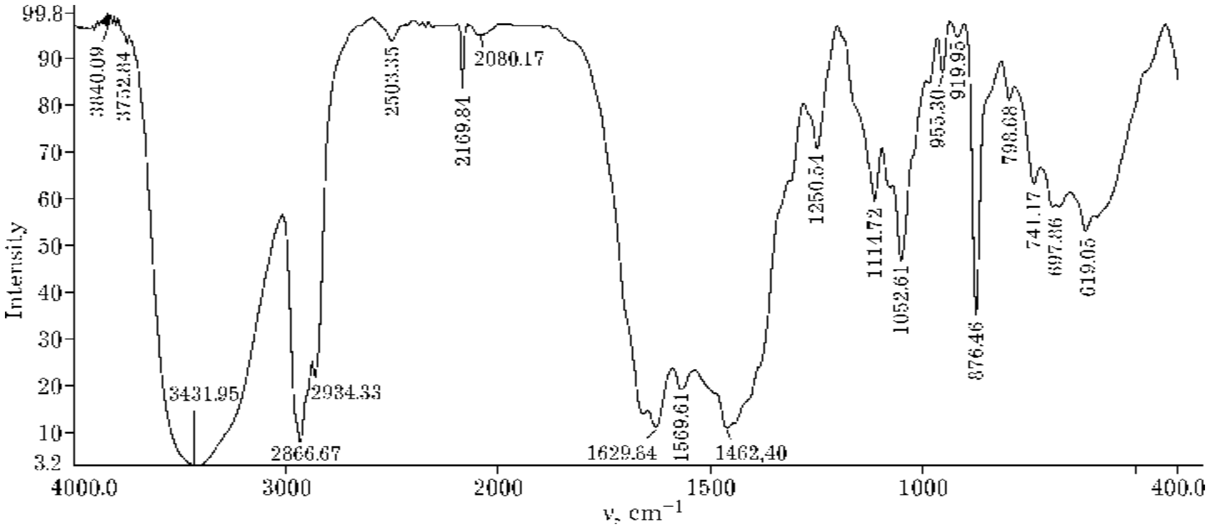

Fig. 3. IR spectrum of bilirubin (pigment of concrements).

muth concentration (32.4 μg/g) were detected in one of the pigment stones. The samples composed mainly of calcium carbonate are enriched with microelements: a stone composed of three polymorphous forms of $CaCO_3$ (sample 35) and the similar in composition black middle zone of stone No. 63 in which Fe, Zn, Sr and Pb are present in high concentrations, and the maximal concentration of Mn is observed. Two other cholesterol-carbonate zones of sample No. 63 are essentially depleted of microelements in comparison with the middle zone in which silver was also found (10.3 μg/g). It should be noted that this was the second stone of the analysed collection that contained silver. In the central part of the stone, elements Mn, Fe, Zn, Sr and Pb are present in the lowest concentrations but only here vanadium and increased amount of titanium were observed.

Gallstones or separate zones of stones composed only of cholesterol exhibit smaller diversity and, as a rule, the minimal amount of microelements. Only two of them (samples 2 and 22) exhibited an increased copper content (101 μg/g).

According to the obtained results, we may assume a reason of aragonite association with the pigment. It is likely that increased concentrations of such microelements as strontium, lead, copper *etc.* in the organism provoke deposition of the pigment because these elements can enter into its structure forming bilirubinates [2, 3]. Under the conditions favourable for the crystallization of carbonates, in the presence of large cations the aragonite structure is formed in which Ca is surrounded by nine oxygen anions, so that its coordination polyhedron has a larger volume than that in calcite and faterite structures in which the coordination number of calcium is equal to 6. In natural processes in the presence of strontium in mineral-forming solutions, aragonite structure is formed, too [8]. Manganese is likely to enter into the structures of all the three polymorphous calcium-containing modifications.

Analysis of the data obtained for the cholesterol gallstones of the Omsk collection revealed a group of 13 elements with the concentration above 10^{-4} mass %. According to the experimental data, the element concentrations in gallstones can be ranged in the following order: Ca>K>Mn>Fe>Cu>Pb>Ti>Zn>V>Ni>Bi>Cr.

We distinguished several gallstones sharply differing in the composition from the mean element concentrations in the samples. In the first sample, lead concentration exceeds the mean value for gallstones by a factor of 50. In the second stone, mercury concentration is 12 times larger than the mean value. In the third stone, we observed the maximal concentrations of copper (290 times as high as the mean value), iron (59 times) and bismuth 310 times). It is interesting to note that nickel concentration is minimal in this stone (10 times lower than the mean level).

It may be concluded on the basis of the data obtained that the features of microelement distribution in separate samples are connected with the specific features of physiological processes in a human organism.

Basing on the data of the comparative analysis, we revealed three groups of stones depending on the percent ratio of manganese, iron and copper in them. These elements were chosen because they are present in increased concentrations in cholesterol gallstones in comparison with other identified microelements. Increased concentrations of Mn, Fe and Cu in gallstones may be due to the ecological situation in the Omsk Region. According to the data of the Ob-Irtysh interregional territorial administration of hydrometeorology and environmental monitoring, the Irtysh River is most heavily polluted with the compounds of manganese (44 MPC), iron (24 MPC), copper (23 MPC), zinc (14 MPC), while the concentrations of manganese and copper in the Om River are higher than 100 MPC [9]. A comparative analysis of the data obtained by us with the literature data on the Transbaikalia was carried out [10] (Table 1). One can see that calcium is the dominant element in gallstones; wide variations in the concentrations and distribution of microelements in gallstones of the collections under investigation are due to the regional features.

It is interesting to compare the concentrations of microelements in the experimental samples and in bile (according to the data of [11]): unlike the experimental data, the element pre-

TABLE 1

Microelement concentrations in gallstones according to the experimental (Novosibirsk, Omsk) and literature data (Transbaikalia)

Element	Novosibirsk			Omsk			Transbaikalia		
	Test number	Mean concentration, mass %	Range of concentration, mass %	Test number	Mean concentration, mass %	Range of concentration, mass %	Test number	Mean concentration, mass %	Range of concentration, mass %
Ca	19	2.1060	–	12	2.0721	0.0525–17.8622	207	6.395	0.01–30
K	19	0.0581	–	12	0.0735	0.0136–0.1000	–	–	–
Mn	19	0.0426	0.0001–0.4993	12	0.0085	0.0006–0.0527	207	0.076	0.001–3
Fe	19	0.0604	0.0013–0.4262	12	0.0058	0.0005–0.0119	207	0.285	0.002–3
Cu	19	0.0433	0.0001–0.3654	12	0.0033	0.0009–0.0077	207	0.018	0.0005–1
Pb	19	0.0011	0.00005–0.0049	12	0.0025	0.0002–0.0224	57	0.002	0.0002–0.01
Ti	19	0.0020	0.0001–0.0085	12	0.0016	0.0009–0.0028	207	0.011	0.003–0.2
Zn	19	0.0055	0.0003–0.0513	12	0.0011	0.0004–0.0034	–	–	–
V	19	0.0011	0.0002–0.0109	12	0.0006	0.0001–0.0012	194	0.0012	0.005–0.01
Ni	–	–	–	12	0.0005	0.0001–0.0020	2	0.0012	0.0005–0.002
Bi	–	–	–	8	0.0005	0.0005–0.0005	8	0.002	0.0003–0.04
Cr	19	0.0039	0.00004–0.0695	12	0.0005	0.00002–0.0017	207	0.018	0.0005–0.05
Hg	–	–	–	4	0.0004	0.0001–0.0014	–	–	–
Br	19	0.0022	0.00008–0.0193	12	0.0007	0.00001–0.0031	–	–	–
Sr	19	0.0015	0.00002–0.0102	12	0.0005	0.00001–0.0044	–	–	–
Se	19	0.0007	0.00001–0.0049	12	–	–	–	–	–

vailing in bile is sodium, then go P, Ca, K, Mg, Fe, Cu.

In the present work we studied element content of bile collected from patients with choleolithiasis during operative stone ablation. Nine elements with the concentrations above 10^{-4} mass % were determined in bile. The abundance ratio of elements in bile is represented by the sequence: Na>Ca>P>K>Mg>Fe>Zn>Cu>Mn. According to the experimental data, the element prevailing in bile is sodium, which is in good agreement with literature data [11]. However, unlike the sequence presented in [11], the second place most abundant element is calcium, next to sodium. The rest part of the sequence is in line with that presented in [11]. The concentrations of such elements as P and K in pathogenic bile are 1.5 times as high as the normal levels, the concentration of calcium is 2.5 times higher, magnesium 4.5 (Fig. 4), while the concentration of iron is 3.5 times lower than the normal level.

So, it may be concluded that the main elements of bile are sodium, calcium and phosphorus, according both to the experimental data and to literature data.

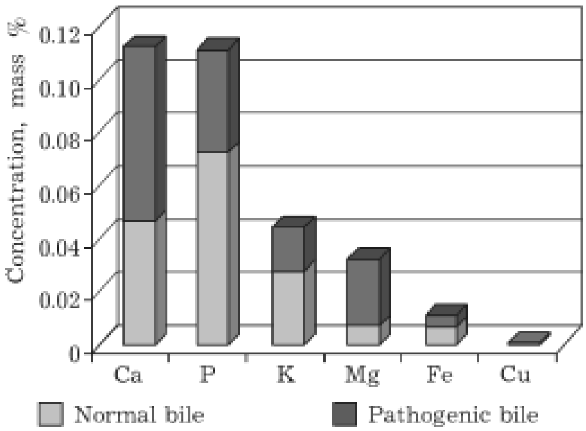

Fig. 4. Element ratios in the normal and pathogenic bile.

CONCLUSIONS

1. It was established by comparing the Novosibirsk and Omsk collections of gallstones that the samples extracted from patients liv-

ing in the Novosibirsk Region are characterized by a wider range of compounds incorporated into choleoliths. Calcium carbonate is present in both collections in the majority of samples; however, for the Novosibirsk samples, the predominant modification of $CaCO_3$ is faterite, while aragonite prevails in the Omsk collection.

2. The microelement composition of gallstones showed that calcium in them is a predominant element; differences in concentration and distribution of microelements in choleoliths from different geographic regions is a regional feature.

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