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Review

Phytate (IP6) is a Powerful Agent for Preventing Calcifications in Biological Fluids: Usefulness in Renal Lithiasis Treatment

FELIX GRASES and ANTONIA COSTA-BAUZÁ

Laboratory of Investigation into Renal Lithiasis, Faculty of Sciences, University of Illes Balears, Ctra. Valldemosa km 7.5, 07071 - Palma de Mallorca, Spain

Abstract. *The extraordinary capacity of phytate (myo-inositol hexaphosphate), a substance present in blood, urine, interstitial and intracellular fluids, to inhibit crystallization of calcium salts (oxalate and phosphate) is discussed. Its role in preventing calcium renal stone formation is specifically presented and discussed. "In vitro" and "in vivo" experiments, as well as clinical studies clearly demonstrated that phytate plays an important role as a crystallization inhibitor of calcium salts in biological fluids and becomes a clear alternative in the treatment of calcium oxalate renal lithiasis.*

The maximum total amount of a product that can remain indefinitely solved in an aqueous solution at a given temperature (the so called "solubility") depends on its chemical nature. Solubility is an equilibrium parameter, independent of time, and thermodynamics demonstrates that it mainly depends on the stability of the crystal lattice and the stabilitv of the formed aqueous solvates (soluble species). Solubility can also be affected by the composition of the media (mainly ionic strength) due to its influence on the reactivity (related to chemical potential) of the solvates. When a system contains higher amounts of solute than that corresponding to the "solubility" (saturated system), the system remains in an unstable state (supersaturated) and sooner or later must evolve to the stable conditions (thermodynamic equilibrium) through the crystallization of the excess of solute. Thus, the driving force that pushes the crystallization processes is the difference between the equilibrium conditions (solubility) and the actual ones. These time dependent processes, studied by the kinetics, can occur over seconds or years. The general mechanism of formation of a single crystal can be explained as a result of the combination of two independent steps: nucleation and crystal growth. The time necessary to generate a crystal mainly depends on the nature of the crystal, the supersaturation of the solution, the presence of performed solid particles (the so-called heterogeneous nucleants) and the presence of crystallization inhibitors. These latter are substances that due to their structure interact with the nucleus or the crystal provoking important disturbances in their formation and/or development, preventing as a consequence the crystallization processes, as we will discuss later.

Life itself implies a chain of continuous changes in such a manner that no occasion remains in a situation of equilibrium (from thermodinamic view point). Thus, the majority of the human biological fluids are supersaturated with regard to some substances. Therefore, blood, interstitial liquid and intracellular liquid, due to their pH value (pH > 7.0), free calcium ion concentration and phosphate concentration, are supersaturated with respect to calcium phosphate (hydroxy-apatite). Urine is always supersaturated with respect to calcium oxalate and depending on its pH value, is also supersaturated with respect to uric acid (pH < 5.5) or calcium phosphates (pH > 6.0). In spite of this, normal crystallization processes only take place in biologically controlled situations like in the formation of bone and teeth. Nevertheless, uncontrolled pathological crystallization is also frequent: tissue calcification associated to cancer, calcification of atheroms, calculi formation (renal, biliar, sublingual,) etc. The question is, why does crystallization not take place indiscriminately in all human fluids and yet only appears in pathological situations? The answer is now clear: there are three main aspects that must be considered to explain pathological crystalllzation: supersaturation higher than usual of the crystallizing substance, and/or the presence of heterogeneous nucleants (crystallization inducers), and/or deficit of crystallization inhibitors. Thus, in healthy conditions, the presence of crystallization inhibitors at adequate concentrations prevents the development of solid concretions. In fact, the crystallization inhibitors act to delay the crystallization of supersaturated substances, avoiding crystallization taking place before the renovation of the corresponding fluid (it must be remembered that life implies a continuous change and renovation).

The first indications of the existence of the so-called crystallization inhibitors occurred in the 60's. In 1965

Correspondence to: Prof. Dr. F. Grases, Laboratory of Investigation in Renal Lithiasis, Faculty of Sciences, University of Illes Balears, Ctra. Valldemosa km 7.5, 07071 - Palma de Mallorca, Spain.

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Bliznakow demonstrated that some molecules were able to clearly reduce the rate of crystal growth of a given substance, due to their adsorption on the growing surfaces [1]. The first biological inhibitor to be recognized and investigated was inorganic pyrophosphate, which inhibits precipitation at very low concentrations and is present in blood, urine and joint fluid at effective concentrations for blocking the growth of hydroxyapatite. Since alkaline phosphatase is known to have pyrophosphate activity, this enzyme might act by locally destroying the circulating inhibitor, thus allowing mineralization to take place. Pyrophosphate in urine also acts as a powerful crystallization inhibitor of calcium oxalate [2, 3]. Thereafter, a variety of urinary contained substances have been described as crystallization inhibitor molecules, some of them of low molecular weight such as magnesium and citrate [4-8] and others with high molecular weight like glycosaminoglycans, Tamm-Horsfall glycoprotein [9-12], nephrocalcin [13-14], and osteopontin [15]. It is very interesting to observe the evolution of ideas about the importance of crystallization inhibitors in urine during recent decades. Thus, around the 70's, after the identification of some urinary inhibitors, such as pyrophosphate, and the study of their *in vitro* effects, it was accepted that these substances can play an important role in preventing renal lithiasis. At the end of that decade and during the 80's the existence of a potent inhibitor of calcification present in normal urine, acidic and of relatively low molecular weight, was supported. The substance was originally thought to be a small peptide but this work was later disproved [16]. Phosphocitrate was also cited as a potent inhibitor [17], but no conclusive evidences was subsequently supplied. In the last decade, it seems that the tendency has been to consider that protein inhibitors of stone formation play the major role in the natural defence against nephrocalcinosis [18-19]. Nevertheless, *in vitro* experiments seem to be inconclusive on the inhibitory capacity of these macromolecular substances, some authors defending their inhibitory capacity, others demonstrating crystallization promoter properties [20]. In fact, there is no unequivocal evidence that any single macromolecular substance or group of macromolecular substances are directly involved in preventing stone formation as crystallization inhibitors, although their action as antiadherent substances (lubricants) on preventing the adherence of solid particles on the uroepithelium seems clear [21-22], yet this is a different action that must not be mistaken with the authentic behaviour as a crystallization inhibitor.

Recently, it has been demonstrated that phytate (myo-inositol hexaphosphate), a substance of relatively low molecular weight, present in blood, urine, interstitial and intracellular fluids [23-26], exerts a potent action as crystallization inhibitor of calcium salts (oxalate and phosphate), the aim of this paper being to present and discuss the present knowledge about the action of phytate on renal stone formation.

The development of renal stones, that is, urolithiasis, is a very painful disease, which has afflicted a wide sector of the human population since ancient times. At present, approximately 10% of the human population are affected.

Broadly, renal stones comprise the following substances: calcium oxalate (~70%), infectious components (~15%), uric acid (~10%), calcium phosphates (~2%), cystine (~1%) and medicament components (~1%). All of these can be organized in a variety of crystalline phases, morphologies and microstructures, thus that more than 20 types of calculi have been classified [27].

In nearly all cases, the formation of renal calculi must be attributed to the combination of multiple factors. These can be classified into two main groups: a) factors related to urine composition; and b) factors concerned to renal morphology.

Urine is a metastable system, in which several substances capable of crystallizing and generating renal calculi coexist. Normally, these substances are in supersaturated conditions and, as it is noted above, the ease of crystallization depends on a) the degree of supersaturation, b) the presence of heterogeneous nucleants: solid substances able to induce formation of new crystals of another composition on their surface; and c) the level of inhibitors of crystallization.

The main factors linked to renal morphology that favour calculus formation are: a) the presence of renal cavities with low urodynamic efficacy that, as a consequence, retain urine for long periods of time in the upper urinary tract; and b) alteration of the epithelium that covers the renal papillae (for example, damaged or reduced anti-adherent glycosamino-glycan layer, necrosis, *etc.*).

The development of a renal calculus generally depends on the coexistence of several of these factors and, in most cases, eliminating some of them is enough to avoid the development of a new calculus.

In vitro studies

Poor understanding of renal stone development in a substantial number of cases is, to a considerable extent, caused by the difficulties in the direct observation of the various steps of the full *in vivo* process. Moreover, it is extremely difficult to reproduce the natural conditions of human stone development in animals due to the multifactorial nature of this disease, particularly also owing to the formation period of the most frequent calculi (calcium oxalate) being 3-5 years. In fact, the majority of hypotheses related to mechanisms of stone formation have been based on results from *in vitro* experiments.

The relevance of *in vitro* experiments for studying urolithiasis depends on the degree of correspondence between the experimental conditions and those prevailing in the stone-forming kidney. To provide useful data, *in vitro* method must reproduce some of the stages of the real biological process. A variety of common laboratory crystallizers were used to study calcium oxalate crystal

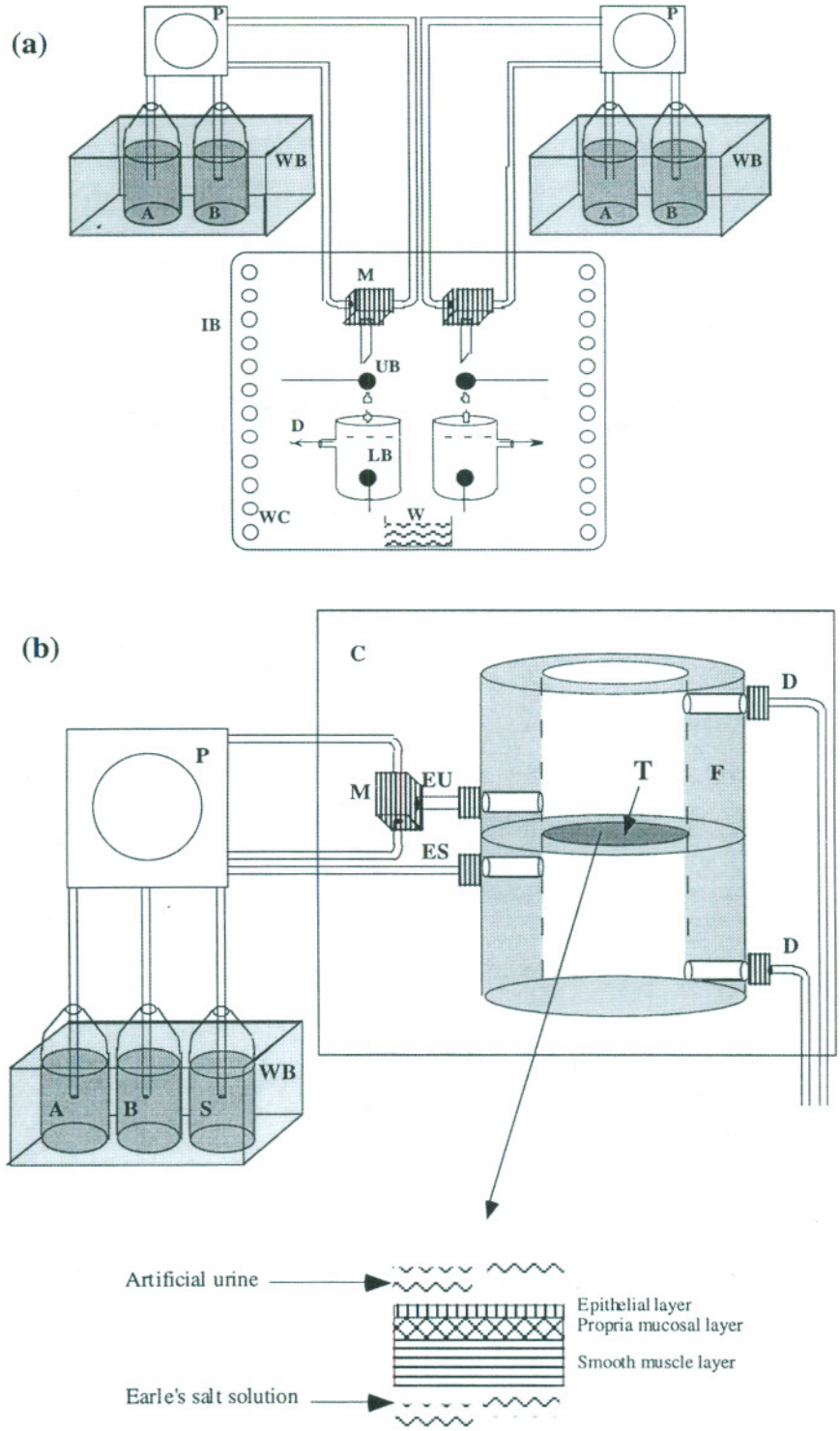

Figure 1. *(a) Schematic diagram of a model used to simulate renal stone formation on inert substrates. (IB) Closed insulating box. (A) Solution A for synthetic urine preparation. (B) Solution B for synthetic urine preparation. (WB) Constant temperature water bath. (P) Peristaltic pump. (M) T-type mixing chamber. (UB) Upper wax ball. (LB) Lower wax ball. (D) Drainpipe. (W) Water to maintain 100% humidity. (WC) Water circulating coil with constant temperature. (b) Schematic diagram of the model used to simulate renal stone formation on pig urinary bladder tissue. (A) Solution A for synthetic urine preparation. (B) Solution B for synthetic urine preparation. (S) Aerated Earle' s physiological solution. (WB) Constant temperature water bath. (P) Peristaltic pump. (C) Temperature-controlled chamber. (F) Methacrylate cylindrical flask. (M) T-type mixing chamber. (EU) Entrance for synthetic urine. (ES) Entrance for physiological salt solution. (D) Drainpipes. (T) Piece of tissue. Note: these schemes are not a scale drawings of the different parts.*

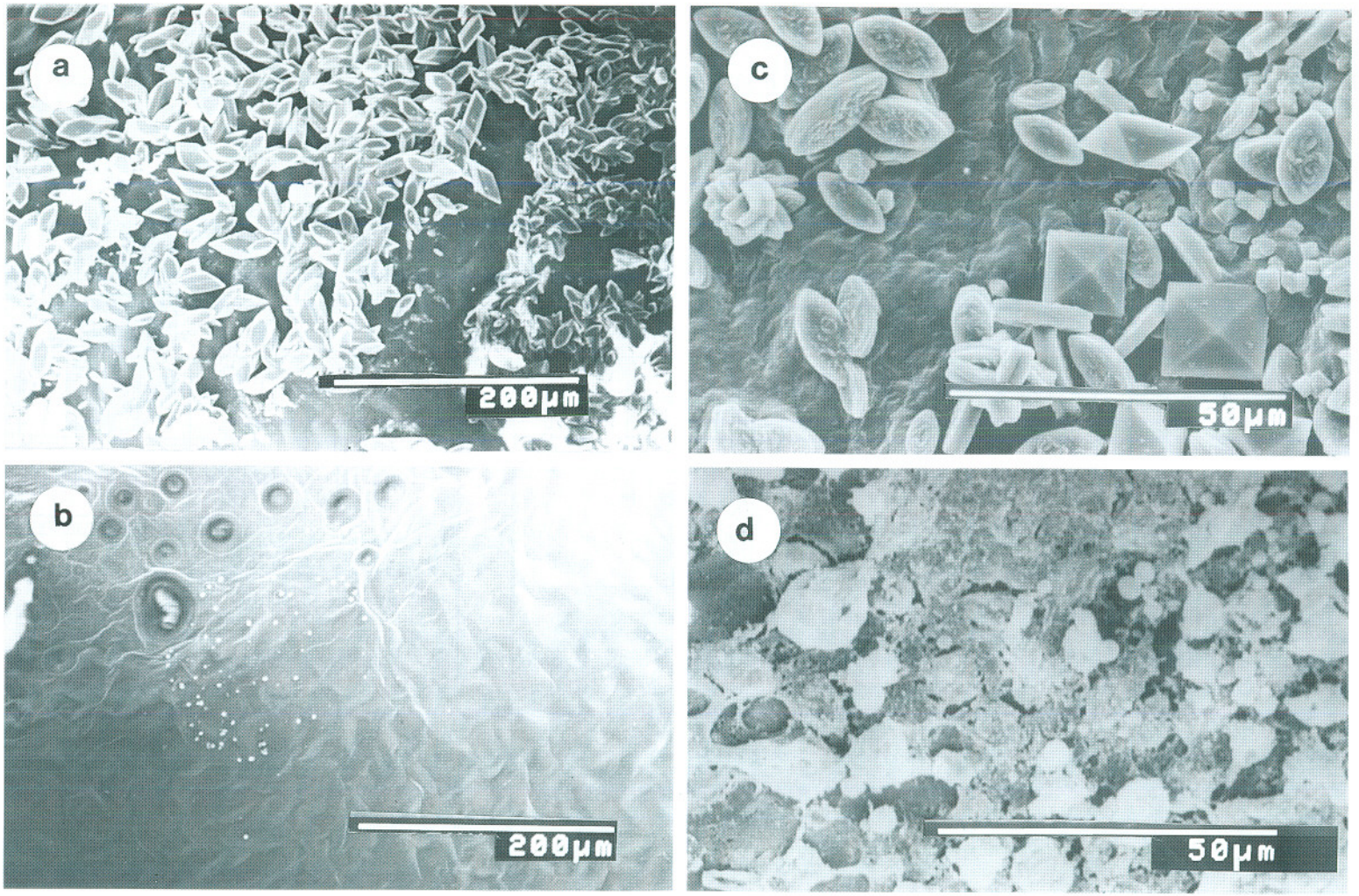

Figure 2. *SEM images of (a) calcium oxalate monohydrate crystals developed after 78 h on the surface of an inert substrate (paraffin) when using the system represented in Figure la with idiopathic (125 mg/L Ca^{2+}, 25 mg/L oxalate) synthetic urine at pH = 5.5 in absence of phytate; (b) surface of the inert substrate when the experiment corresponding to the image (a) was performed in the presence of 4 mg/L of phytate, on which no crystals were observed. (c) Calcium oxalate monohydrate and calcium oxalate dihydrate crystals developed after 8 h on the urothelium of pig urinary bladder with injures induced by desiccation, when using the system represented in Figure lb with hypercalciuric (350 mg/L Ca^{2+}) and hyperoxaluric (50 mg/L oxalate) synthetic urine at pH = 5.5 in absencc of phytate; (d) surface of the pig urinary bladder when the experiment corresponding to the image (c) was performed in the presence of 0.5 mg/L of phytate, on which no crystals were observed.*

formation, nevertheless, all these systems are far from the real conditions of stone formation in the kidney. Recently, new experimental devices that closely simulate these conditions of stone development in the kidneys have been developed. Some of these systems used non-living substrates, as the devices shown in Figure 1a. But systems that permit the use of living tissues on which stone development is studied have been also used. Thus, in Figure 1b one of these systems that uses urothelium of pig urinary bladder is described. These studies showed the important role that substances naturally found in human urine, such as phytate, can have as crystallization inhibitors preventing the development of COM crystals [22, 28-30]. As shown in Figure 2, phytate caused a significant inhibition of calcium oxalate crystallization on inert material (paraffin or calcium phosphate) and also the total inhibition of calcium phosphate crystallization on pig bladder epithelial tissue. Thus, whereas in the absence of phytate considerable amounts of calcium oxalate, brushite an hydroxyapatite were detected on the inert material, the epithelium with the reduced GAG layer and the necrosed epithelium, the presence of 1.0 μg/mL phytate totally prevented deposits of calcium oxalate and/or of calcium phosphate from forming. This inhibitory action must be related to the affinity of phosphate groups to the calcium, and particularly to the chemical ring structure of the phytate that, through adsorption processes, causes significant disturbances of calcium oxalate and calcium phosphate crystal nucleation and growth.

In vivo studies

Since phytate is naturally present in urine at similar concentrations to those used in the *in vitro* studies [25] and urinary phytate concentration also depends on dietary intake, this demonstrates the potential beneficial therapeutic effects of phytate on the treatment of calcium renal lithiasis in preventing calculus development.

The effects of phytate on urolith development in a nephrolithiasis animal model using ethylene glycol were studied [31]. Urine analysis were carried out to determine the levels of calcium, oxalate, citrate, Zn(II) and phytate in control and phytate treated groups. At the end of the experiment all kidneys were removed and macroscopically and microscopically examined for possible crystal/stone locations and the total calcium amount of the renal papillary tissue was evaluated. In the group of rats treated with phytate, the number of calcifications on the papillary tips and the total calcium amount of the papillary tissue, were significantly reduced when compared with the control group treated exclusively with ethylene glycol.

Recent studies have demonstrated that phytate is also naturally present in human urine and normal levels oscillated between 0.5 and 6 mg/L [25, 26] being the urinary concentrations found in a group of calcium oxalate active stone-formers significantly inferior to those found in a group of healthy people [32]. It has also been found that urinary phytate mainly depends on dietary intake in such a manner that when it is totally eliminated from the diet, the urinary levels immediately fall and after several days become undetectable [32, 33]. Ingestion of phytate clearly and significantly reduced the urinary risk to develop calcium stones in humans [34]. Thus, a clinical study using 36 calcium oxalate active stone-formers with positive urinary risk to develop calcium stones was performed. In a subgroup of 19 stone- formers the urinary risk to develop calcium stones was newly evaluated after 15 days. The other group of 17 stone formers was treated with phytic acid (120 mg of phytate/day) during 15 days and then the urinary risk was newly evaluated. Other urinary lithogen parameters were also determined. The obtained results showed that whereas the ordinary urinary lithogen parameters were not modified by ingestion of phytate, the urinary risk to develop calcium stones was significantly reduced, this demonstrates an interesting efficacy of the therapy using phytate as crystallization inhibitor. The urinary risk factor was evaluated using a test specially developed and validated for this purpose [35].

It is important to remark that phytate was previously used in the treatment of renal lithiasis. Thus, in 1958, Henneman *et al* [36] used high doses of phytate (8.8 g/day) to treat stone-former patients with idiopathic hypercalciuria. Nevertheless, the objective and basis of such treatment was clearly different to that proposed here. Thus, high doses of phytate were supplied to hypercalciuric patients with the aim to form non soluble complexes in the intestinal tract, to prevent the absorption of dietary calcium and as a consequence provoke the decrease of its urinary excretion. However, the low doses supplied in the new proposed treatment has as a main finality to raise the urinary excretion of phytate, increasing the inhibitory capacity of urine against calcium salts crystallization (oxalate and phosphate). Precisely, this treatment exhibited the maximum efficacy in non-hypercalciuric oxalocalcic stone-formers, for whom it is recommended. Consequently, from all *in vitro* and *in vivo* results, it can be clearly concluded that phytate plays an important role as crystallization inhibitor of calcium salts in biological fluids and becomes a clear alternative in the treatment of calcium oxalate renal lithiasis, in the treatment of calcium oxalate renal lithiasis, that has to be added to other therapeutic properties of phytate, like anticancer agent [37-40] or antioxidant [41].

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