Journal of Optoelectronics and Advanced Materials Vol. 6, No. 1, March 2004, p. 261 - 268

GROWTH AND DISSOLUTION OF CALCIUM OXALATE MONOHYDRATE (COM) CRYSTALS

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Calcium oxalate monohydrate (COM) crystals were obtained by different methods. The tendency of COM to form splices, twins, spherulites and dendrites was demonstrated. The mechanism and kinetics of growth of two out of three basic faces were investigated by *in situ* atomic force microscopy. The rate of COM dissolution was revealed to increase in the presence of aluminum and iron ions. Substitution of Aqua RX water for distilled water also increased the dissolution rate.

(Received September 23, 2003; accepted February 19, 2004)

Keywords: Calcium oxalate monohydrate, Atomic force microscopy, Nucleation, Surface structure, Step kinetics, Dissolution

1. Introduction

Investigation of the nucleation process, the mechanism and kinetics of growth and dissolution of substances constituting renal stones is important for understanding the etiology of urolithiasis and working out the methods of treatment. Homogeneous nucleation of crystals is known to occur at very high supersaturation and the nucleation probability at a moderate supersaturation is much greater on a particular surface. Therefore it is useful to know what kinds of material particles promote nucleation. Second, it is possible to inhibit the growth of a crystal by impurities adsorbed on its surface. These impurities hinder the moving growth layers and at a sufficient concentration create a supersaturation "dead zone" where no growth occurs at all. Third, it is desirable to find a solvent that would dissolve the formed crystals faster than water does.

The present paper is devoted to crystals of calcium oxalate monohydrate. This compound is a basic component of most renal stones. Hundreds of publications deal with COM formation, but only in two of them atomic force microscopy (AFM) was applied [1,2]. Today it is impossible to understand and describe quantitatively the processes occurring on the crystal surface without using this device. The cited papers can be considered as merely a beginning, we continued these investigations in the present work.

$CaC_2O_4 \cdot H_2O$ is a poorly soluble compound. In water at 25 °C its solubility product is $K_{sp} = 2.0 \times 10^{-9}$ mol^2/l^2 [3] and at 37 °C $K_{sp} = 2.8 \times 10^{-9}$ mol^2/l^2 [4]. Accordingly, under these conditions the solubility is equal to 0.00065 mass% ≈ 4.5×10^{-5} mol/l and 0.00077 mass% ≈ 5.3×10^{-5} mol/l. Therein lies the difficulty of producing relatively big crystals. Pouring together solutions of $CaCl_2$ and a readily soluble oxalate (e.g., $K_2C_2O_4$) with the concentrations of a few mass % results in a high supersaturation and the COM crystals of nanometer size are formed.

The crystalline structure of the COM natural analog – mineral whewellite – is well studied, faceting of crystals is described too [5,6]. Crystals of whewellite relate to the prismatic class of monoclinic syngony, they have one symmetry plane and the second-order axis (*b)* is perpendicular to the symmetry plane. The elementary cell has the symmetry space group $P2_1/c$ with the parameters

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a = 6.290 Å, b = 14.583 Å, c = 10.116 Å, β = 109.46°. The cell contains 8 formula units. The {100} pinacoid is usually well developed, the crystals also have the {010} pinacoid and the {021} prism.

2. Materials and methods

We used the high purity Merck reagents: $CaCl_2$, $K_2C_2O_4$, KCl, $MgCl_2$, $AlCl_3$, $FeCl_3$ and the acetate buffer with pH = 5.6. Distilled water passed through the Milli-Q ion-exchange column, and Aqua RX water (Penta Water, research grade [7]) with the specific resistances of 16 and 2 MOhm·cm, respectively, were used. When producing Aqua RX water, jets of purified water collided with each other under high pressure and this process heated water to 60 °C. On cooling down to 4 °C the water was saturated with oxygen.

Crystal preparation

COM crystals were grown in urine, in different gels (by the counter diffusion method) and by direct pouring of reagent solutions together. In urine the crystals took several days to grow up to 30-50 μm in size without any impurities and up to 1 mm in size with addition of calcium chloride and potassium oxalate. Note that crystal preparation and increase in size in urine have recently been used in Ref. [8]. This indicates that the solution in kidneys is always supersaturated with respect to COM crystals and the supersaturation level is characterized by the COM crystal growth rate in urine. In our experiments such crystals contained unknown inclusions and had a bad surface. Crystals grown in gelatin, agar-agar, agarose and acidulous sodium silicate gels frequently generated splices, spherulites, dendrites and various twins. The size of dendrites reached 1 mm. Usually 4% potassium oxalate solution was poured into a tube filled with a gel, and this column was put in $CaCl_2$ solution of the same concentration. Formation of crystals began near the boundary between the gel and calcium chloride. Dendrites mainly appeared there. Twins and crystals of the typical COM tablet shape were produced sequentially higher in the gel column. The tablet-shaped COM crystals included a great number of spherulites and twins. It is known that well shaped crystals may also be obtained in such a gel. The size of such crystals can reach 0.5-1 mm [9]. The shape variety of obtained crystals and crystal splices is shown in the photographs in Fig. 1.

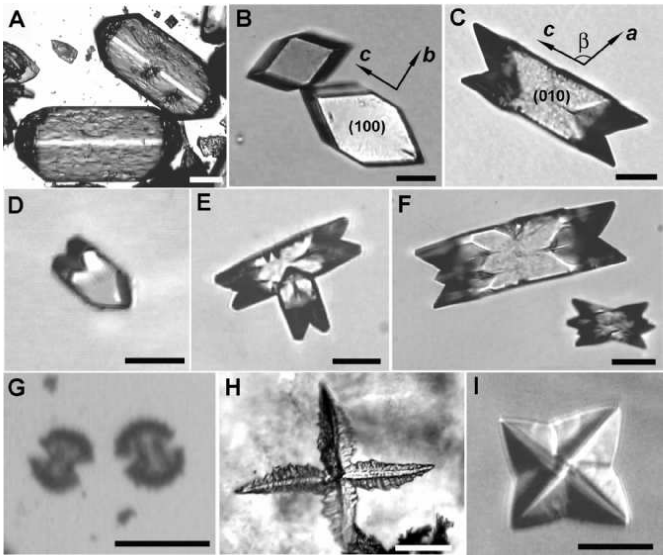

Fig. 1. COM crystals obtained by different methods. (A) – crystals grown in urine; (B-D) – crystal forms obtained by mixing solutions of $CaCl_2$ and $K_2C_2O_4$ in the buffer at pH = 4÷6; (C) - penetration twin; (D) – contact twin; (E, F) – conjoined crystals; (G) – spherulite formed in gelatin gel; (H, I) – dendrite formed in agarose gel. Bar, μm: (A, H, I) – 100; (B-F) – 20; (G) – 5.

For AFM experiments it was desirable that crystals should nucleate and grow on a substrate that could be installed directly in the device. Furthermore, we wanted to observe the whole crystal, therefore its size ought to be of the order of 10-50 μm. Such crystals were obtained by the method described in [1]. Three solutions were prepared: (i) the acetate buffer with the concentration of 0.05 or 0.1M containing 0.1M of KCl; (ii) the same solution with 6 mM of $CaCl_2$; (iii) the same solution with 2 mM of $K_2C_2O_4$. The glass or mica substrate was placed on the bottom of the vessel and three above mentioned solutions were poured into this vessel so that the final solution contained 4 mM of calcium chloride and 0.2 mM of potassium oxalate. It was important that potassium oxalate should be added by drops. It took the crystals 1-7 days to grow. About 10% of the crystals were twins. Note that in the solutions prepared with Aqua RX water the number of twins was considerably smaller.

Experimental protocol

The experiments were made at the temperature of 25±1°C in the Digital Instruments Nanoscope III atomic force microscope and the liquid cell (with the volume being about 25 mm^3) of the same manufacturer. The cantilevers with silicon nitride tips (the vertex angle being 72°) and the 13 μm scanner were applied, the images were recorded in the contact mode. The Femtoscan program [10] was used for subsequent image processing. The supersaturated solution or water was pumped by the peristaltic pump through the cell at the rate of 0.3-0.5 ml/min. This provided the independence of the growth (and dissolution) rates from the fluid flow velocity. It was possible to change quickly the composition of the flowing fluid

The growth solutions with different supersaturations, s, were prepared from the three solutions listed above, the concentration of potassium oxalate being always lower than that of calcium chloride.

The solution supersaturation, s, was calculated as follows:

$$s = f\{[Ca][C_2O_4]/K_{sp}\}^{1/2} - 1,$$

where the square brackets indicated the molar concentrations of calcium and oxalate, and the activity coefficient f was determined from the expression $-\log f = Az^2[I^{1/2}/(1 + I^{1/2}) - 0.3I]$. Here A = 0.5115 – the Debye-Huckel constant, $I = \Sigma z^2 c$ – the ionic strength, z – the valency, and c – molarity, totaled for all ions in solution. The typical supersaturation was greater than 1-2, since at smaller s no crystal growth was observed. At s > ~8 new crystals spontaneously nucleated in the solution, fell down on the surface under investigation and reduced the supersaturation in an uncontrolled way.

3. Results

Crystal nucleation

Usually we used mica (muscovite) as a substrate. Before experiments the mica surface was refreshed through scotch sticking and removal. We found that crystals develop only on non-refreshed part of surface (Fig. 2a). We proposed two hypotheses. The first: the fresh surface is negatively charged because some quantity of muscovite cations departed from the surface into the solution and this effect prevents crystal nucleation. The second: some impurities were adsorbed on the original (non-refreshed) mica surface and activate COM nucleation. In order to check these hypotheses, the mica was cleaned by acetone in an ultrasonic bath and rinsed in water and ethanol. After that one half of the mica surface was scratched. As before, no COM crystals nucleated on the fresh part of the mica surface. However, the rinsed part of mica had a much smaller number of crystals than before cleaning. Experiments with glass substrates were also performed. Glass substrates were covered by polymer films: negatively charged sodium 4-styrenesulfonate or sodium salt of methacrylic acid and positively charged polyethylenimin. Then we cut off half of the glass with the polymer film and performed crystallization on both parts. The crystals nucleated on both positively and negatively charged surfaces, but not on the clean glass (Fig. 2b).

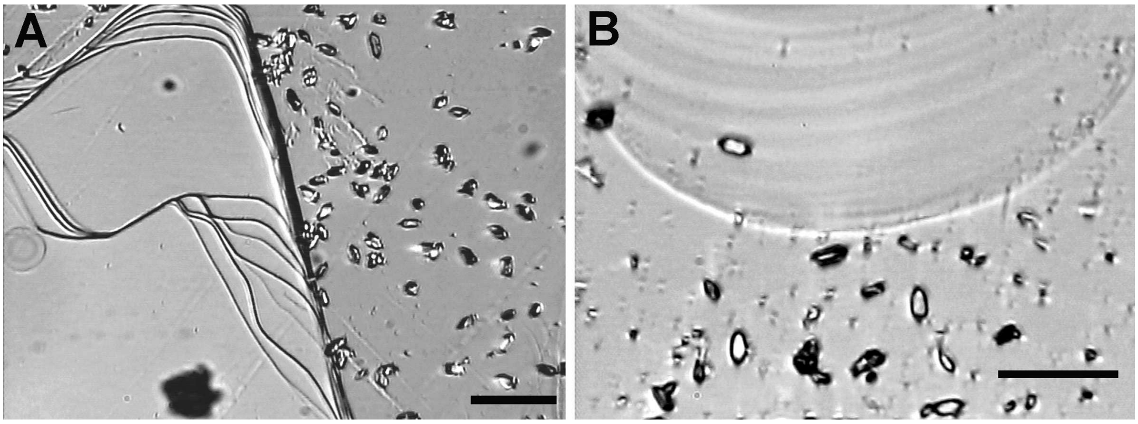

Fig. 2. Heterogeneous COM nucleation on mica (A) and on glass (B). Some layers were scratched at the left part of mica and crystals were not formed there. The glass was covered by a polymer film and then the top part was scratched. Here you can see only one crystal that obviously settled down onto the glass from the solution. Bar – 50 μm.

Mechanism and kinetics of face growth

Dislocation growth sources on the (100) face (the biggest but slowly growing face) were rarely observed. The (100) face with one dislocation hillock is represented in Fig. 3. Sometimes, as in [1], the (100) face contained several dislocation hillocks. Usually the step height was equal to 1 - 1.5 unit cell parameter *a*. The step velocity at s ~ 6 was equal to 2 nm/s and the hillocks slope was ~ 10^{-3}. More frequently, the macrostep (its height being equal to ~ 50 nm) appeared along the perimeter of the (100) face and slowly covered the face surface (Fig. 4). We observed the formation of this macrostep. Two-dimensional nucleus appeared at the corner between [001] and [021], then it grew quickly along the crystal edges and moved slowly towards the face center. New nuclei appeared at the same place and the macrostep became higher. This process can take place at a low supersaturation (s ~ 2.5). Evidently, the nuclei formed on the crystal defect. When this defect was overgrown, the nucleus formation stopped and the face growth finished [11]. In many cases, as in [2], we were not able to see growth layers on the (100) face and the face did not grow.

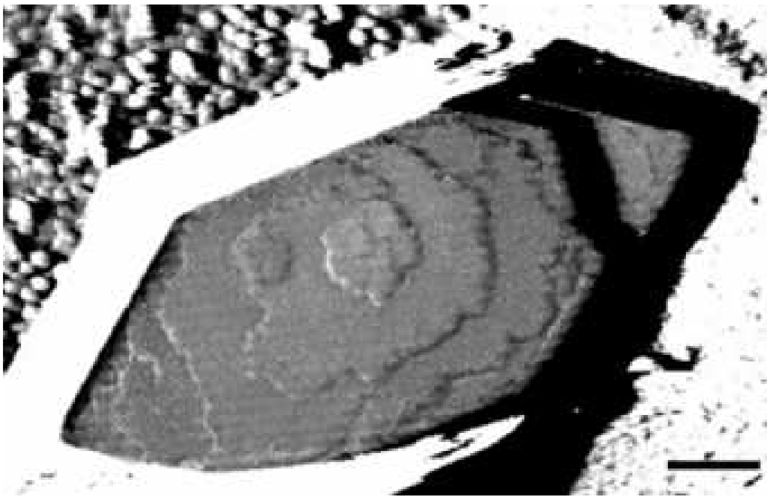

Fig. 3. Dislocation hillock on the (100) face. Bar – 0.5 μm.

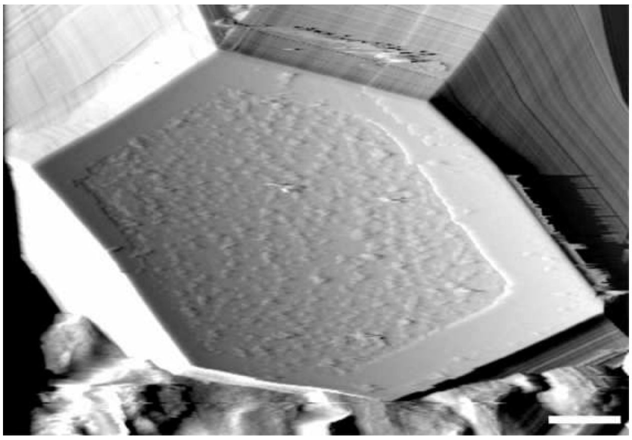

Fig. 4. The (100) face grown at the supersaturation s ≈ 3. Growth layer (thickness ~ 50 nm) seen at the perimeter of the crystal arose by two-dimensional nucleus formation in the right upper corner of the face. Nucleus formation continued and the layer moves slowly to the face center. The origin of particles covering the part of the face that is not occupied by a new layer is now unknown. The heights of these particles are various (less than 30 nm) and they are easily removed by the AFM tip. Bar – 1 μm. Note that the shape of side faces was disfigured on all AFM images; this shape and visible details characterize the AFM tip profile.

Many particles were found on the surface of the (100) face (Fig. 4). The dimension of these particles was up to 30 nm. These particles could be displaced by the microscope tip across the surface and, in some cases, were removed from the face. It can hardly be assumed that the particles were those of dust or an impurity because the reagents were pure and the glassware used in experiments was thoroughly cleaned. Evidently, those particles were clusters of substances included in the growth solution.

It was possible to study the (010) face only when a thick enough twin lied on this face. The twinning boundary was always marked by a row of screw dislocation hillocks. However, the density of screw dislocations and hillocks was very high on this face even far away from the twinning boundary and was equal to $10^6 - 10^8$ cm^{-2} (Fig. 5). The step heights were equal to 0.5 or 1 unit cell parameter along the *b*-axis. The dependence of the step velocity (v) on the supersaturation was linear and $v/s \approx 0.3$ nm/s. At $s = 4 \div 6$ the hillock slope $p \approx 10^{-2}$ and consequently the normal growth rate of the face $R = pv \approx 1.5 \times 10^{-2}$ nm/s ≈ 1.3 μm/day.

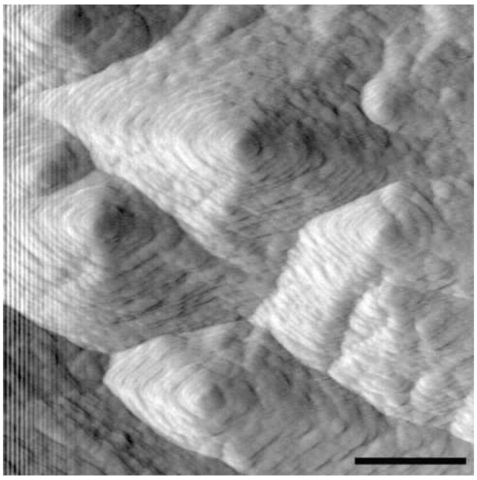

Fig. 5. Dislocation hillocks on the (010) face far from the twinning boundary. Bar – 1 μm.

It was shown that as a result of adding 1 mM of $MgCl_2$ to the solution the step velocity became about two times lower.

Crystal dissolution

Etching pits (described in [2]) appeared on the (100) face on dissolution. At first, these etching pits had a shape similar to that of the (100) face, then they combined into grooves elongated along the [001] direction (Fig. 6).

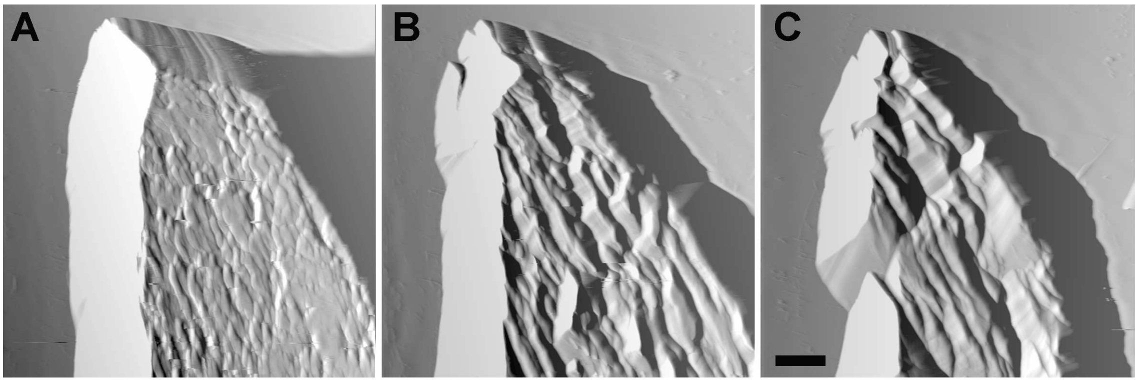

Fig. 6. Crystal dissolution in Milli-Q water. Time, minutes: (A) – 9; (B) – 58; (C) – 142. Bar – 1 μm.

Dissolution of the (010) face started from the formation of etching pits on the top of dislocation hillocks and was accompanied by step movement toward the hillock center at the velocity $v = 1.1$ nm/s (in Milli-Q water). This velocity (at $s = -1$) was approximately 3 times higher than at $s = 1$.

The crystal dissolution rate was calculated by measuring the changes in the crystal size. At the first stage of dissolution (until the crystal retained its own shape), the dependence of the crystal size on time was linear. The normal dissolution rate along [010] was equal to $R_{010} = 0.16$ and 0.47 nm/s, the former value refers to Milli-Q water, the latter - to Aqua RX water. Thus, the dissolution rate in Aqua RX water was 3 times higher than that in Milli-Q water. The unusual properties of Aqua RX water were found earlier in [12,13], but the reason for these properties was not established. With time the crystal shape became irregular and the dissolution proceeded more rapidly.

We tried to find impurities that inhibit growth step movement. For this purpose two impurities were tested: trivalent ferric and aluminum ions. These impurities are known as strong inhibitors for KH_2PO_4 crystals. It was established that presence of these ions results in an intensive dissolution of the crystal instead of reducing the step velocity. It was found that these impurities induce an intensive dissolution even at a high supersaturation of COM solutions. This effect is obviously connected with the formation of readily soluble complex chemical compounds. The dissolution process is shown in Fig. 7. The dissolution effect of aluminum was stronger than that of iron. In aluminum nitrate the dissolution proceeded much more rapidly than in aluminum chloride. The normal dissolution rate along direction [001] was found to be as follows:

Solution	R_{001}, nm/s
Milli-Q water	0.15
Aqua RX water	0.42
Milli-Q water + $AlCl_3$ (1mM)	0.58
Milli-Q water + $Al(NO_3)_3$ (1mM)	1.42

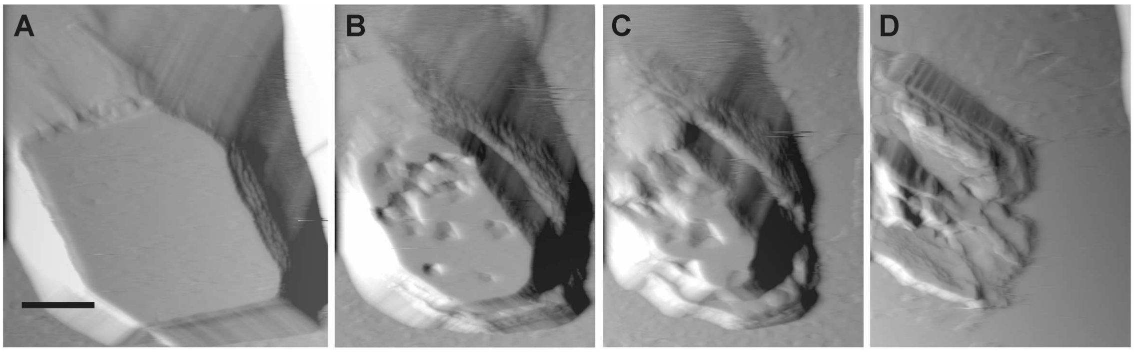

Fig. 7. Dissolution of crystal in solution containing 10^{-3} mol/l of $FeCl_3$. The solution is supersaturated with respect to COM: $s \approx 4$. Time, minutes: (A) - 0; (B) - 13; (C) - 23; (D) - 43. Bar – 1 μm.

4. Discussion

The tendency of COM crystals to form contact twins, penetration twins, spherulites, dendrites and splices (which is likely to take place in human kidneys) is apparently due to imperfections of the crystalline structure. This fact can be confirmed by abnormally high dislocation density. The formation of defects may be connected with alternation of positively and negatively charged growth layers that are parallel to the (100) face [6]. The clusters of COM (COD or another associates) molecules are transferred from the solution to the surface of the (100) face, then they travel across the surface (Fig. 4) and, since the surface is charged, they can be incorporated in a disoriented way into the crystal structure. In this case, it is difficult to obtain a high resolution and observe elementary unit cells on the crystal faces [11].

COM crystals do not nucleate on a fresh mica surface in the presence an "old" surface and on fresh glass (Fig. 2). We consider that these facts are to be studied in future. This study could result in finding substances that promote COM nucleation and formation of renal stones.

It is important to investigate the possibility of more rapid dissolution of COM crystals in the presence of Aqua RX water and with addition of an aluminum salt into the water. However, such work would be possible only in collaboration with a medical institution.

5. Conclusions

Using *in situ* atomic force microscopy it was shown that the (010) face of COM crystals grows by the dislocation mechanism, while the (100) face more frequently grows by forming two-dimensional nuclei. The thickness of elementary growth layers was measured and the dependence of their velocity on the solution supersaturation was determined. This dependence was found to be linear with a very low angle coefficient (~ 0.3 nm/s). Magnesium ions inhibit growth.

It was found that dissolution of COM crystals in Aqua RX water was more rapid than in distilled water. The dissolution rate was much higher in the presence of trivalent iron and aluminum ions.

Acknowledgements

The authors are thankful to Bio-Hydration Research Lab, Inc. (San Diego, U.S.) for the samples of AquaRX water for the experiments. The authors are indebted to Dr. O.A. Shustin and T.G. Chernevich for their help in experiments, Dr. Olga Konstantinova (Urology Institute, Moscow) for consultation about COM formation in kidneys. This work was supported by Grant of Russian

Foundation for Fundamental Research № 03-02-16113 and Grant of the President of Russian Federation № MK-669.2003.02.

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