A Review of New Concepts in Renal Stone Research

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A REVIEW OF NEW CONCEPTS IN RENAL STONE RESEARCH

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Abstract

Clinical and basic research in the field of urolithiasis has developed rapidly in recent years. Progress in extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PNL) has brought about a revolution in the surgical treatment of urolithiasis and research at the cellular and molecular level is now expanding. In spite of these advances, however, clinical treatment of urolithiasis remains far from satisfactory. Stone recurrence in many patients cannot be predicted and is beyond control of urologists mainly because the mechanisms of stone formation are still not fully understood. It is necessary to study the process of stone formation more intensively at the cellular and molecular level, and to strengthen the links between basic and clinical research in the field.

In this review, the processes involved in the formation of stones are compared with those involved in normal bio-mineralization and a model of urolithiasis is put forward based on modern systems science. Attention is concentrated on: (a) Directions of research based on physico-chemical theories of stone formation; (b) The role of renal tubular defects in urolithiasis; (c) The role of free radical reactions in stone formation; and (d) Macromolecular abnormalities and their correction.

Key Words: Urolithiasis, bio-mineralization, non-equilibrium thermodynamics, nephrocalcin, Tamm-Horsfall protein, Band-3 protein, glycosaminoglycans, free radical, oxalate degrading enzyme.

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Introduction

Although there has been considerable progress in the field of urolithiasis research during the last decade, the precise mechanism of stone formation is still obscure. It is necessary, therefore, to re-investigate the processes of stone formation at the cellular and molecular level. Within the same time scale, rapid developments have taken place in systems science. These developments have to be incorporated in the model of stone formation. In this review, new concepts in stone research, such as systems science, will be introduced.

Bio-mineralization and urolithiasis

The concept that "urolithiasis can be considered as a syndrome that is related to pathological bio-mineralization" has generally been accepted [80]. Bio-mineralization is the process that occurs in the body and results in formation of bio-minerals. Because bio-minerals can only be formed under physiological conditions, some highly ordered structures and special means of organization are essential. Bio-mineralization is associated with a series of specific properties and physiological functions (Table 1).

Bio-mineralization can be divided into two types: (a) physiological mineralization (e.g., normally growing bone, teeth and other hard tissues), and (b) pathological mineralization. There is a striking similarity in the chemistry between the two types of mineralization. The differences between them lie in the sites where the processes take place and in the degree of organization of the mineral concerned. Various types of pathological mineralization are listed in Table 2.

Demineralization is the opposite process to mineralization. From the viewpoint of chemistry, both mineralization and demineralization can occur simultaneously and depend upon each other. From the viewpoint of biology, mineralization and demineralization are both "controlled" by cells. In this context, these are two aspects to "control": (1) the rate of the chemical reaction is controlled by various kinetic factors, and (2) the equilibrium between mineralization and demineralization is controlled by thermodynamic factors.

The introduction of the concept of bio-mineraliza-
tion into the field of urolithiasis has necessitated taking into account the following: the cellular metabolism, cellular activity, and the participation of macromolecules and matrix in stone-formation. Cells are considered to provide the required micro-environment and control for conditions necessary for mineralization, including synthesis and regulation of macromolecules and matrix materials. Recently, Cœ et al. [29] listed common protein inhibitors of crystallization in various vertebrates (Table 3). The authors proposed the idea that the problem of crystallization and its modulation by inhibitors has been a general problem for nature throughout evolution. Therefore, the concept of bio-mineralization could be useful in increasing our understanding of the mechanisms of stone-formation.

Modern systems science in urolithiasis

The concept of "strong inference" as a strategy in science was first introduced into stone research by Finlayson [22]. To understand the mechanisms of stone-formation at the cellular and molecular level, it is necessary to introduce various elements of modern systems science, namely: (1) system theory, (2) cybernetics, (3) information theory, (4) bio-self-organization, and (5) non-equilibrium thermodynamics.

First, we would like to give a brief introduction of several elements of system science; second, we will try to establish a link between systems science and the pathological process of urolithiasis; last, we will explain the mechanisms of stone-formation based on systems science.

System theory. The human body may be considered to be a large system composed of many sub-systems in which many complex reactions occur simultaneously and depend on each other. Its main feature is that it comprises an open reactive system that has to exchange materials and energy between each sub-system and its surrounding environment. Compartmentalization of the human body includes a series of reactions which are demonstrated in physiological and biochemical processes as follows: (a) concentration of materials; (b) separation of reactants; (c) changes in various equilibrium points which depend on the concentration gradient of reactants and other conditions; (d) changes in reaction velocity.

Cybernetics. In cybernetics, the relationship between input and output, and various modulations of these processes within a given system, are studied. The majority of chemical reactions in the human body are carried out under far from equilibrium conditions. A steady-state is established when input becomes equal to output. However, the system must be controlled in order to establish homeostasis. Modulation of these systems usually depends on a pair of antagonistic regulators (promotory and inhibitory factors) or on an opposite direction for a process (change-over switch). It should be emphasized that a non-equilibrium state can produce orderly structures in time (chemical vibration) and in space (dissipation structure and other sequence structures) [90].

Table 1. The characteristics of bio-mineralization.

<table>
<thead>
<tr>
<th>Structure</th>
<th>High level of order in bio-minerals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>Bio-minerals are formed in special media and are embedded in an organic matrix.</td>
</tr>
<tr>
<td>Functions</td>
<td>The constituent ions of all bio-minerals participate not only in the processes of mineralization and demineralization, but also in various cellular reactions. Bio-minerals can only be formed during biological metabolism.</td>
</tr>
</tbody>
</table>

Information theory. Information can be defined as a state and style of movement of an event. Information processing has to be controlled, irrespective of whether it happens between a human being and its natural environment or between cells and their surrounding micro-environment. The process of transforming information has a material base such as a message carrier, transmission media, and receptors, etc. There is a relationship between the sequence of events and the information capacity: the larger the information content, the higher the sequence. For example, the selectivity of an enzyme for its substrate depends upon the size of the molecule and its spatial configuration, the distribution of the electron charge and its hydrophobic parameters. The more specific an enzyme, the larger its information content. It should be emphasized that modulation of homeostasis can only be obtained when based on a highly ordered structure in the body, and that this modulation possesses a material base.

Bio-self-organization. Bio-self-organization is the basis of the maintenance of biological systems. Many human organs, tissues and cells exhibit this phenomenon, which guarantees the stability of each biological entity. Bio-self-organization is the process of forming an orderly structure. This can be considered to be a very important characteristic of the biological universe.

Non-equilibrium thermodynamics. The human body can be thought of as a highly ordered structure being at a far from equilibrium state. Between this open reactive system and its surrounding environment, exchange takes place, not only of materials, but also of energy. Non-equilibrium thermodynamics studies the thermodynamic behaviour taking place under far from equilibrium conditions which can lead to the formation of a dissipative structure that is stable, orderly and dynamic. This occurs under the following conditions: (1) the system must be an open reactive system; (2) various elements within the system should have non-linear correlations; (3) when the system becomes unbalanced, a new stable structure with orderly characteristics is formed through fluctuations of the system. It should be noted that the dissipative structure is a living structure which is quite different from an equilibrium death structure.
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Table 2. Various pathological bio-mineralization processes.

<table>
<thead>
<tr>
<th>Tissue mineralization</th>
<th>Calculus</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Calcification of necrotic tissue</td>
<td>(1) Dental calculi</td>
</tr>
<tr>
<td>(2) Ectopic mineralization</td>
<td>(2) Gallstone, gastric and pancreatic calculi</td>
</tr>
<tr>
<td>(3) Crystal membranolysis</td>
<td>(3) Renal, ureteral and bladder calculi</td>
</tr>
</tbody>
</table>

Table 3. Known crystallization inhibitions in vertebrates.

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Target crystal</th>
<th>Inhibition *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrocalcin</td>
<td>Kidney/Urine</td>
<td>Calcium oxalate</td>
<td>G/N/A</td>
</tr>
<tr>
<td>Tamm-Horsfall protein</td>
<td>Kidney/Urine</td>
<td>Calcium Oxalate</td>
<td>A</td>
</tr>
<tr>
<td>Antifreeze protein</td>
<td>Fish Blood</td>
<td>Ice</td>
<td>N/G</td>
</tr>
<tr>
<td>Statherin</td>
<td>Saliva/Salivary gland</td>
<td>Brushite/Apatite</td>
<td>N/G</td>
</tr>
<tr>
<td>Pancreatic stone protein</td>
<td>Pancreas</td>
<td>Calcite</td>
<td>N/G</td>
</tr>
<tr>
<td>Gallstone protein</td>
<td>Bile</td>
<td>Calcite</td>
<td>N/G</td>
</tr>
</tbody>
</table>

*G: Growth, N: Nucleation, A: Aggregation.

\[ F : Q \times \Sigma \rightarrow Q \]

\[ G : Q \times \Sigma \rightarrow \Theta \]

**Figure 1.** A response model of stone formation in the urinary tract system in which $\Sigma = \text{set of input signals}; Q = \text{set of metabolic states}; \theta = \text{set of output signals}; F = \text{state transition function}; \text{and } G = \text{output state function. According to this model, an abnormal output signal or state function demonstrated by an individual stone former can be imagined from his initial metabolic state of body and input signals from its surrounding environment. See text for details.}

The modern concept of systems science can be included in our thinking about urolithiasis, in relation to its initiation, progress, treatment and prevention. We can use systems science to answer the question: why do some people suffer from urolithiasis while others, living under the same conditions, do not?

Urolithiasis can be considered to result from an alteration in the state functions. There is a set of state functions representing a set of "n" metabolic states (Q) in the urinary tract system which can be described by

\[ Q = \{ q_1, q_2, q_3, \ldots, q_n \} \]

This system can recognize and interact with a set of "m" input signals (\( \Sigma \)) originating from its environment which can be described by

\[ \Sigma = \{ \sigma_1, \sigma_2, \sigma_3, \ldots, \sigma_m \} \].

The system also produces a set of "p" output signals (\( \Theta \)) that can be described by

\[ \Theta = \{ \Theta_1, \Theta_2, \Theta_3, \ldots, \Theta_p \} \].

Assuming that there is a transition within the system from one internal state to another, following a given input signal, the reaction proceeds according to a set of well-defined rules:

\[ F : Q \times \Sigma \rightarrow Q \]

\[ G : Q \times \Sigma \rightarrow \Theta \]

where "x" indicates interaction, and F is a transition function or a set of rules that pairs each member of set Q x \( \Sigma \) with one or more members of set Q. The system generates an output signal whose character is determined by its internal state according to the rule:

\[ G : Q \times \Sigma \rightarrow \Theta \]

where G is the output function that pairs the internal states plus received signals of the system with an output signal. Above statements are schematically shown in Fig. 1. It can be seen that the abnormal output signals (\( \Theta \)) (e.g., lower urinary inhibitory activity, hypercalciuria, hyperoxaluria, hyperuricosuria and hypocitraturia) and the abnormal output state function (G) as revealed by a dynamic procedure such as a calcium load test, indicate that there may be something wrong with the function of the urinary tract system (Q), and/or that the system cannot tolerate a strong stimulation of environment factors (\( \Sigma \)).

The application of systems science to stone-formation is summarized schematically in Fig. 2. Generally speaking, an input signal must be recognized first, then the body can trigger the control-unit for response. Thus, the body can remain in homeostasis during normal physiological processes. Homeostasis in human body represents a metabolic state with a very low probability value for stone-formation.
do not know where the initial site of formation of the urinary crystals is: in urine itself, within the renal tubular lumen or within renal tubular cells?

It has been demonstrated that a high percentage of large crystals (> 12 μm) is one of the characteristics of recurrent CaOx urolithiasis [99]. The lack of an appropriate reaction against exogenous disturbances, such as overloads of calcium, oxalate, acid, citrate, purine, and protein, in stone-formers suggests defects in their control system in vivo [38, 52, 61, 62, 86, 105, 106]. An epidemiological investigation has shown that stone-formers have a metabolic sensitivity to stimulation by the diet [99]. This aspect of a body process "out of control" may explain the existence of "injuries" to the urinary tract. An "injury" to the organism can arise both at the cellular and molecular levels. The same triggering factor may induce different pathological processes, depending on which sites and which target molecules are affected. Many abnormal phenomena, which are found in stone formers, e.g., pathological alteration of renal tubular cells, formation of Randall's plaques, alteration of the properties of the urothelium, and the secretion of abnormal macromolecular substances, might be attributed to the existence of injury to the renal epithelium.

According to the analysis of pathological processes of urolithiasis from the viewpoint of modern systems science (see Figs. 1-2), it is easy to understand the directions for future research as proposed by the Consensus Development Conference on the Prevention and Treatment of Kidney Stone [32]. Based on these directions, the following questions should be addressed:

(a) What are the triggering factors which induce stone-formation?

(b) How do these factors play a role in stone-formation?

(c) Where are the injuries located at the various levels associated with stone formation? What is the molecular basis of injury?

(d) Can we recognize and correct the molecular and cellular abnormalities to prevent stone formation and its recurrence?

Recently, evidence has indicated that the early events in the process of stone-formation occur in the kidney. These include:

(a) Sites of initial stone/crystal formation and their micro-environment [36];

(b) Injury of renal tubules and crystallization occurring afterwards [60, 69];

(c) Abnormal properties of intrarenal macromolecular inhibitors and stone formation [4, 6, 29, 30, 37, 50, 116, 117];

(d) Abnormal transport of calcium and oxalate in kidney tubular cells and its influence on stone-formation [12, 21, 55, 72, 74];

(e) Interaction between crystals and cells, and the mechanisms of crystal retention, especially investigation of the principle of cellular modulation in stone-formation [120];

Figure 2. General pathological model of urolithiasis deduced by system science. From the viewpoint of system science, urolithiasis can be thought of as a kind of disorder of regulation occurring between human body and its surrounding environment. Homeostasis represents a normal physiological regulation state which can tolerate triggering factors from the environment and remain unchanged. Once the homeostasis is interfered with too strong triggering factors, human body is undergoing a over-control regulation resulting in "injuries" or dysfunction. If the injuries cannot be repaired by the system (human body), the pathological process of stone formation will start. See text for details.
Physico-Chemical Theory of Stone Research

The physico-chemical theory of stone research has given the exact definition of the phases of stone-formation and has indicated the targets for the prevention of stone formation and stone recurrence [40, 81, 85, 96]. In the past, it has been shown that many factors can play a role in stone-formation by the means of thermodynamic and kinetic processes. Therefore, new data have to be linked with this physico-chemical theory. However, to explain the complex situation which exists in vivo, the physico-chemical theory of stone formation needs to be developed in two new directions:

(a) from the use of simple media (artificial or diluted urine) to complex physiological media (whole urine system, intracellular and extracellular fluid, and bio-membranes); and

(b) from classical thermodynamics in a closed system to non-equilibrium thermodynamics in an open reactive system.

It may be necessary to explain why there should be a development from the use of classical thermodynamics to non-equilibrium thermodynamics. This is logical, because stone-formation is the process of forming bio-minerals, occurring under "far-from-equilibrium" conditions in the body which itself is an open system. Hence, application of non-equilibrium thermodynamics in life sciences becomes more and more important.

Until now, no one has been able to produce in vitro bio-minerals such as bone, teeth and renal stones, that possess exactly the same features as their counterparts in vivo, in terms of composition and structure. A satisfactory explanation of the fine structure of the bio-minerals can be made utilizing non-equilibrium thermodynamics, because a stable, orderly and dynamic structure in which the system is transformed from a random to a non-random one, and form a high ordered structure with low entropy.

According to Prigogine’s theory [82] of dissipative structure, the change of entropy (dS) in an open system can be divided into the following two parts:

(a) the change of entropy production (dS<sub>p</sub>) is due to an irreversible process inside the system (e.g., heat transfer, diffusion, chemical reaction etc.); and

(b) the change of entropy current (dS<sub>e</sub>) is due to the exchange of materials and energy with the environment, that surrounds the system.

The total entropy change (dS) in the system is the sum of dS<sub>p</sub> and dS<sub>e</sub> described as equation (3):

\[ dS = dS_p + dS_e \]  

(3)

From the second law of thermodynamics we have, for a non-equilibrium state:

\[ dS_k \geq 0 \]  

(4)

but the value of dS<sub>e</sub> can be positive or negative, therefore, the change of entropy (dS) in the system will be positive or negative depending on the value of dS<sub>k</sub>. When dS is negative the total entropy of the system will decrease with decreasing value of negative entropy current (dS<sub>k</sub>) and the system may constitute a dissipative structure in which the system is transformed from a random to a non-random one, and form a high ordered structure with low entropy.

The concept that a non-equilibrium state is the origin of the sequence, has been recently recognized and accepted [82, 83]. Some questions previously assumed to be insoluble in the field of life sciences, such as biological evolution, self-catalysis, bio-self organization, negative and positive feedback control (which cannot be solved by classical thermodynamics) have been successfully explained [56].

To define the processes of stone-formation at the molecular and cellular levels, it is essential to have a model available. An attempt to incorporate the effects of secretion, re-absorption and flow in the urinary tract in a highly integrated computer-simulation strategy has recently been published [22]. A molecular model of the living cell, developed during Bhopalator (India) congress in 1985, integrates molecular genetics, enzymology and biochemistry within a coherent theoretical framework [56]. It can be supposed that the Bhopalator cell model may be useful for the later investigation of stone-formation. According to the Bhopalator model, the living cell has to be considered as a self-moving, self-regulating and self-reproducing machine that receives information and energy from its environment, processes the information and generates output signals to the environment in order to realize a telonomic-designed function [56].

Traditionally, one thinks that stones can be formed in urine with high supersaturation and low inhibitory activity [97]. This speculation perhaps is partially right, but the initial micro-environment might need a special reaction medium and a matrix, and the influence of a cellular metabolism. This environment is generally ignored. For instance, it has been suggested that minerals and organic matrices are both necessary for the orderly structure observed in urinary calculi. However, the following questions remain to be answered:

(a) Which component plays the decisive role in stone formation: the minerals or the organic matrix?

(b) What is the ordering structure of a stone?

(c) How do the minerals and matrix cling together?

It is necessary to study the mechanisms of stone formation not only in simple inorganic solutions, but also in complex physiological media such as whole urine, cytosol and tissue fluid [91, 104]. In addition, there is now more emphasis on the role played by tubular cells in stone-formation. There are several possible roles:

(a) Cellular compartmentalization may provide the required micro-environment for bio-mineralization;

(b) Cells may induce crystal nucleation on a template by supplying growth sites for crystallization
Renal Tubular Defects and Urolithiasis

The mechanism by which the initiation of calcium oxalate stone occurs is not completely understood. There are two main theories. One theory states that stone-formation is essentially an extracellular process. When the renal tubular concentrations of calcium and oxalate exceed the formation product of that salt, it causes spontaneous crystallization of calcium oxalate within the renal tubule. If within the transit time of urine through the urinary tract, the resulting crystals and aggregates grow sufficiently large to become trapped at some narrow section of the tract, they may go on to form stones [100]. The other theory states that it is an intracellular process. Crystals are formed first within the tubular cells and, if this is excessive, it leads to cell death. The crystals and cell debris extruded from the cell act as nuclei for renal stone-formation by blocking the tubule [93]. Whichever theory is correct, the concept that a cellular metabolic defect in the handling of inorganic ions and/or at in the synthesis of macromolecular substances is the basic problem in stone-formation has generally been accepted [21, 30, 68]. The speculation that renal tubular injury induced by various agents is the cause of urolithiasis has been supported by animal investigation [19, 60], cell culture study [69] and clinical observation [11].

Calcium oxalate stones can be induced in animals by the oral administration or injection of oxalate or one of its precursors, with or without gentamicin, have been indicated. In such models, renal tubular injury has been detected [27, 58-60]. The enhanced excretion of urinary enzymes, including brush border enzymes (such as alkaline phosphatase, γ-glutamyl transpeptidase and leucine aminopeptidase) and lysosomal enzymes (such as N-acetyl-β-glucosaminidase) during the first 24 hours of challenge, suggests that there may be damage to the brush border membrane of the renal tubules and that this correlates with the retention and deposition of crystals in the kidney [60]. Ultrastructural studies have also demonstrated a relationship between crystal deposition and damage to the tubular epithelium. Crystal deposits, formed as a result of hyperoxaluria, have always been associated with degradation of the cell membrane. But in these animal experiments, the following two questions were not answered:

(a) Does the renal tubular injury precede or follow the process of crystal formation?
(b) Is the initial crystal formation an intracellular or an extracellular process?

Deganello et al. [36] reported that, according to their micropuncture data, the tubular fluid in the thin segment of the Henle’s loop is the initial site of stone-formation. Recently, our ultrastructural study of experimentally induced microliths in rat was able to demonstrate not only ultrastructural changes in the proximal tubular cell, but also showed the presence of intracellular crystals in more distal tubular cells, after 4 to 8 days on the lithogenic diet [19].

It is known that a specific metabolic disorder cannot be found in 80% of patients with idiopathic calcium oxalate urolithiasis (ICU). It is not yet clear as to whether ICU is a specific disease or whether it is only the expression of an intrinsic metabolic abnormality, and is ICU a disease of renal cells or does it originate in the urine? Should we switch our research efforts to the biology of tubular cells, or should we continue to address the traditional stone-forming constituents of urine and their physico-chemistry in order to understand the early events of stone disease [107]? In this connection, the following questions need to be answered [32]:

(a) What are the fundamental mechanisms of ICU?
(b) Is renal tubular injury and/or dysfunction involved in the pathogenesis of stones?
(c) Is there a genetic basis for ICU and can individuals at risk of stones be identified using genetic probes?
(d) For defined genetic disorders will gene therapy have practical application?

In this connection, Bianchi et al. [17] found that in erythrocyte membranes the calcium-magnesium-ATPase activity was significantly increased in 38 patients with idiopathic hypercalciuria. They suggested that the abnormalities in erythrocyte calcium-magnesium-ATPase activity may represent an inherited defect in calcium transport related to the cause of idiopathic hypercalciuria.

Baggio et al. [11, 12] reported altered red blood cell transmembrane transport of oxalate in "idiopathic" renal stone formers, they found a higher-than-normal exchange rate in 78% of patients. This observation has since been confirmed by other groups [55, 74]. However, Motola et al. [72] have recently shown that the abnormal values of oxalate self-exchange in erythrocytes is not specific to patients with ICU but may be found in patients with other renal diseases. However, Baggio insists that Motola’s observation does support his hypothesis [43].

The defect in oxalate self-exchange in erythrocytes of patients with idiopathic calcium oxalate nephrolithiasis seems to lie in a Band 3 protein that controls anion transport by phosphorylation and dephosphorylation [13, 14]. Recently, Baggio et al. [15] reported that oral administration of glycosaminoglycans (GAGs) significantly lowered both the rates of Band-3 phosphorylation and erythrocyte oxalate self-exchange, and reduced urinary oxalate in 40 patients with idiopathic calcium-oxalate nephrolithiasis. The mechanism of action of GAGs in correcting the abnormality in the erythrocytes of patients with ICU seems to be through the inhibition...
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of the protein kinase involved in Band-3 phosphorylation. This, in turn, leads to a lower transmembrane oxalate transport.

Since red blood cells are good models for the study of membrane function and transport systems, the above results obtained from red blood cells could logically be extrapolated to other types of cells. If intestinal absorption and renal oxalate secretion occur in a fashion similar to that in red blood cells (via Band-3 or Band-3-related proteins) then the relationship between the common cellular defect and the pathophysiology of idiopathic calcium oxalate nephrolithiasis may be defined. New molecular and biological investigations in the field of Band-3 protein and anion transporters will have to be carried out in order to test the hypothesis that an inherited defect in the cellular transport of oxalate may be a common factor in idiopathic calcium oxalate nephrolithiasis [21]. In order to elucidate the cellular mechanisms underlying the cause of stone disease, Sigmon et al. [114] studied oxalate uptake in suspensions of renal cortical and papillary cells derived from control and stone-forming rats. They found that, in the renal tubular cells from rats, in which stones were induced by administration of ammonium oxalate and gentamicin, there was reduced uptake of oxalate in cortical cells and an enhanced uptake of oxalate in papillary cells compared with the corresponding cells from untreated control rats. This finding suggests that the alteration of oxalate transport in damaged rat renal tubular cells may play a role in the causation of their stones.

In summary, up to now, the fundamental mechanisms of ICU are not yet solved. Although it is not clear yet whether or not there is a common defect in cellular calcium and/or oxalate transport in the patients with ICU, further investigations of searching for any possible genetic abnormality in these patients probably are needed.

Free Radical Reaction in Stone Research

A marked increase in interest in the role of free radicals in biology and medicine has developed during the last 20 years [42, 47]. (A free radical is an atom or molecule with one or more unpaired electrons in the outer orbit such as \( \cdot \text{O}_2 \) and \( \cdot \text{OH} \).) Many free radicals can be detected using an electron paramagnetic resonance spectrum analyzer.

A multitude of endogenous and exogenous sources of free radicals exist. Exogenous sources include oxidative drugs (such as \( \text{CCl}_4 \) and acetaminophen), cigarette smoke, radiation and substances that oxidize glutathione. Considering the situation \textit{in vivo}, cellular sources include the mitochondrial electron transport chain, the microsomal electron transport chain, oxidation enzymes (such as xanthine oxidase and cyclooxygenase), phagocytes, and cellular auto-oxidation of Fe\(^{2+} \) and epinephrine [42].

In general, in a free radical reaction, the following three steps are present: induction \( \rightarrow \) propagation \( \rightarrow \) termination. Once a free radical reaction is induced in cells, the reaction is not only fast, but it may also seriously damage cells. For example, lipid peroxidation occurring on the cellular membrane may disrupt the structural integrity of the lipid bilayer leading to increased membrane permeability and subsequent impaired ion transport, impaired electron transport for the oxidative phosphorylation in mitochondria, and increased lysosomal permeability. Increased lysosomal permeability causes the release of hydrolytic enzymes that further enhance cell injury [44].

Recent evidence indicates that an oxygen and/or hydroxyl radical reaction mechanism can injure renal tubular cells and promote calcium oxalate crystallization within the cells [94]. Bakris et al. [16] reported that the increase in urinary Tamm-Horsfall protein excretion after injection of contrast medium is, in part, mediated by oxygen free radicals and may serve as a marker of renal tubular injury. Walker and Shah found evidence for a role of hydroxyl radical in gentamicin-induced acute renal failure in rats [121]. Selvam and Kurien [109] reported that oxalate can induce lipid peroxidation through inhibition of catalase activity.

Lipid peroxidation of unsaturated fatty acids has been linked with altered membrane structure and enzyme inactivation which may lead to an oxidative cellular damage [39]. Although it was known that deficiency of vitamin B-6 results in increased oxalic acid biosynthesis in liver and leads to hyperoxaluria, very little work has been done on the implications of the hyperoxaluria induced by vitamin B-6 on lipid peroxidation in subcellular fraction of the rat kidney. Recently, the results presented by Ravichandran and Selvam [94] indicated that calcium and oxalate accumulate predominantly in the subcellular fraction of the kidney, and that these membranes-like materials may be the site for stone-formation through peroxidative damage.

More recently Selvam and Devaraj [110] have examined the effect of lipid peroxidation on oxalate binding in mitochondria. Their finding is that lipid peroxidation, via the hydroxyl radical reaction, led to oxalate binding in rat kidney mitochondria. Therefore, it can be reasoned that the accumulated oxalate, derived from the enhanced oxalate binding reaction under the condition of increased peroxidase activity, may be an important factor for the aetiology of stone formation.

Erythrocyte lipid peroxidation has been found to be elevated in kidney stone patients [3]. Taking into account the fact that the flux rate of erythrocyte oxalate self-exchange is abnormally high in the patients with idiopathic urolithiasis [10], one may speculate that the elevated lipid peroxidation, resulting in injury of the membrane, may be responsible for this finding.

Increasing evidence regarding the role of free radical reactions in the causation of stone-formation suggests that the effects of free radical reactions on oxalate binding, on macromolecular substances secretion and on the oxalate transport should be explored in model systems, both \textit{in vitro} and \textit{in vivo}. A new investigation of
Table 4. Various macromolecular abnormalities in urolithiasis.

<table>
<thead>
<tr>
<th>Name</th>
<th>Abnormality</th>
<th>Effects on urolithiasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine:glyoxylate aminotransferase</td>
<td>Deficiency</td>
<td>Type-I Hyperoxaluria</td>
</tr>
<tr>
<td>D-glycerate dehydrogenase</td>
<td>Deficiency</td>
<td>Type-II Hyperoxaluria</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Deficiency</td>
<td>Urolithiasis (Xanthine)</td>
</tr>
<tr>
<td>Ribose phosphate pyrophosphokinase</td>
<td>Superreactivity</td>
<td>Urolithiasis (Uric acid)</td>
</tr>
<tr>
<td>Hypoxanthine phosphoribosyl transferase</td>
<td>Deficiency</td>
<td>Urolithiasis (Uric acid)</td>
</tr>
<tr>
<td>Adenine phosphoribosyl transferase</td>
<td>Deficiency</td>
<td>2,8-Dihydroxydeninuria</td>
</tr>
<tr>
<td>Inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephrocalcin</td>
<td>Deficiency or abnormal structure</td>
<td>CGI and CAI**</td>
</tr>
<tr>
<td>Tamm-Horsfall protein</td>
<td>Abnormal existing state</td>
<td>CGI and CAI</td>
</tr>
<tr>
<td>Glycosaminoglycans Heparan sulphate and Chondroitin sulphate</td>
<td>Deficiency, Structure and Composition</td>
<td>CGI and CAI; anti-adherence of crystals</td>
</tr>
<tr>
<td>Ribonucleic acid (RNA)</td>
<td>Deficiency or others</td>
<td>CGI and CAI</td>
</tr>
<tr>
<td>Uropontin</td>
<td>Unknown</td>
<td>CGI and CAI</td>
</tr>
<tr>
<td>Crystal matrix protein</td>
<td>Unknown</td>
<td>CGI and CAI</td>
</tr>
<tr>
<td>Inter-α-trypsin protein</td>
<td>Unknown</td>
<td>CGI and CAI</td>
</tr>
<tr>
<td>Water surrounding macromolecules in kidney</td>
<td>Abnormal structure or composition</td>
<td>CGI and CAI</td>
</tr>
<tr>
<td>Anion exchanger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Band-3 protein</td>
<td>Structure</td>
<td>Oxalate transport</td>
</tr>
<tr>
<td></td>
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<tr>
<td>The abnormalities of enzyme, inhibitors and anion exchanger reflect metabolic disorders and cellular injuries or dysfunctions in patients with urolithiasis. **CGI: inhibition of crystal growth; CAI: Inhibition of crystal aggregation.</td>
<td></td>
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</table>

the mechanisms of a free radical reaction in stone formation [111] and an application of a free radical scavenger such as DL α-lipoic acid in stone prevention has more recently been reported [118].

Macromolecular Abnormalities and Their Correction in Urolithiasis

Recently, investigators have concentrated on trying to determine the macromolecular abnormalities associated with urolithiasis and on looking for approaches to correct them. Modern molecular biological techniques are available to clarify the relationship between abnormal macromolecular substances and stone formation. Probably gene therapy of stone disease can be expected in the future. Various macromolecular abnormalities in urolithiasis are summarized in Table 4. Because the listed molecular pathophysiological mechanisms of the enzyme disorders have been clarified, here we will only discuss the following abnormalities of macromolecules:

Nephrocalcin

Nephrocalcin is a glycoprotein isolated and purified by Nakagawa et al. [75, 79]. The nephrocalcin in urine is a macromolecular complex with molecular weight (MW) from 14 to above 200 kD. Cell culture and immunohistochemical examination have indicated that nephrocalcin is present in renal proximal tubular cells and in the thick ascending limb of Henle's loop (TAL) [76, 115].

It is well known that normal nephrocalcin not only inhibits the growth of calcium oxalate crystals, but also the aggregation of such crystals which is a most important risk factor for stone-formation [48, 78, 125]. Nephrocalcin can be adsorbed to calcium oxalate crystals forming a monomolecular layer which inhibits crystal growth. As nephrocalcin is a metabolic product of renal cells, defects in the molecular structure of nephrocalcin and a pathophysiological disorder may be responsible for stone formation. An investigation of the interaction between a cultured cell line of monkey kidney cells and

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calcium oxalate monohydrate (COM) crystals has been done by Lieske et al. [65] who found evidence that nephrocalcin can block the initiated DNA synthesis induced by COM crystals stimulation.

Patients who form kidney stones, are claimed to produce an abnormal nephrocalcin with a very weak inhibitory activity towards calcium oxalate crystallization [77]. The main molecular abnormality of nephrocalcin from patients with calcium oxalate nephrolithiasis is said to be a deficiency in γ-carboxyglutamic acid (Gla). Because Gla in nephrocalcin commonly stabilizes molecules into their final configurations through calcium bridges, loss of Gla leads to reduction of inhibitory activity of nephrocalcin and to loss of its amphility [77, 78]. However, later data do not support the finding [Netzer et al. (1992) and Hughes et al. (1992); abstracts at the VIIth International Symposium on Urolithiasis, Cairns].

Recently some new non-nephrocalcin inhibitors of crystallization including uropontin [116], crystal matrix protein (CMP) [37], Inter-α-trypsin protein [117], and an other, unnamed, glycoprotein [4] have been demonstrated. These inhibitors are listed in Table 4.

**Tamm-Horsfall protein/Uromodulin**

Tamm-Horsfall protein (THP) isolated from human urine by salt precipitation method [119] and uromodulin isolated from urine of pregnant women by lectin adherence columns [73] are the most abundant proteins in normal human urine, although THP and uromodulin may be different names for the same protein since the sequence of cDNA and the amino acids of uromodulin has recently been shown to be identical to THP [53, 89]. The physico-chemical and biological properties of THP have been studied extensively, but its function in various pathological and physiological processes remains obscure [54, 102]. Rindler et al. [95] have used cDNA encoding for THP/uromodulin to study the release of the expressed protein from cultured cells. Their results indicate that THP is one member of lipid-like membrane proteins and is released into the urine after the loss of its hydrophobic anchor, probably by the action of a phospholipase or a protease. Two excellent reviews have recently been published on this topic: one provides background data on the synthesis of THP/uromodulin and on its location and properties [63], the second reviews the roles of THP in calcium oxalate monohydrate crystallization [49].

THP/uromodulin is a major and specific renal glycoprotein which readily polymersizes to form a gel under conditions of low pH, high ionic strength and the presence of certain mono- and divalent ions. It is synthesized by the kidney and localized in the epithelial cells of the thick ascending limb (TAL) of Henle’s loop and of early distal convoluted tubules [64, 112]. THP/uromodulin is released into urine in large quantities in a polymeric form (MW = 7x10^9 Da). Nuclear magnetic resonance (NMR) data and available molecular biological evidence suggest the presence of a single THP gene and multiple lectin binding sites in the tetraantennary chain of THP, which further suggest that there may be many immunomodulatory roles for THP [33, 53, 123].

Based on THP/uromodulin synthesis, localization and molecular structure, this glycoprotein may play the following roles in calcium oxalate urolithiasis:

(a) THP may be considered as a intranephronic inhibitor of crystal growth and agglomeration. It prevents large crystal formation under physiological conditions [30, 108].

(b) Specific intranephronal pathological conditions, such as low pH, high ionic strength, high concentration of sodium and calcium and presence of oxygen free radicals, can stimulate THP self-polymerization in tubules. As a result, THP may be considered as a promoter of stone formation [103].

(c) According to the protective colloidal theory of stone formation [23], THP in normal urine could be bound to crystal surfaces as a stabilizing agent for the urinary colloidal system through a steric stabilization mechanism. Thus, THP can be considered as an inhibitor of stone-formation.

(d) Depending upon the urinary environment, THP in urine may exist in different forms. Non-polymerized THP can be considered as an inhibitor of crystallization; whereas polymerized THP is a promoter [18, 108].

(e) Since mannose-sensitive fimbriated bacteria such as *E. coli* adhere to THP via mannose-containing side chains, it has been suggested that THP may trap urinary pathogens and prevent them from cloning the urothelium [84]. This mechanism of THP may be beneficial for the prevention of stone-formation and stone recurrence.

(f) A free radical reaction may occur in the kidney to induce stone formation, in which intranephronal THP could be polymerized and denatured in a very fast reaction [16].

Although many studies have been carried out on THP/uromodulin gene analysis, molecular structure analysis, immunological characterization and its corresponding physiological functions, we still do not know what is the exact nature of THP from patients with calcium oxalate urolithiasis either in molecular structure of THP or in its function. It has been shown in several studies that THP from stone formers, at low pH in the presence of NaCl and calcium, exhibits an increased tendency towards self-aggregation which reduces its ability to interact with crystals and prevent their aggregation [18, 50]. The tendency may be inheritable, but the molecular basis for the effect is unknown. Further investigation of the molecular defects in THP from patients with urolithiasis may be an important direction for stone research.

**Glycosaminoglycans**

Glycosaminoglycans (GAGs) are polysaccharides

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1. In literature, some confusion exits about GAGs and polysaccharides. Some semi-synthetic polysaccharides such as sodium petosan polysulphate are not real GAGs, they should be named sulphated polysaccharides.
chains composed of repeating disaccharides of identical composition. GAGs including heparan sulphate, chondroitin sulphate, dermatan sulphate and hyaluronic acid are naturally occurring in human urine. GAGs may be present in urine in a free form or in association with proteins, such as glycoproteins. Little is known about the mechanisms involved in the synthesis and excretion of GAGs in man. Although the differences of urinary GAGs in quality or quantity between patients with urolithiasis and normals are not clear yet, there is no doubt that GAGs and a number of semi-synthetic sulphated polysaccharides can modulate calcium oxalate crystallization as is shown in various model systems in vitro and in vivo [26, 51]. The following mechanisms of action of GAGs in calcium oxalate stone prevention:

(a) GAGs can act as inhibitors of calcium oxalate crystal growth and aggregation in vitro [26, 34];
(b) GAGs act as protectors of the urinary tract mucosa and can prevent crystal adherence to the mucosa [45, 88, 120];
(c) GAGs can correct oxalate transport disorders occurring at cellular membranes [15, 21];
(d) GAGs can modify the micro-environment within cells and in urine to prevent stone-formation [25].

It should be noted, however, from a recent study in rats that chondroitin sulphate promotes the growth of stones in the urinary tract [71]. This finding suggests that there is a complex situation in the mechanism of action of both endogenous GAGs and exogenous sulphated polysaccharides in stone formation. Investigations of the mechanisms by which GAGs prevent stone-formation should not be restricted to urines. The correlation between active stone formation and GAGs behaviour in cell should be an important project. Many receptors for sulphated polysaccharides (GAGs) on lymphocytes as well as on other cell types such as macrophages, polymervonuclear leukocytes, mast cells and fibroblasts, have been demonstrated by Chong and Parish [28] and Parish and Snowden [87]. Their studies illustrated that a variety of cell types and cell lines are able to bind certain sulphated GAGs selectively. Clearly, biochemical characterization of these receptor molecules is important for a better understanding of the mechanisms of stone-formation at the cellular level.

Baggio et al. [15] found that GAGs inhibit the phosphorylation of Band-3 protein that may be responsible for oxalate transport. Oral GAGs can correct the abnormality of red blood cell oxalate self-exchange rate and reduce urinary oxalate excretion in patients with idiopathic calcium nephrolithiasis.

In spite of the possible protective effects of GAGs against stone-formation shown in recent experimental results, the possible role for the accumulation of GAGs in stone matrix has to be investigated. It is possible that molecular defects of GAGs caused by abnormal cellular metabolism, have some connection to stone formation. 

**Water-surrounded macromolecules in kidney**

A new approach, at the molecular level, to the understanding of the processes of stone-formation is to investigate the specific pathophysiological and biochemical characteristics of the stone forming environment [6-8]. In these studies, proton-relaxation time measurements were performed on lyophilized urine samples collected from recurrent calcium oxalate stone formers (SF) and normal subjects (N). \(^{1}H\) - and \(^{2}H\)-nuclear relaxation times (T1, T2) which change during rehydration of various lyophilized urines, were measured using NMR. The prolongation of the relaxation times as a function of rehydration was found to differ significantly between SF and N (P < 0.005). Water compartmentalization was calculated according to a fast proton diffusion model. Throughout most of the rehydration process, significantly less water (P < 0.001) was bound to the compounds of urine from SF than that obtained from normal subjects. These results suggest that the macromolecules in the urine of calcium oxalate stone-formers and normals differ in their amount of water binding sites and in the water multilayer thickness surrounding them. Hydrophilic compounds, with a relatively large active surface area, seem to be characteristic of the urine of normal individuals. Azoury’s results [6-8] obtained from lyophilized urinary compounds by NMR are similar to those of Nakagawa et al. [78] who reported that an important feature of the nephrocalcin from patients with urolithiasis is the loss of its amphilicity, which indicates less separated and well-organized hydrophilic and hydrophobic sites.

**Band-3 and related protein**

Increasing evidence indicates that a high concentration of oxalate is a more important risk factor for stone-formation than hypercalciuria [41, 101]. Williams and Wandzilak [124] reviewed the mechanisms involved in the synthesis, absorption, excretion, and transport of oxalate and the factors controlling these processes in man, and gave background data on the clinical syndromes associated with hyperoxaluria and recurrent calcium oxalate stone disease.

In recent years, the possibility that some patients with urolithiasis have a generalized oxalate transport abnormality, has attracted the attention of investigators [11-15, 17, 21]. Band-3 protein is a specific anion transporter which is initially found located on the membrane of red blood cells [5, 24, 31]. It is well known that Band-3 protein is a 91k amino acid protein, and its corresponding gene is located on chromosome 17 [67]. The carboxyl-terminal domain in Band-3 protein is a hydrophobic transmembrane domain to serve the anion transport by phosphorylation and dephosphorylation [20]. Searching for Band-3 and related proteins in non-erythroid cells and other tissue may confirm the concept that a defect in cellular oxalate transport is the fundamental abnormality in idiopathic calcium oxalate nephrolithiasis. Once it is identified that the defect of oxalate transport in the kidney and the gut is similar to that in red blood cells, a logical speculation can be made that if there is something wrong with Band-3 in red blood cell it will be the same for other anion exchangers. Increasing evidence indicates that many anion-exchangers share
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**Figure 3.** Anion exchangers in cell membranes (both basolateral and apical) of renal proximal tubular cells. Circles with shadow represent the exchangers relating to oxalate transport in which a Cr:oxalate exchanger and a possible Na-dependent sulphate exchanger at the luminal membrane surface and a SO⁴⁻(Oxalate):HCO³⁻ exchanger at the contraluminal membrane surface.

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Functional similarities with Band-3 protein. Selected Band-3 proteins related to anion transporters of renal epithelia are listed by Alper [1] in which MCT (medullary collecting tubule), CCT (cortical collecting tubule), CTALH (cortical thick ascending limb of Henle), MTALH (medullary thick ascending limb of Henle), PST (proximal straight tubule), PT (proximal tubule), BBMV (brush border membrane vesicles of proximal tubule), and BLMV (basolateral membrane vesicles of proximal tubule), LLC-PK1 and MDCK are involved. A supposed mechanism of oxalate secretion by the renal proximal renal tubule is illustrated in Figure 3 [46] which has recently been confirmed by the new result obtained from LLC-PK1 cell line [70].

Although human erythrocyte Band-3 is encoded by a single-copy gene [67], and is expressed only by erythrocytes and by a subpopulation of acid-secreting cells in the distal nephron [35] where no oxalate transport occurs, immunological evidence indicates that the genes encoding Band-3 protein of the kidney and the intestine belong to the same family. Anion exchange in non-erythroid cells may be mediated by closely related molecules.

In our stone research laboratory, a new cell culture model has been set up for studying epithelial handling of oxalate and cellular interactions with crystals of stone salts. In the model system, renal epithelial cells cultured in two-compartment Transwell cell culture chambers on collagen-coated permeable supports form highly differentiated polarized monolayers resembling naturally occurring epithelium. Our preliminary result did not demonstrate a significant transcellular transport in LLC-PK1 cell line under the physiological conditions used [120]. However, oxalate transport across the same monolayer of renal proximal tubular epithelial cells (LLC-PK1) as we used, has been shown by several research groups [70, 122]. This phenomenon may have something to do with the following points: (a) The characteristics of anion transport in LLC-PK1 cells may have been lost; (b) extracellular environment including used buffers, temperature, pH and concentration of chloride, sulphate and bicarbonate; (c) the state of LLC-PK1 cells in these experiments; and (d) individual operation program in these experiments. We are now trying to search for cellular oxalate transport in a primary cultures of human renal proximal tubule in our cell culture model.

We believe that a molecular biological approach will provide a powerful complement to immunological and ligand-based methods for describing a class of the anion transport proteins of renal and other epithelia, which would be useful for stone prevention.
Oxalate degrading enzymes

The concept that urinary oxalate is the most important risk factor in patients with calcium oxalate urolithiasis has been generally accepted. Thus, a logical topic for stone research should be to develop effective and safe methods for lowering urinary oxalate, regardless of the specific aetiology of stone disease. Many approaches which may lower urinary oxalate include inhibiting oxalate synthesis in liver and kidney, blocking oxalate secretion into the renal proximal tubule, and decreasing its absorption in the gut. Allison [2] has put forward the idea that urinary oxalate may be lowered by using oxalate-degrading enzymes in the gut. This suggestion is based on the fact that sheep fed with oxalate-rich plants, in presence of sufficient numbers of degrading oxalate bacteria in their rumen (e.g., Oxalobacter formigenes) are able to tolerate increased amounts of oxalate without forming calcium oxalate stones.

Although mammalian cells do not produce any enzymes that are capable of degrading oxalate, two major pathways for the degradation of oxalate in non-mammalian systems are: oxalate oxidase (EC 1.2.3.4) and oxalate decarboxylase (EC 4.1.1.2). Recently two coenzymes (formyl-CoA transferase (EC 2.8.3.4) and Oxalyl-CoA decarboxylase (EC 4.1.1.8) participating in biochemical pathways of oxalate degradation in oxalobacter have been purified and identified [9, 10]. It is obvious that the applications of these enzymes may contribute towards reducing the circulating oxalate levels in vivo. Such treatment could be beneficial for prevention of calcium oxalate stone disease. An early example of application of oxalate degrading enzyme in vivo has been reported by Raghavan and Tarachand [92], who gave rats oxalate oxidase plus peroxidase, loaded in dialysis membranes, by intraperitoneal implantation. In recent years, rapid developments of drug delivering techniques such as liposome formation, erythrocyte loading or conjugating with poly ethylene glycol (PEG), can be expected for the therapy of these oxalate-degrading enzymes. Based on modern molecular biological concepts, an innovative proposal to transfer the gene of an oxalate-degrading enzyme into human cells has been put forward. This might be an extremely effective procedure for reducing the amount of oxalate in the body and in urine but it is a difficult and dangerous technique. Recently, cloning of the oxalyl-CoA decarboxylase gene from the bacterium Oxalobacter formigenes and its subsequent expression in a foreign environment has been demonstrated by Lung et al. [66].

Concerning the correlation of abnormal macromolecular substances (MMS) to urolithiasis, in general, isolation, purification and identification of the MMS is an important topic in the field of stone research. Searching for exact defects of the MMS in stone formers could be the first step to understanding the mechanisms of stone formation. In addition, it should not be forgotten that existing state of the MMS isolated and their properties may or may not represent the actual situation in vivo.

Conclusions

In summary, the clinical treatment of urolithiasis is still unsatisfactory, in spite of the technological advances brought by extracorporeal shock wave lithotripsy (ESWL) and endoscopic surgery. But why has so little progress been achieved in the field of stone prevention? Perhaps, the old concepts on stone-formation need to be challenged and a fresh approach to the problem introduced. In this paper, we have attempted to do this by considering stone-formations a form of bio-mineralization subject to the laws of non-equilibrium thermodynamics and systems science. We firmly believe that the processes involved in the formation of stones need to be studied at the molecular and cellular level and that there needs to be a strengthening of the links between basic and clinical research in the field.

References


New concepts in renal stone research


New concepts in renal stone research


Discussion with Reviewers

H.-G. Tiselius: Oxalate is undoubtedly a very powerful urinary constituent in terms of calcium oxalate crystallization. Which limit of normal oxalate excretion is applied by the authors and according to your experience how great is the fraction of stone formers that demonstrate a hyperoxaluria?

Authors: According to the critical value for defining hyperoxaluria ( > 0.45 mmol of oxalate excreted within 24-hour urine), approximately 20-25 per cent of the patients with calcium urolithiasis were found as hyperoxaluria in our hospital. Due to the limitation of precise analytical methods of oxalate in urine and serum/plasma, no systematic metabolic evaluation of hyperoxaluria has carried out in our hospital so far. Therefore, we just follow the common formula to treatment of the stone patients with hyperoxaluria. However, we would like to emphasize here the following points:

(a). A systematic evaluation for hyperoxaluria, for instance, as Hatch recently described (Urol Res 21: 55-59, 1993), is clearly needed not only for further understanding the pathophysiology of hyperoxaluria, but also for successful treatment.

(b). Although many methods to reduce urinary oxalate can be chosen as listed in your review (Mineral Electrolyte Metab 13: 242-250, 1987), best treatment for individual patients with hyperoxaluria should be in accordance with the classification of hyperoxaluria. Recently, a new classification of hyperoxaluria has been reported in which the term "mild metabolic hyperoxaluria" was much improved (Urol Res 21: 55-59, 1993).

(c). Endogenous production of oxalate contributes to the hyperoxaluria, therefore, finding a way that could significantly reduce endogenous production of oxalate may an important topic.

(d). A link between hyperoxaluria and its molecular and cellular basis should be made in further research.

(e). The viewpoint of system science described in the present review paper is certainly needed in the research on hyperoxaluria. For example, Prof. Dr. Rose
recently showed that excreted oxalate in plasma and 24-hour urine significantly decreases with oral pyridoxine in normal subjects, but does not in idiopathic stone formers with hyperoxaluria (Urol Res 18: 393-396, 1990). This result may reveal a disturbance of system regulation existing in patients which could be connected to a injury/dysfunction of renal tubular cells.

**H.-G. Tiselius:** Although most calcium stones contain calcium oxalate, a significant number of these stones also contain calcium phosphate. Do you have any speculation on the role the latter salt plays in these stones?

**Authors:** This is an interesting question, and also difficult to be answered in a brief way. The concept that urolithiasis is a heterogeneous disorder, with varying chemical composition and pathophysiological background has generally been accepted. Therefore, it is no surprise to see the phenomenon that calcium phosphate can often be found in calcium oxalate stones. The following speculations are listed for discussion:

(a). According to chemical characteristics of the calcium ion, calcium is one of the most active and rich divalent elements in vivo which can easily form insulated salts either in physiological bio-mineralization (e.g., bone, teeth and other hard tissues) or in numerous pathological bio-mineralizations (e.g., urinary tract stones and dentinal stones). Calcium ions can easily connect to oxygen atoms proved by a number of amino acids of protein, phosphoproteins and phospholipids. In addition, calcium ions can combine with polysaccharides and glycoprotein to form stable macromolecules. All those macromolecules containing calcium have biochemical and physiological activity and certain functions in vivo.

(b). From the urinary chemistry, human urine is supersaturated for a number of calcium phosphates including hydroxy apatite (HAP), octacalcium phosphate (OCP), and di-calcium phosphate dihydrate (DCPD).

(c). From the physicochemical viewpoint of stone formation, it is well known that calcium oxalate monohydrate (COM) can precipitate on the surface of seed crystal of DCPD and HAP and that calcium oxalate trihydrate (COT) can induce crystallization of HAP at low levels of supersaturation and HAP was a suitable seed for further COM grow. In addition, stone matrix made by dysfunctional renal tubular cells can combine with HAP as a HAP-sphere that can induce COM heterogeneous nucleation.

(d). From the viewpoint of crystal retention, i.e., the interaction between cell and crystal, glycoprotein, an important component of the cellular membrane, presumably has only two kinds of binding sites for accepting calcium or phosphate, but none for oxalate. Oxalate presumably combines with the glycoprotein through a phosphate bridge. In addition, phosphoproteins and phospholipids located on renal tubular cellular membranes may provide the sites for combining calcium which means these macromolecules provide a connection between a crystal/stone and tissue.

The differences of chemical composition and structures shown in a giving stone may have something to do with the kinetic process of its formation and with disturbances of the patient with urolithiasis. Therefore, studying stone composition and structure may be beneficial for understanding the mechanisms of stone formation.

**H.-G. Tiselius:** It is an interesting idea that crystals form within the cell, from which they subsequently are expelled or released. Would you consider it reasonable to assume such a mechanism even for the crystalluria observed in normal subjects?

**Authors:** Recently the finding of intracellular crystals has been shown in an ultrastructural study of experimentally induced microliths in rat proximal and distal tubules (J Urol 149: 893-899, 1993). Our results strongly suggest that renal tubular cells are the host in stone formation as a pathological bio-mineralization which should be responsible for all earlier events of nephrolithiasis. However, it remains uncertain which process is the first: luminal crystals or intracellular crystals? intracellular changes or intracellular crystals? Therefore, at this moment we can hardly imagine the behavior of intracellular crystals such as their expelling or releasing.

From the viewpoint of systems science and bio-mineralization, the crystalluria observed in normal subjects can be thought of as the result of a physiological regulation against interference with an excess high concentration of stone salts in the urinary system. Crystalluria seen in normal subjects also reflects injury/dysfunction of the renal tubular cells, whereas a series of abnormalities including renal handling ions, renal secretion modifiers of crystallization and epithelium properties will happen resulting in stone formation. Therefore, crystalluria can be considered as a mirror of the environment where crystals and stones are formed.

**J.P. Kavanagh:** Has the use of system science given you new insights into problems of urolithiasis and do you think it will help in the future as greater understanding of stone formation at the molecular level develops?

**Authors:** The answer should be "yes". This review paper represents our own thinking on current stone research. System science as a tool and a strategy in urolithiasis research has been introduced by Prof. Finlayson about 20 years ago [41]. In fact, many applied methods and achieved results in stone research are more or less connected with the theory. You yourself have also applied this viewpoint to explain the high crystal nucleation rate you found in normal subjects [57]. Each investigator should have the systematic viewpoint when he performs his study. Many examples have been presented in our manuscript above.