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ROLE OF AGGLOMERATION IN THE EARLY STAGES OF PAPILLAR STONE FORMATION

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Abstract

Possible effects of crystal agglomeration on the early stages of calcium oxalate papillar stone formation are evaluated. The collecting ducts are filled with liquid that flows laminarly as established through hydrodynamical and physicochemical considerations. Under such conditions, agglomeration due to laminar shear forces proceeds. Agglomeration of calcium oxalate monohydrate crystals present in urine at a concentration typical for clinically observed crystalluria cannot result in the formation of a particle sufficiently large enough to be retained in the Bellini's duct and become a papillar stone nidus (nucleus). Formation of such an aggregate during the passage time of urine through the duct requires an unrealistically high concentration of crystals in urine, one that exceeds the normal content of urinary oxalate by several orders of magnitude. Aggregates obstructing the Bellini's duct as assumed in the free particle theory cannot represent a major factor in stone formation. This conclusion is corroborated by experimental results and other observations.

Key Words: Calcium oxalate monohydrate, papillar calculi, mechanism of stone formation, agglomeration of crystals.

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Introduction

Though considerable effort has already been devoted to the problem of renal stone formation, no definitive solution is available yet. Many different theories of stone formation, put forward during years of research, are often built on speculations rather than solid experimental and theoretical grounds. According to one of the few solid theories proposed by Finlayson [7, 8], the possible mechanisms of renal stone formation can be broadly divided into two groups, namely, the mechanism of the free particle and the mechanism of the fixed particle.

The mechanism of the free particle assumes that crystals nucleated in urine acquire a sufficient size during residence time in the Bellini's ducts or in the inner kidney, a size which precludes their being washed away from the upper urinary tract. That is, crystals must become large enough either to obstruct the Bellini's duct or to settle in the pelvis in order to serve later as a stone nucleus. However, the crystal growth rate ensuring that nucleated crystals can reach a required size within the available residence time exceeds the value established for conditions prevailing in the kidney by several orders of magnitude. This conclusion is also confirmed by an experimental model of the Bellini's duct showing that formed crystals are far too small (of the order of micrometers) to be trapped in the urinary tract [2]. Therefore, only in rare cases are crystals formed in the urine retained in the kidney to later become a stone nidus (nucleus).

The fixed particle mechanism is based on the idea that crystals are either nucleated in a liquid and subsequently attached to the urinary tract wall or are formed directly on the wall. Particles attached to the wall are prevented from washing away from the kidney and can constitute a stone nidus. The observations that more than 30 [10] or 60% [6] of calcium oxalate monohydrate (COM) stones show distinct signs of attachment to the wall lends support to this theory. These facts, together with the very low probability of the free particle mechanism resulting in a renal calculus, suggest that the fixed particle mechanism represents the prevailing course of renal calculi formation.

Symbol Table

A	constant
A_D	cross section area of the Bellini's duct
d	diameter of a capillary
d_D	diameter of the Bellini's duct opening at termination on papilla
d_e	Stokes diameter, i.e., diameter of a sphere settling with the same rate as a particle in question
g	the acceleration due to gravity
G	shear rate
k_v	volume shape factor
L	capillary elevation
L_D	length of the Bellini's duct
m	mass
N	number of crystals
N_o	number concentration of separate particles at t = 0
N(i)	number concentration of entities composed of i particles
P	total number of Bellini's ducts per kidney
Re	Reynolds number
r	particle radius
t	passage time
u	liquid flow rate in the Bellini's duct
V_D	a volume of liquid passing through one kidney
α	collision efficacy factor
γ	interfacial tension on the liquid-vapor interface
μ	liquid dynamic viscosity
ρ_l	liquid density
ρ_S	solid density
τ	the half time of agglomeration
θ	wetting angle

However, the conclusion of the free particle theory was recently called to question based on various experimental results concerning crystal agglomeration [13, 15, 17] since this process was not, in fact, considered in the original Finlayson's theory. Based on experimental observations, it was suggested that agglomeration could account for a rapid increase in a solid particle size to a dimension necessary for obstructing the Bellini's duct. Notwithstanding apparent plausibility of this theory, possible effects of agglomeration on the size of final particles have not been assessed quantitatively based on the theory of agglomeration.

This paper presents a theoretical estimation of effects caused by agglomeration on the size of particles appearing in the Bellini's ducts and their conceivable impact on the early stages of renal calculi formation. All considerations herein are confined to papillar calculi containing COM as a predominant component.

Hydrodynamics of Bellini's Ducts

According to Finlayson and Reid [8], in the human kidney, the number of Bellini's ducts per papilla ranges from 13 to 86 (mean: 39.5), and the number of papillae ranges from 4 to 13 (mean: 7.9). Hence, the total number of Bellini's ducts per kidney, P, varies approximately between 50 and 1120 (mean: 310). The length of a Bellini's duct is $L_D = 2.2 \times 10^{-2}$ m, and the diameter of its opening at its termination on the papilla is $d_D = 2 \times 10^{-4}$ m [8]. Assuming a duct opening of circular shape, the cross-sectional area of the duct opening is $A_D = \pi \times (1 \times 10^{-4}\ m)^2 = 3.14 \times 10^{-8}\ m^2$. Taking two liters as the volume of liquid passed through both kidneys per 24 hour period and assuming a constant liquid throughput, the liquid volume passing through all the Bellini's ducts in one kidney per second is $V_D = \{1 \times 10^{-3} / (24 \times 60 \times 60)\} = 1.16 \times 10^{-8}\ m^3 \cdot s^{-1}$.

The flow rate of liquid can be determined as the liquid volume passed per unit time through a given cross-sectional area. The flow rate in the Bellini's duct, u, is thus:

$$u = \text{liquid volume} / (\text{no. of ducts} \times \text{cross-sectional area}) = V_D/(P \times A_D). \quad (1)$$

Using already established values, the maximum flow rate is:

$$u = 1.16 \times 10^{-8}/(50 \times 3.14 \times 10^{-8}) = 7.4 \times 10^{-3}\ m \cdot s^{-1}, \quad (2)$$

and similarly, the minimum and the mean flow rate are 3.3×10^{-4} and $1.2 \times 10^{-3}\ m \cdot s^{-1}$, respectively. Implicit assumptions introduced into this calculation are that (i) all ducts are completely filled with liquid and (ii) the Bellini's duct adopts the form of a regular cylinder.

The assumption (i) can be justified as follows: the capillary elevation, L, i.e., the height to which a liquid rises when one end of an open capillary with diameter d is immersed into the liquid, is given by [1]:

$$L = 4\ \gamma \cos\theta / dg\ \Delta\rho, \quad (3)$$

where θ: is the wetting angle, γ: the interfacial tension on the liquid-vapor interface, g: the acceleration due to gravity, and $\Delta\rho$: the difference between density of the liquid and gas phase. Assuming that urine wets the

Bellini's duct wall similarly as or better than water wets the surface of glass, then $\theta < 10°$, and therefore, $\cos\theta \cong 1$. Substituting the interfacial tension value of water ($\gamma \cong 7 \times 10^{-2}$ kg · s^{-2} at 37°C [22]) for the unknown value of urine and assuming $\Delta\rho \approx \rho_l$, the capillary elevation of urine in a Bellini's duct is:

$$L = (4 \times 7 \times 10^{-2}) / (2 \times 10^{-4} \times 9.81 \times 1015) = 0.14 \text{ m}. \quad (4)$$

Since the calculated L exceeds the duct length by nearly one order of magnitude, i.e., $L >> L_D$, enough space is left to accommodate uncertainties in both θ and γ and still remain in the range $L > L_D$. Therefore, a duct once-filled with liquid for its upward position, would remain so even when its position is reversed. Every collecting duct incidentally adopts an upward position with time and becomes permanently filled with liquid as a result. Thus, the liquid does not form a sort of film on the duct wall but fills the entire duct and flows through its whole cross-section.

Assumption (ii) does not correspond fully to reality. The collecting duct of the human kidney is shown in the literature [e.g., 19] as a truncated circular cone with its diameter increasing towards its opening situated on the papilla. Nephrons are connected to the collecting duct along its length, and therefore, the volume of liquid passing through the duct increases towards its end. As this situation is difficult to quantify, a simplifying assumption (ii) was introduced in order to make further calculations possible.

The character of the flow in the Bellini's duct can now be established. The flow of liquid can either be laminar or turbulent. Laminar, or streamline, flow means that layers of liquid move relative to one another without macroscopic intermixing, i.e., there is no fluid flow across lines parallel to the duct walls. Turbulent flow, on the contrary, results in intensive intermixing due to formation of eddies that move across lines. Whether the flow is laminar or turbulent can be resolved on the basis of the Reynolds number (Re) defined for a tube as [14]:

$$Re = \rho_l \cdot u \cdot d_D/\mu, \quad (5)$$

where μ represents the liquid dynamic viscosity. If the calculated Reynolds number is smaller than the critical value Re = 2100, the liquid flow is laminar. Using ρ_l = 1015 kg · m^{-3} and $\mu = 7 \times 10^{-4}$ kg · m^{-1} · s^{-1}, the Re number for a liquid flow in the Bellini's duct varies within the limits 0.1 and 2.1. Since these values are substantially smaller than the critical value (2100), the flow in the Bellini's duct is laminar (laminar flow of urine in the Bellini's ducts was also assumed in [8]).

The time of liquid passage through the Bellini's duct, t, assuming that the whole volume of liquid passes the entire length of the duct, i.e., all nephrons enter the duct at its lower end, is:

$$t = \text{duct length / flow rate} = L_D/u. \quad (6)$$

The minimum passage time is:

$$t = (2.2 \times 10^{-2}) / (7.4 \times 10^{-3}) = 3 \text{ s}, \quad (7)$$

and similarly, the maximum and the mean passage time are 67 and 19 seconds, respectively. Due to the laminar flow, the passage time is equal to the mean residence time in the Bellini's duct of both the liquid and the possibly-present crystals.

Due to viscous forces acting in a moving liquid, the velocities of individual liquid layers are not equal and increase from the tube wall towards the tube centre. Though a typical velocity profile is parabolic, in further calculations, the linear velocity profile is used for simplification. Zero velocity is ascribed to the liquid layer adjacent to the wall, and the full flow velocity u (estimated previously) is ascribed to the tube axis. The shear rate, G, expresses the gradient of velocity in the tube. For the linear velocity profile, the shear rate becomes:

$$G = \text{flow rate / duct radius} = u / (d_D/2). \quad (8)$$

As a result,

$$G = (7.4 \times 10^{-3}) / (1 \times 10^{-4}) = 74 \text{ s}^{-1} \quad (9)$$

is the maximum shear rate for the linear velocity profile, and, calculated in a similar manner, 3 and 11 s^{-1} are the minimum and mean shear rates, respectively. A similar shear rate varying between 3 and 5 s^{-1} was estimated in [12].

If a particle settles in a quiescent liquid with rate u, its so-called Stokes diameter, d_e, is [5]:

$$d_e = [18 \mu u/(\rho_S - \rho_l) g]^{1/2}. \quad (10)$$

The Stokes diameter gives the diameter of a perfect sphere that would settle at the same rate as the particle in question. A particle of diameter $d > d_e$ remains in an upward-pointing duct (the duct's opening puts upward) through which an ascending current of liquid flows with the rate u, whereas a particle with $d < d_e$ is washed away from the duct. Using ρ_S = 2200 kg m^{-3} as the density of COM [22] and the u values calculated above, the Stokes diameter can be estimated as $d_e = 8.9 \times 10^{-5}$ m and 1.9×10^{-5} m for the fastest and the slowest liquid flow rate in the Bellini's ducts, respectively.

Table 1. Flow characteristics for the rabbit kidneys.

			OMCD	IMCD	DOB
$ux10^3$	ms^{-1}	a	0.6	10.7	60[c]
		b	0.4	1.6	2
$d_D x10^5$	m		3-3.5	3.5-6	6-10[c]
t	s	a	28	1	0.1[c]
		b	42	4	2.5
Re	-	a	≈0.03	0.5-0.9	5.2-8.7
		b	≈0.02	0.028-0.14	0.2-0.3
G	s^{-1}	a	34-40	357-611	1200-2000
		b	23-27	53-91	40-67

OMCD: outer medullary collecting duct;
IMCD: inner medullary collecting duct;
DOB: duct of Bellini;
a: 16 l urine per day; b: 0.6 l urine per day.
[c]data from reference [16].

Used in accordance with Finlayson and Reid [8], the dimensions of the collecting ducts appearing in the preceding calculations are not generally accepted. In a recent paper by Kok and Khan [16], the available data for human kidneys were considered so incomplete that data for rabbit kidneys, which are believed to compare reasonably well to human kidneys, were used instead. As we do not feel qualified to make a decision whether the collecting duct geometry assumed by Kok and Khan [16] corresponds better to reality than the geometry described by Finlayson and Reid [8], calculations will be carried out for both alternatives.

Reynolds number and the shear rate calculated with the values quoted by Kok and Khan [16] employing the expressions given above are listed in Table 1. Calculations were performed for two limiting situations in daily urinary volume: 16 (a) and 0.6 (b) liters per day as considered in [16].

It is evident from Table 1 that the liquid flow is laminar in all parts of the collecting duct of the rabbit kidney for any conceivable liquid throughput.

Agglomeration of Crystals

During crystalluria, 7.2×10^9 particles with a maximum size of 2.5×10^{-6} m can be expected in 1 m^3 of urine [8]. Let identical efficacy of all nephrons in the kidney be assumed. Then, supersaturation of urine is uniform in all collecting ducts at any given time, and formed crystals are evenly distributed through the whole volume of liquid retained in all ducts. Hence, the number of crystals present in one Bellini's duct during the passage time is given by:

$$N = \{7.2 \times 10^9 \times t\} / \{(24 \times 60 \times 60) \times P \times 10^3\}. \quad (11)$$

The factor 10^3 in the denominator converts m^3 to liters. The passage time used in calculation must correspond to the number of ducts in the kidney, that is t = 3 s corresponds to P = 50 ducts and similarly 19 s to 310 ducts and 67 s to 1120 ducts. The ratio of passage time to the number of ducts, t/P, is a constant for a given set of V_D, L_D and A_D values characterizing the kidney. In our case, this ratio equals 0.06. The number of crystals present in one Bellini's duct during the passage time calculated from this expression is approximately 5 and the maximum crystal size was evaluated to be 2.5×10^{-6} m by Finlayson and Reid [8]. Even if all these crystals were to form a single aggregate, its size reaching:

$$(5)^{1/3} \times (2200/1500)^{1/3} \times 2.5 \times 10^{-6} \approx 5 \times 10^{-6} \text{ m} \quad (12)$$

would be far too small to cause an obstruction of the duct. The following formula [16] was used to calculate the aggregate diameter:

$$d(\text{aggregate}) = d(\text{crystal}) \times (N)^{1/3} \times (2200/1500)^{1/3}, \quad (13)$$

where N represents the number of crystals forming an aggregate and 2200 and 1500 are densities of COM and an agglomerate, respectively. In as much as the lowest estimated Stokes diameter, $d_e = 19 \times 10^{-6}$ m, exceeds the size of the formed particle, such an agglomerate is not retained even in an upward-pointing duct. The agglomerate is invariably washed out. All particles smaller than the duct diameter, around 2×10^{-4} m, are automatically washed away from downward-pointing ducts. As shown above, an agglomerate composed of 5 crystals, even if formed, cannot be retained in either an upward- or downward-pointing duct, and it is always washed away.

A recent evaluation by Kok and Khan [16] obtained 24.1×10^9 m^{-3} as the number of crystals present during crystalluria. In such a case, 17 crystals are present in one Bellini's duct during the passage time. An agglomerate consisting of all these particles has a diameter of 7.3×10^{-6} m, considerably smaller than the Stokes diameter. Therefore, a nidus of a papillar calculus cannot be formed in a kidney containing urine with a crystal concentration corresponding to that of clinically observed crystalluria.

Table 2. Variation of (G x t) for the kidneys of rabbit.

Gxt	OMCD	IMCD	DOB
a	952-1120 (1036)	357-611 (484)	120-200 (160)
b	966-1134 (1050)	212-364 (288)	100-167 (133)

Values in brackets represent the arithmetic means; for abbreviation definitions see Table 1.

Aggregation of all crystals present in a given volume of liquid into just one particle due to laminar shear force-affected agglomeration cannot be accomplished within a reasonable time, if at all. Agglomeration proceeds through the gradual formation of dimers, trimers, etc. from monomers, and as a result, the particle size distribution widens and shifts towards the larger sizes. The larger the concentration of individual crystals, the shorter the time required for the formation of a particle of a given size.

Thereafter, a concentration of crystals ensuring the formation of a critical aggregate in the Bellini's duct will be evaluated. Such a task can only be tackled through kinetic considerations.

The kinetics of agglomeration are controlled by the mechanism of particle collisions. Without collisions between particles there would be no agglomeration whatsoever. Particles suspended in urine that flows laminarly through the Bellini's duct acquire different velocities due to laminar shear forces. These particles collide and form aggregates; therefore, in this case, agglomeration kinetics is controlled by the laminar shear rate. Under these conditions, the number concentration of entities (agglomerates) composed of i particles, N(i), formed during time t in a unit volume of suspension initially containing N_o separate particles is [20]:

$$N(i) = N_o \, (t/\tau)^{i-1} \, / \, (1 + t/\tau)^{i+1}. \qquad (14)$$

The half-time of agglomeration, τ, representing the time necessary for halving the number of separate particles in a unit volume of suspension, is defined as:

$$\tau = 1 \, / \, AN_o \qquad (15)$$

The A term acquires the form [21]:

$$A = 2\alpha G r^3 \, / \, 3k_v \qquad (16)$$

where r is the particle radius, α is the collision efficacy coefficient ($\alpha \leq 1$) and k_v represents the volume shape factor. Taking $r = 1.25 \times 10^{-6}$ m, $\alpha = 1$ (i.e., each collision is successful), G = 3 to 74 s^{-1} and $k_v = 4\pi/3$:

$$A = (9.3 \times 10^{-19} \text{ to } 2.3 \times 10^{-17}) \; m^3 \, s^{-1}. \qquad (17)$$

Let it be assumed that a full-sized stone gradually develops from an aggregate of a diameter that exceeds the Stokes diameter. Then, the critical (smallest) aggregate that is retained in the Bellini's duct consists of:

$$N = (19 \times 10^{-6}/2.5 \times 10^{-6})^3 \times (1500/2200) = 300 \qquad (18)$$

crystals. For the fastest flow rate, a sphere with Stokes diameter consists of $\{(8.8 \times 10^{-5})/(2.5 \times 10^{-6})\}^3 \times (1500/2200) \approx 3 \times 10^4$ crystals. However, it will be further assumed that any particle composed of at least 300 crystals is retained in the Bellini's duct.

The crystal concentration necessary for forming the critical aggregate within the passage time can now be evaluated. At least one critical aggregate must appear in the volume of all of the Bellini's ducts in one kidney during the passage time, i.e., $P \times L_D \times A_D = 310 \times 6.9 \times 10^{-10} = 2.1 \times 10^{-7}$ m^3, if the mean P value is considered. That is, the number concentration of N(300) particles must reach $N(300) = 1/(2.1 \times 10^{-7}) = 4.7 \times 10^6$ m^{-3}. Substituting these values into the previously given expression for N(i) with t = 3 s and $A = 2.3 \times 10^{-17}$ yields:

$$4.7 \times 10^6 = \{N_o \, (3 \times 2.3 \times 10^{-17} \times N_o)^{299}\} \, / \, \{(1 + 3 \times 2.3 \times 10^{-17} \times N_o)^{301}\} \qquad (19)$$

from which:

$$N_o \approx 2.2 \times 10^{17} \; m^{-3}. \qquad (20)$$

In these calculations, an aggregation time of 3 s was used since it corresponds to the employed value of A that was calculated for the shear rate 74 s^{-1}.

From preceding calculation, it is evident that a nidus of papillar calculus can develop solely by the agglomeration mechanism taking place in the Bellini's duct only if at least 2.2×10^{17} crystals with size 2.5×10^{-6} m are present in one m^3 of urine. This calculated minimum concentration of crystals necessary for forming a critical aggregate is larger by seven orders of magnitude than the concentration encountered during crystalluria. Furthermore, if these crystals are mono-disperse and form a 0.1×10^{-6} m thick, 1×10^{-6} m wide and 2.5×10^{-6} m long prism, the mass of COM that must precipitate from 1 m^3 of urine is:

$$m = (1 \times 0.1 \times 2.5 \times 10^{-18}) \times 2.2 \times 10^{17} \times 2200 = 121 \text{ kg} \qquad (21)$$

This value exceeds the normal concentration of oxalate in urine by approximately four orders of magnitude.

The agglomeration rate is governed by the product G x t as can be derived from the preceding expressions.

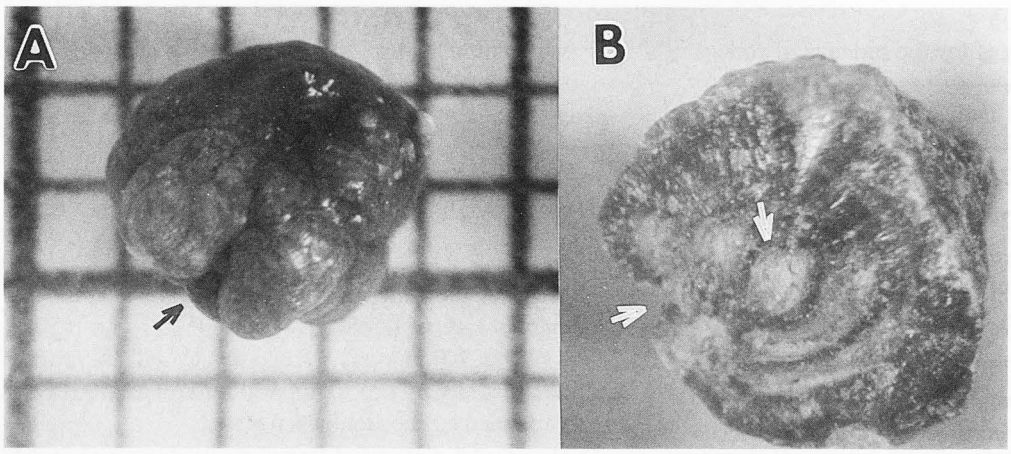

Figure 1. Papillar calculus composed of calcium oxalate monohydrate. (**A**) Overall appearance, the site of attachment to papilla is denoted by arrow; and (**B**) fracture plane passing through the site of attachment to papilla; the stone core and the site of attachment to papilla are denoted by arrows. Calculus size: 3 mm x 3 mm x 3 mm.

This product, however, is determined mainly, and under the simplification used herein exclusively, by the Bellini's duct geometry. Variation in the G x t numerical value calculated for rabbit kidneys using values given in Table 1 is shown in Table 2. From Table 2, it follows that the shear rate decreases along the collecting ducts and acquires the lowest value in the Bellini's duct. However, let the greatest value of G equal to 1050 s^{-1} be used for following calculations.

The total volume of collecting ducts for one kidney varies between 2.296×10^{-6} and 3.152×10^{-6} m^3 if the ducts are approximated as regular cylinders with the dimensions given in Table 1 [16]. Thus, the number concentration of critical aggregates should reach a value between 3.2×10^5 and 4.4×10^5 m^{-3} if a stone is to be initiated. Taking $\alpha = 1$, $k_v = 4\pi/3$ and $r = 1.25 \times 10^{-6}$ m:

$$A = 2 \times 1050 \times (1.25 \times 10^{-6})^3 / 4\pi = 3.3 \times 10^{-16}. \quad (22)$$

using this value, letting t = 48.5 s and assuming that the critical agglomerate consists of 300 crystals,

$$N_o = 5 \times 10^{15}\ m^{-3}. \quad (23)$$

Such a concentration of crystals implies that m = 2.75 kg of COM must have precipitated from 1 m^3 of urine. According to Kok and Khan [16], around 0.05 kg of calcium oxalate monohydrate can precipitate from 1 m^3 of urine. Hence, the calculated m value is about 55 times higher than the amount of COM available in urine.

Let the initial assumption that introducing similar efficacy of all nephrons be dropped, and variation in urine composition among different collecting ducts be recognized. If the crystal concentration required for a critical aggregate formation should be established in the kidneys, then all of the calcium oxalate present in urine in excess of equilibrium solubility must be confined to (310/55) $\approx$ 6 collecting ducts. In other words, if all urinary supersaturation with respect to COM is produced in 6 collecting ducts, a critical aggregate that can be retained in the Bellini's duct can be formed. Variations of urinary composition among different ducts less than that stated above do not result in a critical aggregate formation. However, a situation when just 6 out of 310 collecting ducts are producing all supersaturation can hardly be imagined. Therefore, it can be safely concluded that a concentration of COM particles which would ensure the formation of an aggregate sufficiently large enough to be retained in the Bellini's duct and later serve as a renal stone nucleus cannot occur under physiological conditions.

Discussion

Legitimate objections can be raised against performed calculations on the following grounds:

(**i**) the von Smoluchowski agglomeration kernel [21] is at the limit of its applicability due to the consideration of a large final agglomerate. Thus, the expression for calculating the N_o required for the formation of a critical aggregate during the passage of urine through collecting ducts yields only a rough estimate of the actual value;

(**ii**) the number of crystals forming an aggregate is difficult to establish if their spatial arrangement is not taken into consideration. Therefore, the formula employed herein only approximates the actual number;

(**iii**) the size of the critical aggregate is to some extent questionable since the existence of a Bellini's duct with a considerably distorted opening cannot be excluded; and

(**iv**) quantifying variations of fluid flow, namely in places around a nephron termination into a collecting duct, is beyond the reach of contemporary fluid dynamics.

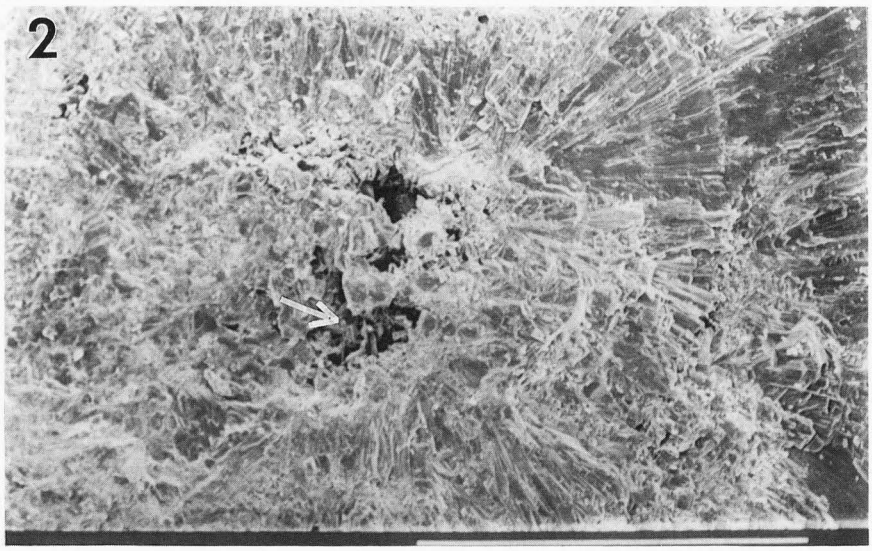

Figure 2. Fine structure of the stone core and adjacent body. The calculus body growing on the core is composed of radially arranged columnar crystals. Arrow indicates the position of area enlarged in Figure 3. Bar = 500 μm.

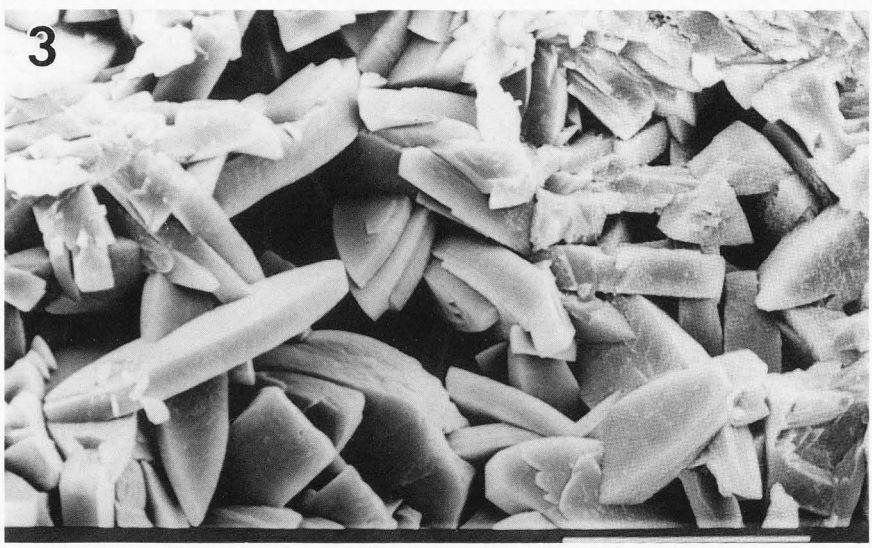

Figure 3. Detailed view of crystal arrangement in stone core from location indicated by the arrow in Figure 2. Bar = 20 μm.

In order to counteract all of the factors which diminish the reliability of our accomplished results, the most favorable conditions for agglomeration, such as the lowest number concentration of critical aggregates, the highest encountered shear rate, the maximum passage time, etc., were considered in our calculations. Even then, reported results can only be regarded as a first approximation of the agglomeration kinetics taking place in the human kidneys.

Based on our results which concern the kinetics of agglomeration, it appears highly unlikely that agglomeration of crystals present in urine during its passage through the collecting ducts can be a decisive factor in the early stages of stone formation, mainly in stone nidus formation. A particle sufficiently large enough to be retained in the Bellini's duct and serve as a nidus for a papillar calculus cannot be formed solely by the agglomeration of crystals present in urine under the conditions (concentration and size of crystals, hydrodynamics, the passage time) prevailing in human kidneys.

The preceding paragraph should not be understood as conveying the idea that agglomeration does not take place in collecting ducts. Quite the contrary, such a process is certainly active in collecting ducts and results in the formation of small aggregates. Hence, urine leaving the Bellini's duct contains both aggregates and individual crystals, but both entities are far too small to be retained near the opening inside the Bellini's duct. An agglomerate retained incidentally in an upper part of a collecting duct, i.e., far from the duct's termination at the papilla, due to the appearance of a sharp bend or a sudden change of diameter, can hardly serve as a nidus for a papillar calculus as transpires from the following reasoning.

Most papillar calculi acquire a shape similar to that shown in Figure 1A. A point of attachment to the papilla can clearly be distinguished in most cases. Calcium phosphate particles and abundant organic matter are frequently encountered in this area and occasionally even calcified tubules can be found [4]. This matter located in the concave zone of the papillary calculus can hardly be considered as a stone nidus responsible for urolith origin. If the opposite were the case, then it would have played a relevant role in the calculus crystalline organization which has seldom been observed. A core developed around a nidus is always situated inside the calculus (Figure 1B) and from the fine structure of the calculus, it is evident that a stone body is grown on this core (Figure 2). The basic geometrical considerations indicate that nidus was originally situated on the papilla surface and not deep in a collecting duct. The latter position would not permit the development of a calculus into its actual shape. Furthermore, the calculus core is invariably composed of intergrown and twinned COM crystals (Figure 3). Hardly any crystal within the core is unconnected by twinning to other crystals. Such a structure can result from crystal growth but not from the physical agglomeration of already grown crystals. The difference between agglomeration through intergrowth and twinning (also termed primary agglomeration) and a physical agglomeration through accretion of individual particles into a new entity (also termed secondary agglomeration) is succinctly explained Grases *et al.* [11].

The results of experimental studies on calcium oxalate crystal agglomeration also indicate a minor role of agglomeration in stone nidus formation. A suspension of calcium oxalate crystals was prepared from solutions imitating urine by batch [18] and continuous (realized in the mixed suspension mixed product removal, MSMPR, reactor) precipitation [12]. After 40 minutes of agglomeration at $G = 5\ s^{-1}$, the size of the largest particle formed was approximately 15×10^{-6} m, and the mode of particle size distribution was about 4×10^{-6} m. This distribution shifted slightly towards larger sizes, its mode reached 10×10^{-6} m, and the maximum particle size was around 25×10^{-6} m after an additional 80 minutes of agglomeration [18]. In [12], the mean residence time of suspension in the MSMPR reactor was 10 minutes. This suspension, already subject to agglomeration in the reactor, contained particles with maximum size of 30×10^{-6} m with the mode of size distribution 15×10^{-6} m. After further agglomeration for 10 minutes under the shear rate $23\ s^{-1}$, the distribution mode shifted to 25×10^{-6} m, and the maximum particle size reached 38×10^{-6} m. Both studies were executed in suspensions containing no urinary organic compounds that promote agglomeration. In the presence of these compounds, the shift of the size distribution would have been more pronounced. However, results of both studies indicate that calcium oxalate crystals precipitated from urine cannot form a particle sufficiently large enough to be retained in the Bellini's duct even for a period substantially exceeding a physiologically reasonable time.

A clinical observation by Brenner and Rector [3] that stones are formed mostly in only one and always the same kidney of a particular stone former further undermines any confidence in a major role of agglomeration during the early stages of stone formation. Both kidneys of a person normally produce urine identical in composition [3]. Thus, there is no reason to expect crystalluria to be confined to just one kidney. Hence, the crystal concentration in both kidneys has to be reasonably similar. Under identical conditions, i.e., identical shear rate and identical initial concentration of particles, prevailing in the kidney, the agglomeration rate must be similar. Therefore, the critical aggregates would be formed in both kidneys. If these aggregates, retained in the Bellini's ducts, develop into papillar stones, then stones would have to be formed in both kidneys simultaneously. As this is rarely the case, some other mechanism than the agglomeration, probably the Finlayson's fixed particle mechanism or its appropriate modification [9], must be responsible for the papillar stone nidus formation. However, the possibility of attaching an aggregate formed in the collecting ducts to the papilla surface is far from being excluded. From this point of view, the mechanisms of the fixed and of the free particle are not mutually exclusive.

Furthermore, the formation of a critical aggregate and its retention by a duct within the passage time implies that such a process is active until urine composition changes due to various external factors. Thus, during a period of conditions favorable for agglomeration, a new critical aggregate representing a stone nidus would appear in intervals equal to the passage time. Hence, approximately every 60 seconds a new duct would contain a stone nucleus and would later become obstructed. Therefore, an average kidney containing around 300 ducts could become totally blocked in a matter of few hours. This has never been observed in a human though a number of papillar stones may appear in the kidney at the same time. This fact also indicates a minor role of crystal agglomeration in stone nidus formation under physiological conditions.

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Discussion with Reviewers

M. Marković: The theoretical model developed in this paper was used to estimate the agglomeration of calcium oxalate crystals in urines. This model could be applied to the agglomeration of different particles independent of their composition; therefore, a comment about general applicability of the model and its possible limitations could be made.

Authors: The expressions used for description of agglomeration in a laminar shear field are based on the following assumptions: (**1**) there is a laminar shear field; (**2**) spherical particles are small enough to be carried by a liquid; (**3**) the number concentration of monomers is not significantly reduced due to progressing agglomeration; and (**4**) the size of final agglomerates is not too large. The more conditions prevailing in the agglomerating suspension deviating from these assumptions, the less reliable results can be expected from theoretical expressions. However, many experiments show that expressions for von Smoluchovski [21] agglomeration yield reasonable estimates of reality even far from the region of their rigorous applicability.

M. Marković: Could you suggest the experimental model system appropriate for the simulation of agglomeration in Bellini's ducts?

Authors: The system described in the paper Söhnel *et al.* (1993) can easily be adapted to simulating agglomeration in a collecting duct. We prefer not to go into detail here since a similar study is being prepared.

R.W. Hartel: How good is the assumption of perfectly cylindrical geometry? Slight variations in geometry may have dramatic effect on these calculations.

Authors: For a geometry differing from regular cylinder, the parameter d_D in the Reynolds number formula must be substituted by the four multiples of the ratio of

the cross-sectional area of the conduit to the wetted perimeter (Knudsen and Katz, 1958). Assuming an elliptical cross-section in accordance with [16], this parameter becomes:

$$d_D = (2)^{3/2} \times a \times b/(a^2 + b^2)^{1/2}, \qquad (24)$$

where a and b are the long- and short-axis of ellipse, respectively.

Taking values quoted in [16], i.e., a = 3 to 5 x 10^{-5} m and b = 3.7 to 12 x 10^{-6} m and u = 0.06 m s^{-1}, the Reynolds number varies between 0.9 and 2.9. This value is again far smaller than the critical value 2100. Therefore, the flow rate in collecting ducts is laminar regardless of any possible modification of their geometry.

R.W. Hartel: Many think that proteinaceous material may enhance agglomeration. How would such a binding force for agglomeration affect these calculations?

Authors: Proteinaceous material increases the efficacy of collisions but not the frequency. Selecting an efficacy coefficient equal to unity implies that every collision is successful and results in the attachment of the colliding particles. Therefore, an influence of the material promoting agglomeration is accommodated by the value of the α coefficient employed in calculations.

M. Daudon: The authors emphasized the reported theoretical results are the most favorable for the crystallization and aggregation in the kidney. On this basis, how do the authors explain that heavy crystal formation in tubules and extended nephrocalcinosis may occur in both kidneys of patients having primary hyperoxaluria, a metabolic disorder in which oxalate concentration rarely exceeds ten times (i.e., one order of magnitude) the oxalate concentration usually found in urine from idiopathic stone formers?

Authors: The notable increase in oxalate excretion due to primary hyperoxaluria will also increase the nucleation rate of calcium oxalate crystals. As a result, the number of crystals in urine will considerably increase compared to normal conditions. An increase in crystal concentration must lead to an enhancement of the aggregation rate, and consequently, final aggregates will be larger than normal. Nevertheless, this does not imply that the final size of these aggregates will reach dimensions comparable to the collecting duct diameter and cause obstruction of Bellini ducts. It must be taken into consideration that primary hyperoxaluria develops early in life, and its clinical manifestations are those of recurrent urinary calculus, hematuria and pyuria. All these processes undoubtedly cause tubular damage that is followed by the rapid development of chronic renal insufficiency. It is not rare that the larger formed aggregates are anchored in tubules where they grow and later cause nephrocalcinosis.

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