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RENAL STONES: CLASSIFICATION, STRUCTURE, METHODS OF ANALYSIS

A. V. Kustov, A. I. Strelnikov, E. V. Lyalyakina, M.A. Moriganov, T. V. Kustova

Kustov, A.V., Doctor of Chemical Sciences
Joint Physical-Chemical Center for Solutions, Krestov Institute of Solution Chemistry of RAS, Akademicheskaya ul., 1, Ivanovo, Russia, 153045
E-mail: kustov@isuct.ru

Strelnikov, A.I., Doctor of Chemical Sciences, Professor
Department of Urology and Faculty Surgery, Ivanovo State Medical Academy, Sheremetevskiy ave., 8, Ivanovo, Russia, 153012
E-mail: strelnikovprof@gmail.com

Lyalyakina, E.V., Moriganov, M.A.
Ivanovo Regional Hospital, ul. Lubimova, 1, Ivanovo, Russia, 15300
E-mail: okb-lab@ivanovo.ru

Kustova, T.V., PhD of Chemical Sciences
Ivanovo State University of Chemistry and Technology, Sheremetevsky ave., 7, Ivanovo, Russia, 153000
E-mail: kustova_tv@isuct.ru

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This review deals with the analysis of our own and most important published data, focuses on physical chemical methods for studying the structure, quantitative chemical and mineralogical analysis of renal stones and the relationship of their physicochemical characteristics with the risk factors of urolithiasis, metabolic and genetic disorders occurring for a particular patient. We have shown that renal stones reveal a complex organization, can contain many chemical compounds, a number of which form stable mineralogical phases of a certain structure. The main types of urinary stones are considered and it is pointed out that the methods of analytical chemistry, elemental and X-ray spectral microanalysis can be used for studying the structure of renal calculi. However, they do not provide the information about their mineralogical composition and microstructure, which is important for diagnostic tasks. In contrast, the methods of scanning electron microscopy as well as the methods of polarization microscopy, FTIR spectroscopy and X-ray phase analysis reveal undeniable advantages for analyzing these species and allow to show how the information of texture of the stone surface and the appearance of certain mineralogical phases helps to clarify the cause of stone formation in the body and prescribe an appropriate treatment.

Introduction

Urolithiasis or kidney stone disease (KSD), associated with the formation and growth of organo-inorganic deposits in the urinary system, continues to be an important medical and social problem, affecting, it is believed, from 1 to 20% of the world's population [1]. In many industrially developed countries of Europe, the incidence of urolithiasis exceeds 10% [1, 2], and epidemiological studies in recent years have noted a persistent trend towards an increase in the incidence of urinary stones among the population. In particular, the number of newly diagnosed cases of kidney stone disease per 100 thousand people in the Russian Federation in the period from 2002 to 2014 increased by 25% [2], and in the USA, Western Europe and, especially, in the countries of the Middle East, it is still even more significant [1, 3]. An increase in the incidence of urolithiasis and an increase in recurrence, averaging 50-75% over a 10-year period [1, 4], significantly increase the costs of outpatient and inpatient treatment of patients, calling for conceptual changes in the strategy of treatment of urolithiasis. Taking into account that the kidney stone disease has a pathogenetic relationship with nephrocalcinosis, diabetes, atherosclerosis [5], and, judging by recent data, with bladder tumors [6], the absence of simple and effective approaches to diagnosis and conservative therapy of urolithiasis, based on information about the mineralogical and chemical composition of stones, features of their structural organization daily excretion of inhibitors and promoters of stone formation, creates objective difficulties in identifying the causes of the disease, prescribing an adequate approach to treatment and correction of metabolic disorders and risk factors in order to reduce the frequency of recurrent stone formation and the threat of development of the above comorbid pathological conditions [5].

The cornerstone of all diagnostic measures is the quantitative determination of stone composition [7], which in domestic clinics is either not performed or limited to semi-quantitative determination of the main anions (phosphates, oxalates, urates) that make up the stone salts. As the results of many studies show, in most cases it is not enough to establish the causes of stone formation and prescribe the appropriate metaphylactic treatment [1, 7]. Firstly, as can be seen from Table 1, the stones may contain several minerals simultaneously giving urate, phosphate or oxalate ions in solution, and thus it is impossible to establish the composition of salt, knowing only the anion.

Secondly, even knowledge of the chemical composition of the concrement tells very little about the metabolic abnormalities and KSD risk factors that caused the formation of the concrement. Indeed, hydroxyapatite (HA)-based phosphate stones form predominantly in slightly acidic or neutral urine and are often associated with renal tubular acidosis (7, 8). In contrast, struvite and carbonatapatite stones (also phosphate) formed in severe alkalinization caused by infection of the urinary tract with urease-producing bacteria [7,8]. It is not difficult to demonstrate that a similar picture is observed in the analysis of urate and calcium-oxalate stones [9-12].

Table 1. Varieties and chemical composition of the main minerals most commonly found in urinary stones [3]

Mineral	**Chemical Name**	Formula
	Uric acid	$C_5H_4N_4O_3$
	Uric acid dihydrate	$C_5H_4N_4O_3 \cdot 2H_2O$
	Ammonium dihydrogenurate	$C_5H_7N_5O_3$
	Sodium hydrogenurate monohydrate	$NaC_5H_3N_4O_3 \cdot H_2O$
	Cystine	$C_6H_{12}N_2O_4S_2$
	Xanthine	$C_5H_4N_4O_2$
	2,8 – dihydroxyadenine	$C_5H_5N_2O_2$
Whewellite	Calcium oxalate monohydrate	$CaC_2O_4 \cdot H_2O$
Weddellite	Calcium oxalate dihydrate	$CaC_2O_4 \cdot 2H_2O$
Whitlockite	Tricalcium phosphate	$Ca_3(PO_4)_2$
Hydroxyapatite	Pentacalcium hydroxyphosphate	$Ca_5(PO_4)_3OH$
Carbonate apatite	Basic calcium phosphate with carbonate	$Ca_{4,75}(PO_4)_{2,65}$ $(OH)_{0,85}(CO_2)_{0,35}$
	Octacalcium phosphate	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$
Newberite	Magnesium hydrophosphate trihydrate	$MgHPO_4 \cdot 3H_2O$
Struvite	Magnesium ammonium phosphate hexahydrate Magnesium ammonium phosphate monohydrate	$MgNH_4PO_4 \cdot 6H_2O$ $MgNH_4PO_4 \cdot H_2O$
Brushite	Calcium hydrophosphate dihydrate	$CaHPO_4 \cdot 2H_2O$
Calcite, waterite, aragonite	Calcium carbonate	$CaCO_3$
Opal, tridymite	Silicon dioxide	SiO_2
Gypsum	Calcium sulfate dihydrate	$CaSO_4 \cdot 2H_2O$
Bobierite	Trimagnesium phosphate octahydrate	$Mg_3(PO_4)_2 \cdot 8H_2O$
Gopeite	Zincphosphate hexahydrate	$Zn_3(PO_4)_2 \cdot 4H_2O$
Monetite	Calcium hydrophosphate	$CaHPO_4$
	Trimagnesium orthophosphate pentahydrate	$Mg_3(PO_4) \cdot 5H_2O$
Gannaite	Trimagnesium ammonium phosphate octahydrate	$Mg_3(NH_4)_2$ $H_4(PO_4)_4 \cdot 8H_2O$
Monohydroxicalcite	Calcium carbonate monohydrate	$CaCO_3 \cdot H_2O$
Humboldtite	Ironoxalate dihydrate	$FeC_2O_4 \cdot 2H_2O$

Recent data (see [10-12] and references there), including our own clinical and laboratory studies [12], show that the relationship of a number of metabolic disorders with the mineralogical composition and texture of calcium oxalate mono- and dihydrate-based stones is established quite definitely. In particular, studies in France, Italy and Russia have reliably established that stones containing large amounts of calcium oxalate dihydrate are typical for younger patients and are associated with severe hypercalciuria and hypocytraturia (rapid crystallization), whereas in patients with calcium oxalate monohydrate stones the deviations are markedly weaker (slow crystallization) and are often associated with hyperoxaluria. Thus, it is obvious that the quantitative determination of all mineralogical phases of calculi is extremely important for proper diagnosis of the stone formation causes and development of an effective scheme of metaphylactic therapy to prevent recurrence of urolithiasis.

Classification and structure of urinary stones

So, urinary stones are solid organo-inorganic formations of biological origin localized in the urinary system [8, 13-15]. The vast majority of urinary stones consist of crystals of organic

or inorganic nature, bound by the so-called matrix [8, 15, 16], consisting of organic substances soluble in urine - proteins, glycosaminoglycans, phospholipids, etc. [13-16]. Usually, the proportion of matrix, additionally binding crystallites, varies at the level of 2-5% of the stone weight and very rarely reaches higher values [15, 16].

Although in the domestic clinical practice the simplified terminology is still used, classifying the concrements according to the salt anions and dividing them into oxalates, phosphates, urates and their mixtures (see above) [2, 8], nowadays the mineralogical classification of urinary concrements is generally recognized [13-15], according to which the stones are classified by the main mineral contained in the stone. The most common chemical compounds of organic and inorganic nature, which are the structural elements of human urinary stones, are listed in Table 1. One of the leading experts in the study of the structure of urinary tract deposits H. Schubert has analyzed more than 100 000 urinary stones and discovered 145 different combinations of minerals [13, 15] that make up the concrements. However, it turned out that only 25 combinations (types) of stones have a frequency above 0.1% and only 13 above 1% [15]. Calcium oxalate hydrate stones, often with an admixture of apatite, are the most common in the human population. More than 75% of patients with KSD have them. Most of these stones are either pure calcium oxalate monohydrate (COM) or monohydrate with some amount of calcium oxalate dihydrate (COD) and/or hydroxyapatite.

2.1. Stones based on calcium oxalate hydrates

Calcium oxalate hydrate stones are the most common type of urinary stone. As seen in Fig. 1 a, b, this type of stones has a dense touch, round or irregular shape, often with a spiky surface, and in many patients, they are stained due to adsorption of blood heme decomposition products in the urine.

Among calcium oxalate hydrates it is customary to distinguish two forms of minerals (see Table 1): Whewelite (calcium oxalate monohydrate, COM, CaC_2O_4-H_2O) and weddellite (calcium oxalate dihydrate, COD, CaC_2O_4-$2H_2O$). A number of studies have reported the presence of calcium oxalate trihydrate (COT) in a number of stones, but this view is still not generally accepted [15]. Nevertheless, in the X-ray phase analysis of one stone in a patient from Ivanovo region, we clearly observed a sufficiently large amount of CCH in a mixed calcium-oxalate stone. Thermo-dynamically stable COM. Often in mixed COM + COD stones there is a situation where the unstable COD loses one water molecule over time, gradually changing to the more stable COM., markedly dominates the prevalence of the two forms of calcium-oxalate stones. This transformation can to some extent complicate the diagnosis of metabolic disorders, based on the mineralogical composition of the stone, because different metabolic disorders are responsible in principle for the formation of stones based on COM and COD [10-12].

2.2. Stones based on calcium and magnesium phosphates

Among phosphate stones there are stones associated and not associated with urinary infection [7, 8, 15, 17]. The most dangerous for the urinary tract is infected struvite stones (Fig. 1, d), often mixed with carbonate apatite, which are formed due to pathogenic microorganisms that produce urease and break down the urea in the urine.

Struvite stones are formed only in an alkaline environment, when the pH of the urine does not fall below six (8, 17). Due to infection, the urine is oversaturated with magnesium, ammonium, phosphate and often calcium. Because of a significant shift in pH to the alkaline region, deposits are precipitated as crystals of phosphorus- and ammonium salt - struvite, often together with carbonate apatite [17, 18]. Struvite is formed only in infected urine (the most complete list of gram-positive, gram-negative bacteria and yeasts is given in [17]), it increases in size very quickly, often forming coral stones (see Fig. 1, d). Sometimes when staying in the urinary system for a long-time struvite is partially or completely transformed into newberrite [15]. There have been many clinical cases in which urease-producing bacteria have led to the formation of coral stones occupying the entire renal pelvis within one to two months [17]. Treatment of struvite urolithiasis, along with antibiotic therapy and urine acidification, involves the mandatory removal of all stone fragments from the urinary tract [7, 8, 17]. Struvite stones are most common in Great Britain and, oddly enough, in Belarus [3].

Fig. 1. Morphology of the most common urinary stones: a - KOM; b - KOD; c - brushite; d - coral-shaped stone based on struvite and carbonate apatite (CA); e - uric acid stone (AK) [15].

As for carbonate apatite, its presence in concrements is not always associated with urinary infection and may be a consequence of distal renal tubular acidosis (RCA) [8, 18]. Nevertheless, recent studies have shown [18] that there is a linear relationship between the degree of carbonization of apatite and the number of bacterial imprints per surface unit of concrements. In many cases, the authors [18] managed to identify the microorganisms contributing to stone formation. In particular, E. coli was found not to break down urea and not to alkalize the urine, but the presence of bacteria significantly reduces the level of citrate ions in the urine and contributes to the appearance of hydroxyl and carbonate apatite crystals [18]. It is concluded that bacterial infection, often latent in the past, regardless of the ability of bacteria to produce urease, contributes to the formation of phosphate stones based on apatite with varying degrees of carbonization. This largely explains the fact that apatite stones are much more common in women than in men, in whom urinary infection is simply less common [18]. Thus, the presence of carbonate apatite in the stone in many cases indicates a pre-existing or current urinary infection, which makes it necessary to investigate the urine culture for pathogenic microflora.

Hydroxyapatite, and especially brushite stones (see Fig. 1, c) are formed in more acidic urine (brushite in general in a narrow range of pH = 6.4-6.8 [8]) and are usually not associated with urinary infection, although as noted above, E. coli can cause nucleation and aggregation of hydroxyapatite crystals. The most probable reasons of bruchite stones formation (see Table 1) are PKA and hyperparathyroidism [7]. It became fashionable to explain formation of apatite and mixed phosphate-oxalate stones for the last years by nanobacteria activity (see [8, 19, 20]). Brushite stones are found in only 1-2% of patients, but their frequency continues to increase [8, 15] and, most importantly, these concrements show an extremely high recurrence rate. In this regard, the presence of even small amounts of brushite in a stone should serve as a trigger for extended diagnostic and metaphylactic measures aimed at preventing recurrence of KSD [7]. Either the other phosphate minerals listed in Table 1 practically do not form independent urinary stones and occur as products of transformation of the chemical compounds described above, or as small admixtures to apatites or calcium oxalate hydrates.

2.3. Stones of uric acid and its derivatives

Concretions consisting of uric acid, its hydrates or salts are commonly referred to as urates. Urate stones (see Fig. 1,e) are observed in 10-15% of patients and belong to organic deposits, because there are no such deposits outside the body [8, 13, 15]. The main mineral of urate stones is uric acid; its monohydrate is extremely rare [21]. Dihydrate stones or stones with dihydrate admixture to anhydrous uric acid are most common in very acidic urine [22]. With the exception of infected ammonium urate [8, 15] stones from uric acid salts are very rare; sometimes uric acid salts are present as peripheral impurities in stones from uric acid. Because uric acid crystals often act as a matrix for the formation of calcium oxalate hydrate stones, promoting the precipitation of COM, uric acid or its salts are often found in the core of calcium oxalate concretions [23]. In most cases, urate stones are yellowish or yellow-red, round, slightly roughened stones of dense consistency. Sometimes they form coral-like structures. The common reasons for the formation of uninfected urate stones are increased excretion of uric acid (disorders of purine metabolism), high osmolarity of urine and, most importantly, its daily pH profile sharply shifted to the acidic area (uric acid diathesis) [24].

2.4. Cystine stones

Cystine stones, which are of protein nature, are very rare. They are found in approximately 1% of patients and are usually detected in childhood [3, 27]. The etiology of cystine stones is based on a genetic disorder, a hereditary disorder of the tubular reabsorption of four major amino acids: cystine, ornithine, lysine and arginine [8, 25]. Ornithine, lysine and arginine are highly soluble, whereas cystine is poorly soluble, and in the presence of hypercalciuria and cystinuria over 200 mg per day is the main cause of cystine stones [8]. The disease is inherited by autosomal recessive type and is more typical for men (70% of patients) [8]. High urine density, high intake of fatty protein and salty foods increase urinary cystine excretion.

2.5. Xanthine and dihydroxyadenine stones

The main constituents of these extremely rare observed concrements are xanthine and dihydroxyadenine, products of purine metabolism in the body. The color of the stones varies from light brown to dark brown. Both stones are due to an autosomal recessive type of purine defect (genetic defect). In both cases family diagnoses are possible [8].

2.6. Stones formed due to medication

The last type of stone we will consider in this review is stones formed under the influence of medications taken [8, 26]. They are also quite rare and occur in less than 2% of patients [26]. Two mechanisms lead to the formation of such concrements. In the first case, the stones consist of either the drug itself, such as indinavir or triamterene, or their metabolites. In the second case, there are deposits that do not contain the drug or its metabolites, but formed as a result of a shift in homeostasis under the influence of the drugs taken. For example, long-term intake of high doses of ascorbic acid (more than 4 g per day) or calcium/vitamin D-containing supplements is thought to lead to stone formation from calcium oxalate hydrates [26]. The most detailed list of medications found in urinary stones is given in [26].

Analysis of the composition and structure of urinary stones

For qualitative and semi-quantitative analysis of the chemical composition of urine stones classical methods of analytical chemistry have been used for a long time. In particular, one of the methods described in the literature stipulates mineralization of a part of a product by the method of "dry" ashing in order to separate an organic component, the subsequent visual analysis of a stone residue and determination of an algorithm of actions to define the organic or inorganic substances of a stone [8]. Possible methods of qualitative definition of substances present in urine stones are described in detail in the work [8], and we are not going to dwell on them. Although these methods are still used in the domestic clinical practice and give some information about the composition of concrements, they do not allow to obtain the necessary information about the quantitative mineralogical analysis of a stone [13, 15]. In particular they do not allow to distinguish mono- and dihydrate calcium oxalate, uric acid from its salts or hydrates, infected (struvite, carbonate apatite) from non-infected (brushite, hydroxyapatite) phosphates, etc. Even the use of modern analyzers and atomic absorption spectrometers capable of quantitative and highly accurate determination of the content of chemical elements in

various samples does not solve the problem of quantitative analysis of the chemical and mineralogical composition of concrements.

Indeed, the number of chemical elements of which material bodies are constructed is limited to a number only slightly exceeding a hundred. In the case of urinary stones, the number does not exceed ten. However, complex substances formed as a result of combining the elements with each other, as can be seen from Table 1, are counted in tens, and if we consider compounds of organic matrix, metal ions and other minor components - in hundreds. These complex substances have very diverse properties and the difference of these properties is due not only to the differences in the chemical composition of the substances but also to the differences in the mutual packing of their atoms and fragments in the condensed phase. Thus, to characterize urinary stones, which are often a mix of different mineralogical phases with different types of molecular packing, it is necessary to use methods capable to study not only the atomic composition but also the supramolecular structure.

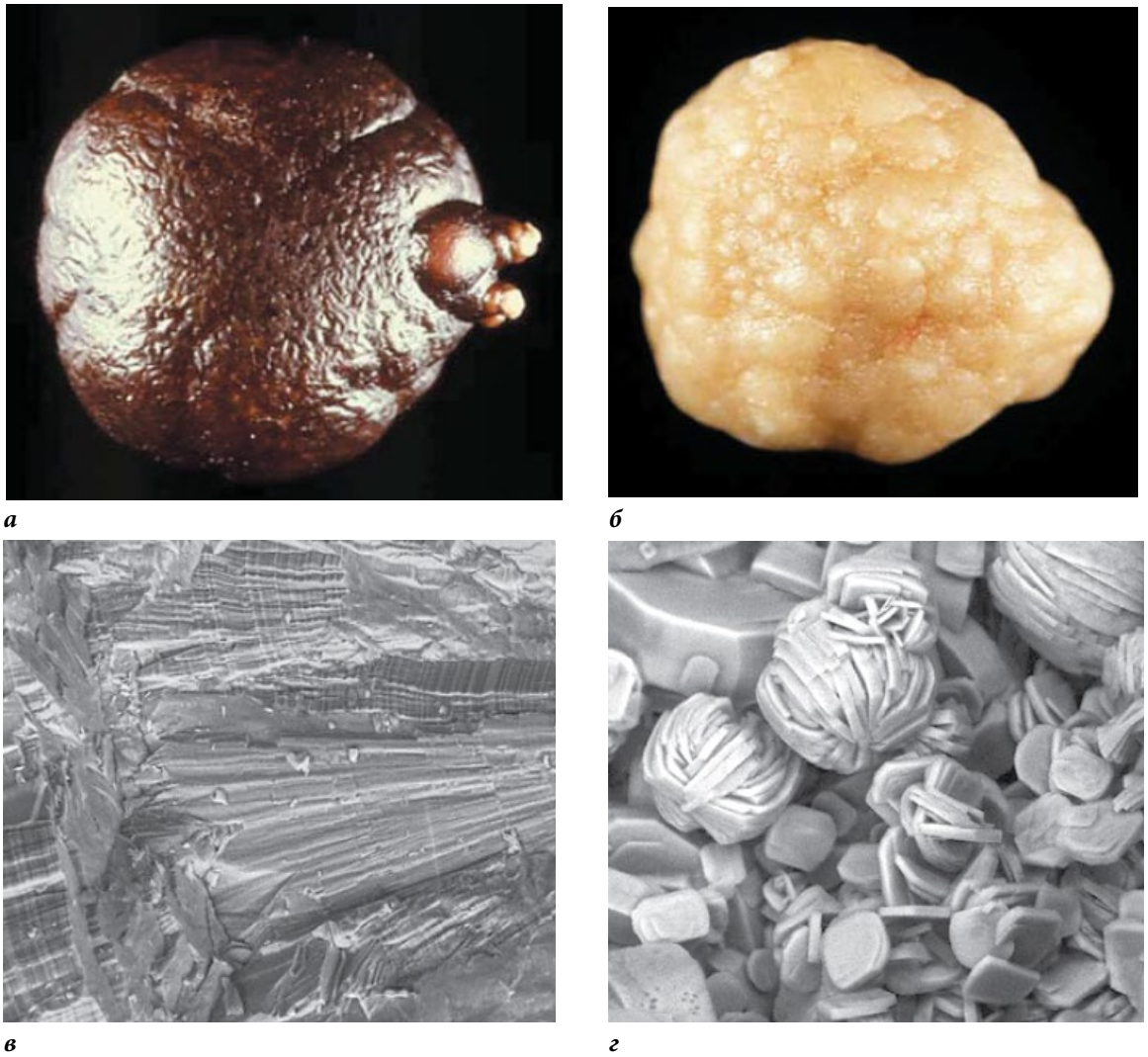

Fig. 2. Different types of stones based on COM [11, 27]: a - slow crystallization due to high osmolarity of urine; b - fast intratubular crystallization due to type I hyperoxaluria; a, b - appearance, c, d - SEM data

Scanning electron microscopy (SEM) and X-ray microanalysis (XRM) can be used to study the morphology, texture and surface composition of urinary stones. The results of these studies are often very useful for understanding the causes of stone formation, as well as for the

targeted selection of litholytic solutions to dissolve the stones (see, in particular [11, 27-31]). Indeed, relatively recently it has been discovered that severe genetic disease of hyperoxaluria I type is accompanied by formation of stones on the basis of COM of a completely definite microstructure [11, 27]. Rapid crystallization leads to the formation of weakly ordered crystallites, which can be clearly seen in the analysis of the stone by SEM (Fig. 2, d). This makes it possible to use SEM data for rapid detection of this disease without resorting to an extremely invasive liver biopsy.

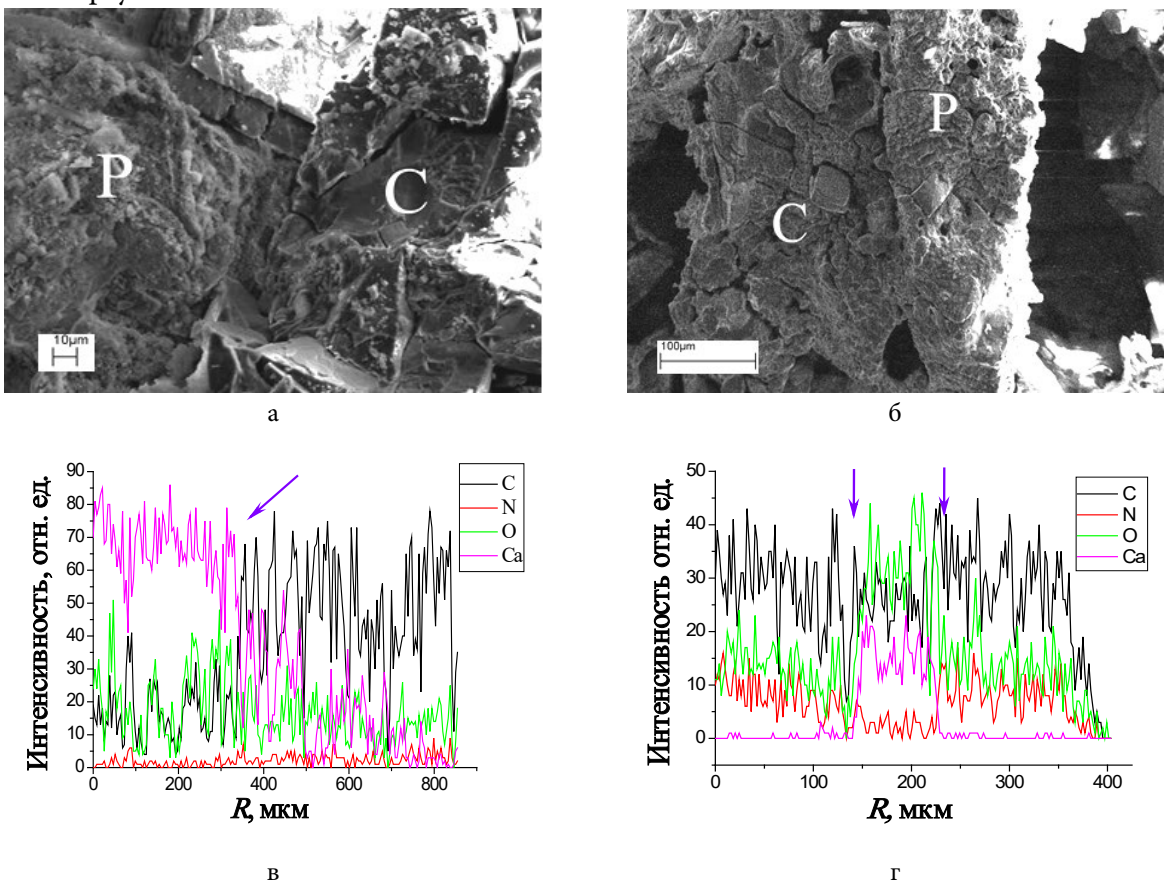

Fig. 3. SEM of calcium oxalate hydrate stone with an admixture of HA (oxalate/phosphate ratio ~ 3): a - the initial sample; b - after 4-hour etching with % aqueous solution of dinatrium salt of EDTA [28]. Symbols C and P designate areas with high content of COM and GA, respectively; c, d - study of stone surface composition by PCA method [30]: c - scanning of calcium-oxalate stone sample surface. The arrow shows the boundary between untreated and Na_2H_2Edta-treated areas where Ca concentration drops sharply; d - scanning of a section of urate stone having a layer of calcium oxalate hydrates inside (shown by arrows)

The joint use of SEM and PCA to study the mechanisms of litholysis of oxalate-phosphate and phosphate-oxalate stones allowed us to reveal interesting law-determinants in their structure and its transformation under the action of litholytic reagents [28-30]. As can be seen from Fig. 3, a, the surface of the oxalate-phosphate nodule is extremely heterogeneous. The data allow to distinguish on it the areas composed either mainly by hydrates of calcium oxalate (symbol C), or by hydroxyapatite (symbol P). This heterogeneity significantly affects the interaction of Na_2H_2Edta-based litholytic solutions with the stone surface [28]. Fig. 3, b shows that the dissolution of the concrement proceeds predominantly along the regions composed of HAs. In this case, visually noticeable cracks and caverns are formed in the stone, reducing the hardness

of the stone and facilitating its destruction by high-energy waves. The concentration of calcium ions on the surface according to PCA data in the C area decreases from 9 to 4% at., and in the P area from 18 to 7% at. [28]. However, the PCA and SEM data do not allow to determine how much and what mineral was before and after etching. Moreover, the given information usually concerns only to a surface of a concrement which composition can essentially differ from composition of a stone as a whole. In particular, for 25% of urinary stones, the core of the stone has a different composition from the periphery [15].

Methods of differential thermal analysis, electron diffraction, laser and electron beam microzoning, polarization microscopy, FTIR spectroscopy and X-ray phase analysis were and are widely used for investigation of urinary stones [15]. However, only the last three, as well as the neutron diffraction method, proved to be effective for quantitative mineralogical analysis of the composition of concrements [11, 15, 31-33]. The method of polarization microscopy is based on the interaction of polarized light with crystals included in the stone [15], and allows one to obtain information on the morphology and mineralogical composition of the concrement (see Fig. 4, a, b).

Advantages of this method are: (a) relative speed of the analysis; (b) low cost of research; (c) possibility to analyze the content of minor components in the samples [15]. In turn, the disadvantages of the method are the need for extensive experience in such studies, the difficulty of quantitative determination of the composition of multicomponent stones, especially in the case of mixtures of uric acid and its hydrates. The method of IR-Fourier spectroscopy is based on the analysis of the interaction of infrared light and the molecules that make up the stones [15]. Light excites various kinds of oscillations in molecules and their fragments, which causes a decrease in the intensity of radiation that has passed through the sample. The observed absorption does not occur in the entire spectrum of incident radiation, but only at wavelengths, whose energy corresponds to the excitation energies of vibrations in the molecules under study. Therefore, wavelengths at which maximum IR absorption is observed testify to the presence of certain functional groups and other required fragments in the sample molecules. This is widely used in various chemistry fields for the structure identification of compounds. The use of the total reflection method in modern models of FTIR spectrometers has greatly simplified the analysis of urinary stones [15], but in many cases laboratories use FTIR spectrometers of old samples.

Advantages of the FTIR spectroscopy method are: a) low cost of analysis; b) rapid determination of composition using automatic Fourier transform of the spectrum; c) the possibility of studying small quantities of the sample and, importantly, amorphous phases. Nevertheless, the method has a number of certain drawbacks, such as: 1) duration of sample preparation when using conventional spectrometers; 2) difficulty in determining the composition of stones in the case of analysis of mixed concretes containing close structure components, in particular mixtures of different phosphates or derivatives of KSD. The analysis is also difficult in the case of small amounts of mineralogical phase, which structurally insignificantly differ from the main component of the stone, for example, small additions of COD to COM-based stones (see, for example, Fig. 4, c).

The method of radiographic analysis (XRF) is the study of the interaction of X-rays with the crystal lattice of the studied sample [15]. Its lattice, a certain chemical composition and a

certain distribution of atoms on the lattice unit cell characterize every crystal substance. The geometry of any lattice defines a set of interplanar distances and, hence, Bragg angles for diffraction of X-rays, electrons, or neutrons at a given wavelength (Fig. 4d, e). Individuality and distribution of atoms determines the intensity of diffracted rays. Thus, the diffraction pattern is a kind of "passport" of a chemical compound, which can be used to determine which of the previously known compounds the obtained X-ray diffraction pattern corresponds to.

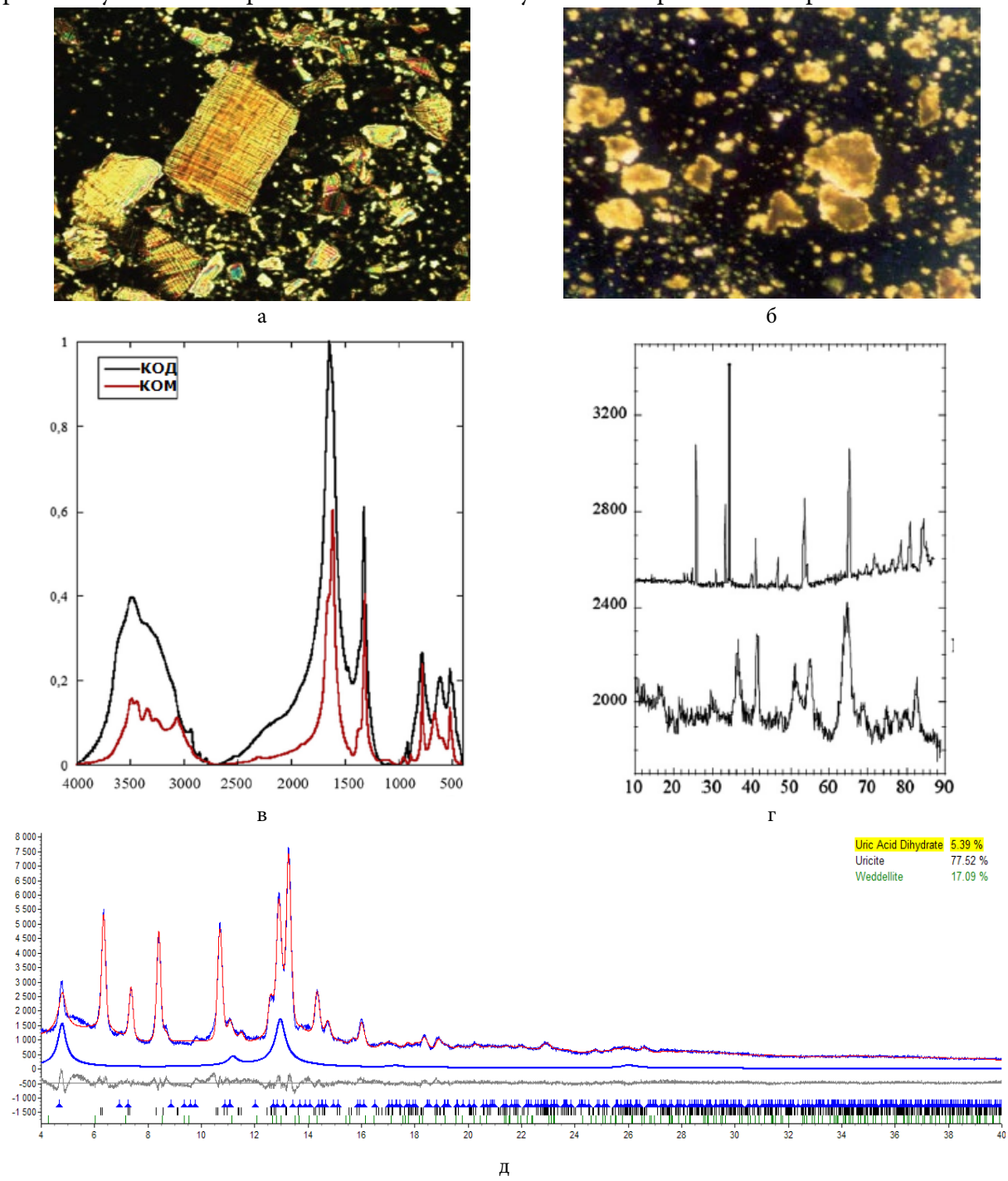

Fig. 4. Study of stone composition by physical and chemical methods [15, 18, 30, 31]: a, b - polarization microscopy of stones based on COM and HA, respectively; c - IR spectrum of stones composed of COD (upper curve) and COM (lower curve); d - neutron diffraction on stone samples from struvite (upper curve) and CA (lower curve); e - quantitative XRD analysis of three-component stone from KSD (78 % wt.), its dihydrate (5% wt.), and COM (17% wt.)

Advantages of the XRF method are quite obvious and include the following: a) the preparation is easily prepared and is not destroyed in the analysis, which is performed automatically; b) a small amount of substance is required for the analysis; c) there is no need to grow and orient single crystals of the compound; d) the use of appropriate computer programs and databases allows for quantitative analysis of di-fractograms even in case of complex mixtures. Quantitative X-ray diffraction analysis using Rietveld procedure and appropriate software allows: determining quantities of crystalline phases in a mixture (see Fig. 4, e); determining average crystallite sizes, functions of their size distribution, by analyzing the profile of lines; studying texturing, i.e., the nature of preferential orientation of crystallites. The disadvantages of the RFA method are the high cost of equipment and the inability to analyze amorphous materials.

All three methods described above have their advantages and disadvantages. The most commonly used in scientific research methods of FTIR spectroscopy and XRD are almost identical in accuracy, although for the analysis of mixtures of different crystalline phases the XRD method is more preferable [15]. Ideally, the laboratory should be able to use all three methods to analyze the composition of concrements, although this is obviously possible only in large diagnostic centers.

As for research of an organic matrix of a stone, consisting of lipids, glycosaminoglucans and proteins, as it was already noted, it usually makes 2-5 % from weight of a dry sample. More than 60% of the matrix consists of different proteins of urine, of which there were no more than 30 until 2004 [16, 34]. With the advent of new methods of investigation, such as high-performance liquid chromatography with tandem mass spectrometry, time-of-flight matrix mass spectrometry with laser ionization (MALDITOF) and understanding of the diagnostic role of urine proteins as markers of various diseases, their number has increased to 70 in just a few years. There is no doubt that matrix plays an important role in the formation of urinary stones. Depending on the change of protein globule structure the same proteins can act both as promoters and inhibitors of concrements formation and growth [34], and attachment of deposits to the renal epithelium occurs only after its damage, that is destabilization or destruction of the double lipid layer. Thus, we come to an understanding of the important role of active oxygen species in the processes of "pre-organization" of the matrix as one of the "triggers" of stone formation. We are confident that in the future this research will con-tribute significantly to the understanding of the pathogenesis of urolithiasis.

Conclusion

Over the past thirty years, improvements in remote shockwave and contact laser lithotripsy techniques, modern endoscopic techniques and expulsive therapy have largely limited open surgical interventions in the treatment of KSD. In this regard, for many patients, rapid and minimally invasive removal of concrements proved to be preferable to long-term metaphylaxis of recurrent stone formation associated with changes in lifestyle, dietary restrictions and taking appropriate drugs [7]. However, unrepaired KSD risk factors aggravated by certain metabolic disorders often lead to very adverse consequences associated not only with the formation of new concrements in the urinary tract, but also, as noted above, with the appearance of a whole bouquet of comorbid pathological conditions [5]. In addition, almost 20% of patients with recurrent urolithiasis are thought to develop nephrocalcinosis and chronic renal failure

over time [1]. Obviously, the cost of treating such severe complications is disproportionately higher than the tedious but essential diagnostic and metaphylactic measures for the treatment of urolithiasis [7, 33, 35, 36]. However, carrying out of these measures is largely "tied" on quantitative definition of all mineralogical phases of a stone, and in some cases on research of its microstructure. Only in this case there is an opportunity to establish the true causes of stone formation, to reduce the recurrence of urolithiasis, to improve the quality and duration of healthy life of many patients.

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