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Invited Conference Paper

Precipitation and Interfacial Phenomena in Biological Mineralization. Introductory Remarks*

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A review is presented in which the physico-chemical aspects of biological mineralization are discussed. Normal mineralization of bone and teeth, the formation of dental calculus and caries and urolithiasis are used as examples. These processes involve the deposition or dissolution of calcium phosphates (in all mineralized tissues), calcium oxalates, magnesium phosphates, uric acid and sodium urates (in urinary calculi) within organic macromolecular matrices. The environments from (into) which deposition (dissolution) ensues (blood serum, saliva, urine) are extremely complex, high ionic strength solutions which contain a large variety of ions, small and macromolecules. Problems associated with the mechanisms of the deposition and dissolution of the mineral phases under normal and pathological conditions have attracted the attention of many investigators. A number of physico-chemical problems have been found to be common to all forms of biological mechanisms of the deposition and dissolution of the mineral under pathological conditions. Such problems are identified and methods for their solution discussed in this paper. It is shown how the results of »in vitro« physico-chemical studies relate to and support the findings and theories based on investigations of biological material.

1. INTRODUCTION

Deposition and dissolution of mineral in biological matrices is a wide — spread phenomenon in nature. Human and animal endo — and exoskeletons, teeth, avian shells etc. are formed this way. Under pathological conditions mineralization of soft tissue causes urolithiasis, gout and pseudogout, the deposition of gall stones and other disorders. The formation of tooth caries and calculus also falls in this category.

The mechanisms of the deposition, remodeling and dissolution of biological mineral are closely related to problems concerning the formation, dissolution, stability and transformation of precipitates. Furthermore problems concerning the interaction of biological macromolecules and small molecules at the solid liquid interface are highly relevant. The aim of this Symposium — as

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an integral part of the Conference on the Chemistry of Solid/Liquid Interfaces has been specifically to discuss these aspects of the problem of biological mineralization and thus to show another fruitful and exciting area of research where colloid and interfacial chemistry can be successfully applied.

In the following presentation some of the relevant physico — chemical problems will be pointed out, using several examples of biological mineralization, i. e. the mineralization of bone and teeth, caries and tooth calculus formation and urolithiasis.

1.1. *Composition of Mineralized Tissues and Their Environments*

The main constituents of some normal and pathological mineralized tissues are listed in Table I[1–3]. The table clearly shows the heterogeneity of the mineral components of both normal and pathological mineral deposits, a relatively large proportion being thermodynamically metastable phases. While the major inorganic constituents of bone and teeth are different calcium phosphates, the composition of urinary calculi is even more variable. Calculi may contain one or more of the components listed in Table I. The numbers in the list show the approximate order of frequency of group occurence; the underlined compounds within each group have been most frequently observed. The exact composition of urinary calculi depends on many factors (regional, dietary, occupational, ethiological etc.).

Mineralized tissues are in constant communication with the body fluids (blood serum, saliva, urine), which are extremely complex environments of high ionic strength and contain biological macromolecules, small organic molecules and inorganic ions. Clearly biological systems are dynamic, nonequilibrium systems in which even the concept of solubility is difficult to define.[4]

2. MINERALIZATION OF BONE AND TEETH

Normal calcified tissues of the body can be devided into two essentially different groups: the mesodermally derived tissues (bone, dentin, cementum) and the ectodermally derived tooth enamel. The two groups differ in composition (Table I) ultrastructure and biological and chemical activity.

2.1. *Morphology and Ultrastructure*

In bone, dentin and cementum the mineral phase is deposited as minute needles or platelets (10—60 nm long, 2—6 nm wide) within the collagen fibers so that their long axes line up with the fibers[5]. In contrast tooth enamel is almost fully mineralized (Table I) and the crystals are much larger. An average length, width and thickness of 600 nm, 100 nm and 35 nm respectively have been reported[6]. It is generally agreed that this tissue consists mainly of prisms or rods which contain dense groupings of the apatite crystals. The bonaceous tissues are chemically and biologically much more reactive than tooth enamel which is in accordance with their respective biological purpose i. e. bonaceous tissues serve as a reservoir for the body minerals, while the purpose of tooth enamel is the protection of the inner layers of the tooth.

2.2. *Composition of the Mineral Phase*

Already in the 60-ies Posner and his collaborators[7] demonstrated that mineralized tissue contains a large proportion of amorphous calcium phosphate

TABLE I

The Composition of Several Normal and Pathological Mineralized Tissues in wt. %[1-3]

Tissue	Crystalline components	Organic matrix
Bone, dentin[1,2]	Total inorganic matter 70.9% Carbonate containing calcium phosphates: — amorphous (ACP) — octacalcium phosphate ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$, OCP) — nonstoichiometric hydroxyapatite (HA) Water 8—10%	Collagen 18.6% Mucopolysaccharide--protein complex 0.2—0.4% Resistant protein 1.0%
Tooth enamel[2]	HA 95% Water 4%	Lipid 0.6% Heterogeneous protein 0.3—0.4%
Tooth calculus[2]	Total calcium phosphates: 80% — calcium hydrogenphosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD) — OCP — tricalcium phosphate ($(Ca, Mg)_3 (PO_4)_2$, TCP) — fluorohydroxyapatite	Protein-polysaccharide 11—17%
Urinary calculi[3]	1. Calcium oxalates: — *monohydrate,* whewellite ($CaC_2O_4 \cdot H_2O$, COM) — *dihydrate,* weddelite ($CaC_2O_4 \cdot 2H_2O$, COD) 2. Calcium phosphates: *DCPD,* OCP, TCP, *HA,* carbonate apatite 3. Magnesium phosphates: — *magnesium hydrogenphosphate trihydrate,* newberryte ($MgHPO_4 \cdot 3H_2O$) — *magnesium ammoniumphosphate hexahydrate,* struvite ($MgNH_4PO_4 \cdot 6H_2O$) 4. Uric acid, urates: — *unhydrous uric acid* ($C_5H_4N_4O_3$) — *uric acid dihydrate* ($C_5H_4N_4O_3 \cdot 2H_2O$) — *ammonium acid urate* ($C_5H_3N_4O_3NH_4$) — sodium acid urate monohydrate ($C_5H_3N_4O_3Na \cdot H_2O$) 5. *Cystin,* xanthin and others	Mucoprotein 2.5%

(ACP). At about the same time Brown[8] showed that octacalcium phosphate (OCP) must be a constituent of bone and tooth mineral. A convincing demonstration of the heterogeneity of bone mineral and the changes of its composition with age was given by Quinaux and Richelle[9]. The authors separated small bone particles (< 5 mμ) into fractions of different specific gravity. Low specific gravity fractions were found to contain prevailantly ACP and OCP,

while with increasing specific gravity more and more hydroxyapatite (HA) was found to be present. It was shown that in young rat bone low specific gravity fractions constitute a much larger proportion than in the bone from adult animals. From these findings the following mineralization pattern was deduced[10]: the mineral phase of bone is first deposited as ACP which gives rise to an initial crystalline precipitate (OCP) and this gradually transforms into HA.

A number of »in vitro« kinetic experiments[7,11–16] have indeed shown that ACP appears as a metastable precursor in the neutral and basic pH region. The formation[14], transformation[11–16] and »structure« (on a molecular level)[17,18] of this phase has drawn considerable attention. It has been suggested that ACP contains a basic structural unit consisting of a spherical cluster with the composition $Ca_9(PO_4)_6$[17] or, alternatively, that it contains OCP-like structures[18].

A kinetic experiment showing changes in the pH and solution calcium and phosphate concentrations as a function of time is given in Figure 1 (after ref. 13). Typically in such experiments precipitation proceeded in two distinct steps. The first drop in pH and solution concentrations corresponded to the

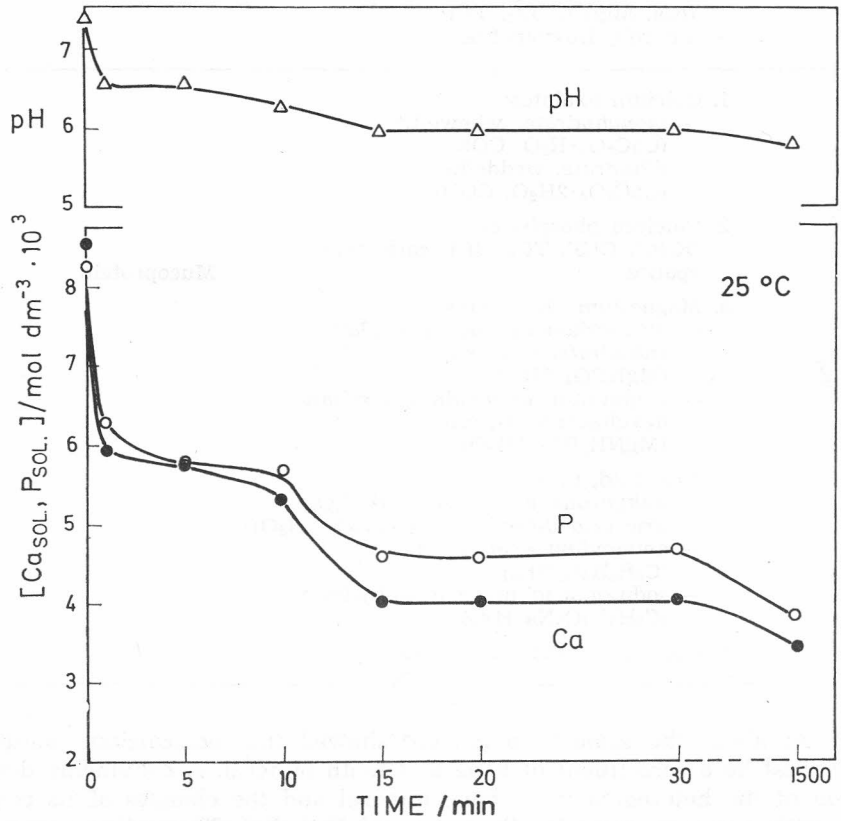

Figure 1. Changes of the pH and the total calcium and phosphate concentrations as a function of time when calcium phosphate is precipitated from equimolar solutions of calcium chloride and sodium phosphate. Initial reactant concentrations: $[Ca] = [PO_4] = 8 \times 10^{-3}$ mol dm^{-3}, $pH_i = 7.4$. (After ref. 13).

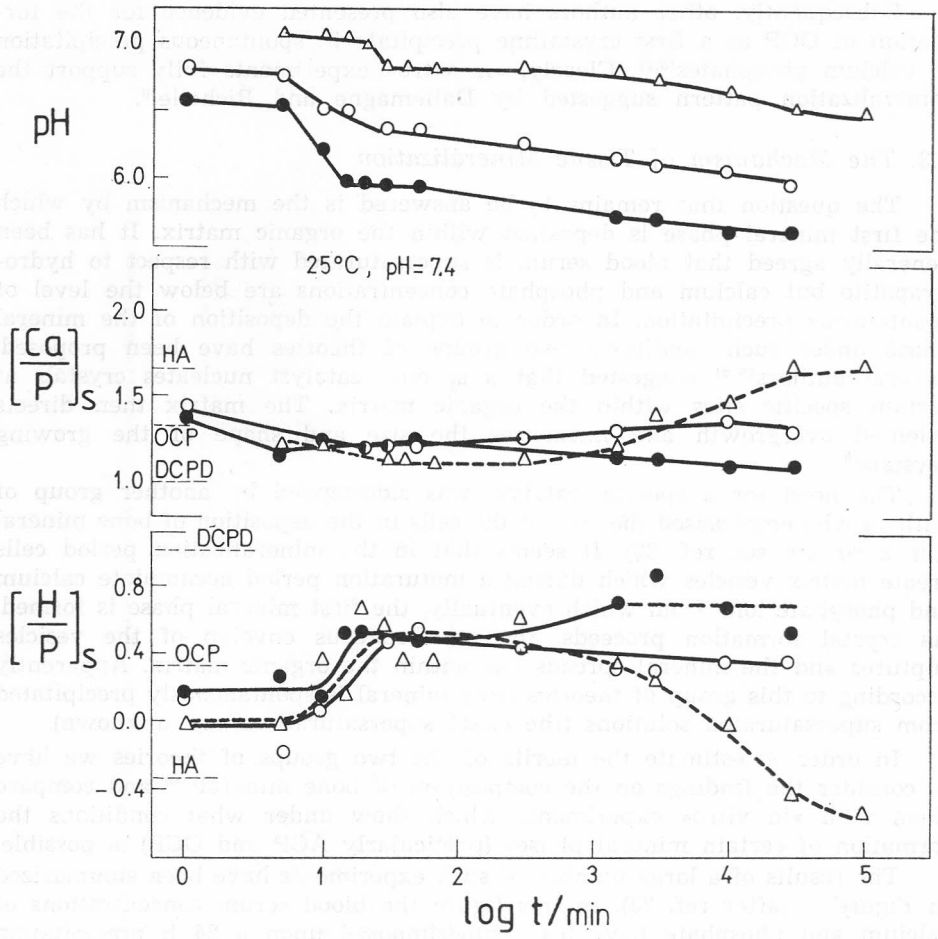

Figure 2. Changes of the molar H/P and Ca/P ratios of calcium phosphate precipitates (calculated from solution analysis) and the pH of the supernatant solutions as a function of time. Initial reactant concentrations / mol dm^{-3}: [P] = 8×10^{-3}; $pH_i = 7.4$; [Ca] = 8×10^{-3} ●, 5×10^{-3} ○ and 3×10^{-3} △. (After ref. 13).

formation of ACP. The precursor usually appeared in the form of chain-like aggregates of spherulitic particles[12,14] which were most probably formed by aggregation of much smaller — primary — particles, initiated by homogeneous nucleation[14]. A second sharp change in the pH and solution concentrations (Figure 1) corresponded to secondary precipitation of a crystalline precipitate which has been identified as OCP by solution and X-ray analysis[12,13]. Further transformation of this precipitate was a solution mediated process which proceeded either towards DCPD or HA, depending on the pH established during secondary precipitation[13]. The process of the formation and transformation of calcium phosphate phases can be illustrated by following changes of the molar Ca/P and H/P ratio of the precipitate (calculated from solution analysis) as a function of time as shown in Figure 2 (after ref. 13).

Subsequently, other authors have also presented evidence for the formation of OCP as a first crystalline precipitate in spontaneous precipitation of calcium phosphates[15,16]. Clearly, »in vitro« experiments fully support the mineralization pattern suggested by Dallemagne and Richelle[10].

2.3. *The Mechanism of Tissue Mineralization*

The question that remains to be answered is the mechanism by which the first mineral phase is deposited within the organic matrix. It has been generally agreed that blood serum is supersaturated with respect to hydroxyapatite but calcium and phosphate concentrations are below the level of spontaneous precipitation. In order to explain the deposition of the mineral phase under such conditions two groups of theories have been proposed. Several authors[19–21] suggested that a specific catalyst nucleates crystals at certain specific sites within the organic matrix. The matrix then directs oriented overgrowth and influences the size and shape of the growing crystals[19].

The need for a specific catalyst was sidestepped by another group of authors who emphasised the role of the cells in the deposition of bone mineral (for a review see ref. 22). It seems that in the mineralization period cells create matrix vesicles which during a maturation period accumulate calcium and phosphate ions from which eventually, the first mineral phase is formed. As crystal formation proceeds, the membraneous envelop of the vesicles ruptures and the mineral spreads out within the organic matrix. Apparently according to this group of theories bone mineral is spontaneously precipitated from supersaturated solutions (the exact supersaturations are unknown).

In order to estimate the merits of the two groups of theories we have to consider the findings on the composition of bone mineral[7–10] and compare them with »in vitro« experiments which show under what conditions the formation of certain mineral phases (particularly ACP and OCP) is possible.

The results of a large number of such experiments have been summarized in Figure 3. (after ref. 23). In this figure the blood serum concentrations of calcium and phosphate have been superimposed upon a 24 h precipitation diagram (pH 7.4, 0.15 mol dm^{-3} NaCl) which shows the composition of the solid phases formed in dependence of the initial reactant concentrations. As expected the physiological concentrations are somewhat lower than the limit of spontaneous precipitation (precipitation boundary) but one can assume that the solid phases formed upon specific catalysts would be similar to those which grew upon nonspecific impurities at very low supersaturations. It has been shown[23,24] that under those conditions (area I in Figure 3) large spherulitic aggregates of OCP (OCP_s) form by direct crystal growth. ACP appeared as a precursor at somewhat higher supersaturations (area II in Figure 3) and transformed into microcrystalline aggregates as previously described (Figures 1, 2 and refs. 12—14).

Considering all evidence it seems that cellular activity might be responsible for tissue mineralization. The corresponding theories visualize the formation of the mineral phase from an environment of higher supersaturations and can thus account for the presence of both ACP and OCP (the latter formed by transformation of ACP) in mineralized tissue. The organic matrix probably plays an architectonical role in controlling the growth pattern and

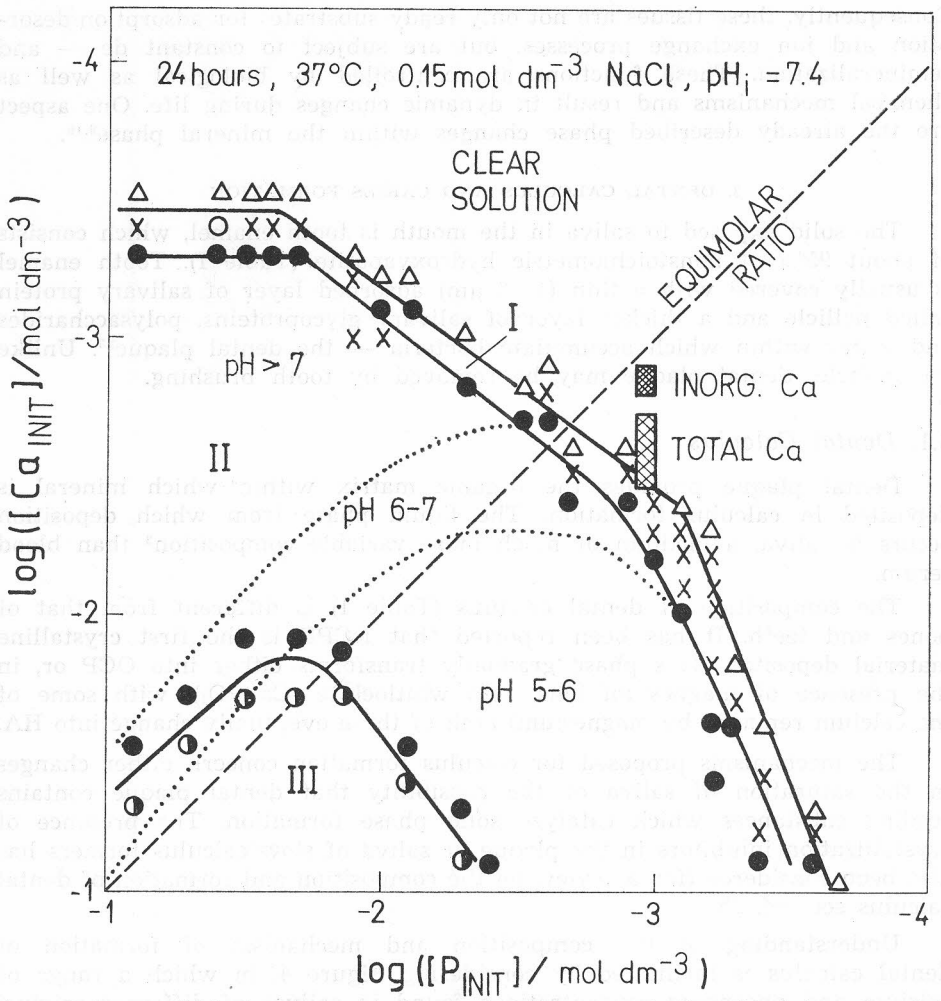

Figure 3. Range of blood serum calcium and phosphate concentrations (shaded quadrangles) superimposed upon a precipitation diagram of the system: calcium chloride — sodium phosphate — 0.15 mol dm^{-3} sodium chloride, Full lines: precipitation boundary and boundaries of concentration regions I, II and III within which different solid phases prevailed. Area I: spherulitic crystalline aggregates of OCP (x) were formed by direct crystallization upon heterogeneous nuclei, area II: microcrystalline aggregates of calcium deficient apatite (DA, ●) were formed via ACP, area III: mixtures of DCPD and microcrystalline aggregates of DA (●) were obtained. Iso-pH curves are represented by fine dots (After ref. 23).

morphology of the mineral phase. While the collagen matrix is quite stable and restricts the growth of mineral crystals in mesodermally derived tissue, the organic matrix of tooth enamel — amelogenin — is gradually destroyed during tissue maturation hence the crystals grow much larger and fill out the interstices[2].

2.4. *Remodeling of Mineralized Tissue*

Once formed, mineralized tissues have many functions: they serve as support and structure to the body, but also for storage of the body mineral.

Consequently, these tissues are not only ready substrates for adsorption/desorption and ion exchange processes, but are subject to constant de — and remineralization. These functions are controlled by biological as well as chemical mechanisms and result in dynamic changes during life. One aspect are the already described phase changes within the mineral phase[9,10].

3. DENTAL CALCULUS AND CARIES FORMATION

The solid exposed to saliva in the mouth is tooth enamel, which consists of about 95% of nonstoichiometric hydroxyapatite (Table I). Tooth enamel is usually covered with a thin (1—3 μm) adsorbed layer of salivary protein called pellicle and a thicker layer of salivary glycoproteins, polysaccharides and water within which accumulate bacteria — the dental plaque[25]. Unlike the pellicle, dental plaque may be removed by tooth brushing.

3.1. *Dental Calculus*

Dental plaque provides the organic matrix within which mineral is deposited in calculus formation. The liquid phase from which deposition occurs is saliva, a medium of much more variable composition[2] than blood serum.

The composition of dental calculus (Table I) is different from that of bones and teeth. It has been reported that DCPD is the first crystalline material deposited. This phase gradually transforms either into OCP or, in the presence of magnesium ions, into whitlockite ($Ca_3(PO_4)_2$ with some of the calcium replaced by magnesium) both of these eventually change into HA.

The mechanisms proposed for calculus formation concern either changes in the saturation of saliva or the possibility that dental plaque contains seeding substances which catalyze solid phase formation. The presence of crystallization inhibitors in the plaque or saliva of slow calculus formers has also been considered (for a review on the composition and formation of dental calculus see ref. 25).

Understanding of the composition and mechanism of formation of dental calculus is facilitated by considering Figure 4. in which a range of calcium and phosphate concentrations found in salivas of different origins[2] is superimposed upon the relevant portions of precipitation diagrams[23] of the calcium phosphate precipitation system.

Clearly at pH 6.5 (the mean pH value of resting, submandibular saliva[2]) a whole set of conditions exists under which calcium and phosphate concentrations are above the limit of spontaneous precipitation. It has been shown earlier[23] that in this concentration region crystal growth is initiated by heterogeneous nucleation upon nonspecific impurties and that intercrystalline mixtures of DCPD and large spherulitic aggregates of OCP (OCP_s) are formed. At pH 6 (the pH of parotid saliva is between 5.2 and 6.2[2]) the limit of spontaneous precipitation is reached only under extreme conditions but crystallization (primarily of DCPD) could be easily initiated by a seeding mechanism. The distribution of solid phases (DCPD + OCP at pH 6.5 and prevalently DCPD at pH 6.0) is consistent with the respective phase diagrams[26] and reflects the distribution of solid phases in early calculus deposits[25].

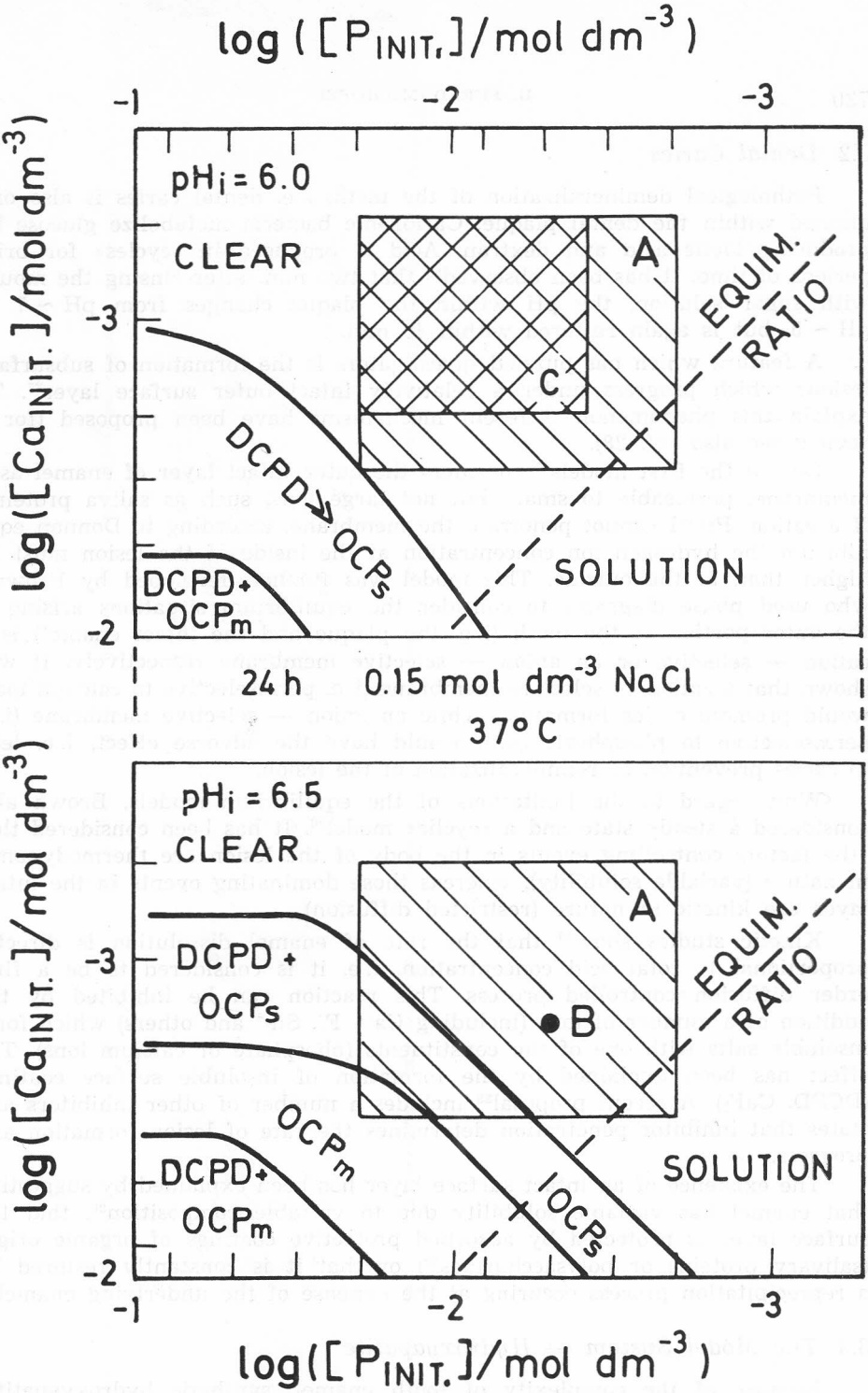

Figure 4. Range of calcium and phosphate concentrations found in resting salivas of different origins (mixed, parotid, submandibular[2]) superimposed upon precipitation diagrams of the system calcium chloride — sodium phosphate — 0.15 mol dm^{-3} sodium chloride, initial pH 6.5 (lower diagram) and 6.0 (upper diagram). Large shaded areas (A) show the whole range of reported salivary concentrations. Point B in lower diagram: mean calcium and phosphate concentrations in submandibular saliva (corresponding mean pH = 6.6). Area C in upper diagram: range of calcium and phosphate concentrations in parotid saliva (corresponding mean pH = 5.8). Solid phases: DCPD, large spherulitic aggregates of OCP (OCP_s) and microcrystalline aggregates of OCP (OCP_m). (Precipitation diagrams after ref. 23).

3.2. *Dental Caries*

Pathological demineralization of the teeth, i. e. dental caries is also originated within the dental plaque. Cariogenic bacteria metabolize glucose by producing lactic acid and dextran. Acid is produced in »cycles« for brief periods of time. It has been observed[25] that two min. after rinsing the mouth with sugar solution, the pH within the plaque changes from pH ~ 7 to pH ~ 5, but is again restored within 40 min.

A feature which has puzzled investigators is the formation of subsurface lesions which progress under a relatively intact outer surface layer[27]. To explain this phenomenon different mechanisms have been proposed (for a review see also ref. 28).

One of the first models[29] considers the outer intact layer of enamel as a membrane, permeable to small, but not large ions, such as saliva proteins. If a cation, PrOH cannot penetrate the membrane, according to Donnan equilibrium the hydrogen ion concentration at the inside of the lesion must be higher than at the outside. This model was further developed by Brown[26] who used phase diagrams to consider the equilibrium situations arising if the outer portion of the tooth (i. e. the plaque and the intact enamel) is a cation — selective or an anion — selective membrane respectively. It was shown that a cation — selective membrane (i. e. permselective to calcium ions) would promote caries formation, while an anion — selective membrane (i. e. permselective to phosphoric acid) would have the adverse effect, i. e. lead to caries prevention or remineralization of the lesion.

With regard to the limitations of the equilibrium models, Brown also considered a steady state and a »cyclic« model[26]. It has been considered that »the factors controlling events in the body of the lesion are thermodynamic in nature (variable solubility), whereas those dominating events in the intact layer are kinetic in nature (restricted diffusion).

Kinetic studies show[30] that the rate of enamel dissolution is directly proportional to total acid concentration, i. e. it is considered to be a first order diffusion controlled process. This reaction can be inhibited by the addition of a number of ions (including Ca^{++}, F^-, Sn^{++} and others) which form insoluble salts with one of the constituents (phosphate or calcium ions). The effect has been explained by the formation of insoluble surface coatings (DCPD, CaF_2). A recent proposal[28] includes a number of other inhibitors and states that inhibitor penetration determines the rate of lesion formation and progress.

The existence of an intact surface layer has been explained by suggesting that enamel has variable solubility due to variable composition[26], that the surface layer is protected by adsorbed protective coatings of organic origin (salivary proteins or polysaccharides[30]) or that it is constantly restored by a reprecipitation process occuring at the expense of the underlying enamel[31].

3.3. *The Model System — Hydroxyapatite*

Because of the complexity of tooth enamel, synthetic hydroxyapatites have been frequently used as models relevant to enamel de — and remineralization[31–33]. The preparation of well defined model supstrates and their characterization is thus of overriding importance.

It seems well established that the mineral phase of tooth enamel was formed by some kind of precipitation process, probably via ACP as a precursor (see also first part of this review). Therefore, preparations of HA obtained by precipitation from aqueous solutions may best reflect the properties of this substance. Temperature programmed dehydration studies of such preparations[34] have shown two major peaks which are due to the evolution of water. The first, at 90 °—100 °C was attributed to desorption of reversibly adsorbed water at the external surfaces of the crystal, the second at 225 °——260 °C to the irreversible removal of tightly bound water (the activation energy associated with the desorption was 26—28 kcal mol^{-1}) from ultrafine pores within the solid. It is interesting that the existence of tightly bound water has also been reported for tooth mineral[35,36] but no evidence for it was found in hydrothermal preparations or in apatite of igneous mineral source[34].

HA prepared by precipitation usually consists of very small crystals with a relatively large specific surface area (60—70 m^2 g^{-1}, ref. 37). In contact with an electrolyte solution the crystals are highly polar and amphoteric[38]. Their isoelectric point lies at pH 7 (obtained by microelectrophoresis,[37] Figure 5), while the point of zero charge (pzc), as determined by potentiometric titration[39] lies at pH = 8.5.

HA is a good adsorbent for a variety of ions, small molecules and macromolecules and has been used in polypeptide and protein separation by chromatography[40]. Using human serum albumines as a model it was shown[37] that the adsorption of proteins on HA depends on the pH and the ionic strength, which is a direct consequence of electrostatic interactions at the protein/HA interface. In these experiments (Figure 5) the pzc was found at pH = 8.8, similar as by potentiometric titrations[39] which shows that the electrostatic interactions at the interface depend on the surface charge rather than the electrokinetic charge.

4. THE FORMATION AND PREVENTION OF URINARY CALCULI

4.1. *Composition and Mechanism of Formation*

Urinary stones are of widely variing composition, consisting either of one or several of the slightly soluble salts which are listed in Table I. As in other mineralized tissues they also contain a macromolecular organic matrix.

The environment from which the solids are formed — urine — is the wastewater of the body and as such contains a large number of ions, small and macromolecules of widely variing concentrations. The pH of urine varies between 5 and 7.8, an average ionic strength may be estimated at 0.3 mol dm^{-3}.

Although it is certain that urinary stones are of different etiologies, there seems to be consensus that crystals are formed from urines supersaturated to the precipitating salt(s). Crystalluria — the voiding of crystals in urine — is not an uncommon phenomenon even in healthy subjects. The problem that needs to be answered is not so much how crystals are formed, but how they are retained within the kidney. In this context a significant observation was made by Robertson and collaborators[41] who showed that patients with recurrent calcium oxalate lithiasis frequently pass large crystals and crystalline aggregates of calcium oxalate dihydrate (COD) which are not formed in normal urines. More recently it has been shown[42] that the composition, morphology and state of aggregation of voided calcium oxalate crystals depends on

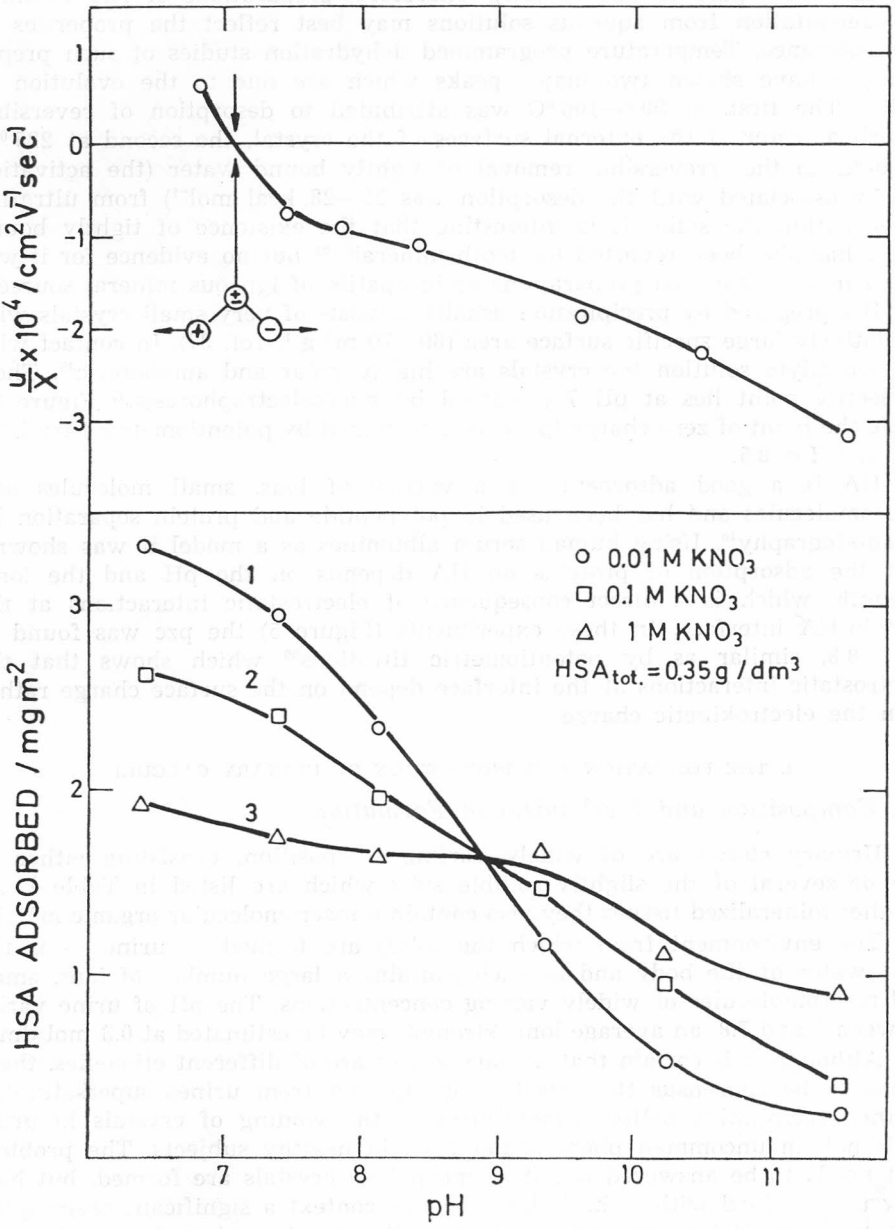

Figure 5. Adsorption of human serum albumin (HSA) on precipitated stoichiometric hydroxyapatite. The electrophoretic mobilities, u/X, of HA particles (upper diagram) and the adsorption of HSA at different KNO_3 concentrations (lower diagram) as a function of pH. (After ref. 37).

the relative supersaturation of the urine. This of course is consistent with the respective precipitation diagram[43].

The fact that urinary calculi often occur as mixtures of two or more mineral phases[44,45] has prompted the interest in mutual interactions of different urinary salts. A number of seeded crystal growth experiments have been performed with the purpose to explore the role of epitaxial growth as a stone forming mechanism[46–48]. It has been shown[46,47] that seeds of sodium urate monohydrate can induce heterogeneous nucleation of calcium phosphate and calcium oxalate and vice versa. Also both hydroxyapatite and brushite can induce heterogeneous nucleation of calcium oxalate and vice versa[46,48].

It would then seem a reasonable hypothesis[49] that the decisive step in urinary stone formation is the retention of large particles or crystal aggregates within some part of the kidney where they serve as seeds for epitaxial growth of either one kind or different urinary salts. Urinary macromolecules may be included either by nonspecific physical adsorption[50] or the matrix may play an active architectonical role as a binding or cementing material[51].

4.2. *Stone Prevention. The Role of Inhibitors*

It has been generally assumed that urines of recurrent stone formers are deficient in some inhibitors which regulate crystal growth and aggregation in healthy subjects. Therefore the effect of a large number of potential inhibitors on the precipitation of calcium oxalates and calcium phosphates has been studied[52–63]. Much attention has been given the inhibitory activity of pyrophosphate[53] which can be formed from the orthophosphate ions present in any biological fluid. The synthetic analogues, multidentate organic phosphonates have also been found effective[54]. Other small molecular inhibitors include citrate, aminoacids, peptides etc.[52]. A number of divalent metal ions have been studied, some were found to inhibit others to accelerate precipitation[55]. Apart from inhibition of crystallization, magnesium ions have been shown to stabilize metastable precursors, weddelite[56], amorphous calcium phosphate[57,58] and whitlockite[59].

A number of investigators concentrated on the isolation of macromolecules from urines and testing of their activity as inhibitors or promoters of crystallization. Glycosaminoglycans (GAGS), glycoproteins, RNA — like material, Tamm — Horsefall protein and others have been studied[52–60]. It has been suggested that the activity of small molecular[61] and macromolecular[62] inhibitors to reduce the rate of crystal growth is due to their adsorption at the active growth sites of the crystal faces. It has also been shown[63] that urinary macromolecules may act as protective colloids or sensitizers which, depending on their concentration, can keep crystalloids in suspension or promote aggregation.

4.3. *Physico-chemical Models Related to Urolithiasis*

The system urine/urinary salts is a complex precipitation system involving the formation of precipitates from a solution supersaturated to more than one crystalline phase. In such systems the properties of precipitates formed depend both on thermodynamic and kinetic factors. The thermodynamic factors, i. e. the degree of saturation of the urine with respect to the urinary salts may

be assessed from the respective phase diagrams. Once the solubility product is exceeded the system may still exist in a state of metastable supersaturation, which indeed is the case for many urines[41]. Precipitation from such solutions can be initiated by the use of specific seed crystals. At supersaturations exceeding the limit of spontaneous precipitation the properties of the precipitates depend on the relative rates of the precipitation processes involved, i. e. nucleation, crystal growth, aggregation and phase transformation. These again are largely determined by the experimental conditions, i. e. the solution composition (reactant concentrations, pH, ionic strength, foreign ions and molecules) the temperature and the reaction time (for reviews see refs. 64, 65). The influence of the solution composition which undergoes large variations in the urinary tract[66] may best be assessed by stationary state experiments. In such experiments the reactant concentrations are changed in discreet steps, which is achieved by mixing together (in a reproducible manner) stable solutions containing different known concentrations of the reactants. The reaction time is kept constant and long enough to allow a stationary state to be established. At the end of this aging time characteristic features of the precipitates are examined and the data are plotted as a function of the initial reactant concentrations as shown in Figures 3 and 4. Such precipitation diagrams[67–69] represent a »map« of the system in a steady-state situation.

Essential parts are the precipitation boundary (showing the limit of spontaneous precipitation under given experimental conditions) and the boundaries between concentration regions within which different kinds of precipitates (with regard to morphology, composition and structure) prevail. Among these the most important is the boundary between the concentration regions within which precipitates are formed prevalently by heterogeneous and homogeneous nucleation respectively. This boundary yields the critical supersaturation for homogeneous nucleation[69] from which the interfacial energy and critical radius of the homogeneous nucleus may be determined.

Precipitation diagrams are especially useful for the understanding of the formation of precipitates from urines supersaturated to more than one of the urinary salts[70]. In such systems mutual interactions of the precipitating components may lead to the formation of inter- and intracrystalline mixtures, stabilization of precursor phases, crystal growth inhibition and other phenomena, which may not be anticipated from thermodynamic considerations (phase diagrams) alone.

The efficiency of potential inhibitors of precipitation is usually assessed by some kind of kinetic experiment. There is, however, some confusion in the literature about the type of experiment to be used and the significance of the results. Some investigators[71] have used turbidity measurements to test the effect of inhibitors on the onset of spontaneous precipitation, others[72] have measured calcium depletion from solution to assay the effect on precipitate formation. Neither of these methods can separate between the individual precipitation processes. This difficulty has been overcome by the use of well defined seed crystals which enabled studies of crystal growth without the influence of nucleation[58,61]. More recently the method was improved by keeping constant concentrations of all ions during the kinetic experiment. Using this »constant composition« technique the action of inhibitors on the rate of crystal growth and phase transformation could be effectively studied[73]. However,

the supersaturations which can be employed in such studies are well below those which are found in urines of recurrent stone formers.

Another dynamic system, the MSMPR (mixed suspension mixed product removal) crystallizer, initially developed for industrial purposes[74] has been recently used to study growth rates of calcium oxalates[75,76] and the action of inhibitors thereon[75]. In these experiments supersaturations are maintained high by continuously supplying calcium and oxalate solutions to the crystallization vessel and therefore crystals are continuously formed by spontaneous precipitation. It has been argued that the MSMPR crystallizer reproduces the situation in the kidney more closely than other techniques.

None of the sofar mentioned techniques gives any information on the process of aggregation. There is however growing awareness that it is this process which is responsible for the formation of particles large enough to be retained in some part of the kidney. This awareness lead Robertson and collaborators[54] to propose the use of the Coulter counter as a tool to follow growth and aggregation of calcium oxalate seed crystals. The method was further developed by Ryall and collaborators[77,78] who defined indices of inhibition of growth and aggregation by comparing the change in total particle volume and in total particle number in the presence and absence of inhibitors.

An alternative, kinetic approach which may be applied to spontaneously precipitating systems is being developed in our laboratory[79,80]. It has been shown[65,80] that the rate of spontaneous precipitation may be described by the general equation:

$$\mathrm{d}\,\alpha/\mathrm{d}t = K\,N^{1/3}\,\alpha^{2/3}\,(c_t - c_s)^p \qquad (1)$$

where α is the fraction of solute precipitated, N is the number of particles, c_t and c_s are solute concentrations at time t and at equilibrium and K and p are constants. A condition for the validity of equation (1) is that the precipitate has a selfpreserving distribution of sizes and shapes of particles, so that an average surface area may be defined. For $N \sim$ const. equation (1) reduces to

$$\mathrm{d}\,\alpha/\mathrm{d}t\;\alpha^{-2/3} = K'\,(c_t - c_s)^p \qquad (2)$$

which is the equation generally used to describe crystal growth controlled by a surface process.

According to eqns. (1) and (2) a log/log plot of the rate v. s. supersaturation curve must give a straight line if the number of particles is constant (i. e. for crystal growth) while deviation from linearity occurs when the number of particles changes, i. e. during nucleation and/or aggregation.

Figure 6 (after refs. 79, 80) shows a typical progress curve and corresponding log rate v. s. log supersaturation curve obtained by following the depletion of solution calcium when calcium oxalate trihydrate (COT) was precipitated from 0.3 mol dm^{-3} sodium chloride solutions. The initial surge of the rate up to a maximum (section A in Figure 6) was attributed to simultaneous nucleation and particle growth. The subsequent linear part of the log rate v. s. log supersaturation curve (section B) indicates that during the corresponding period of time crystal growth prevails and the results can be interpreted in terms of any of the existing crystal growth theories. Subsequent deviation from linearity (section C) shows that in addition to crystal growth another process (dis-

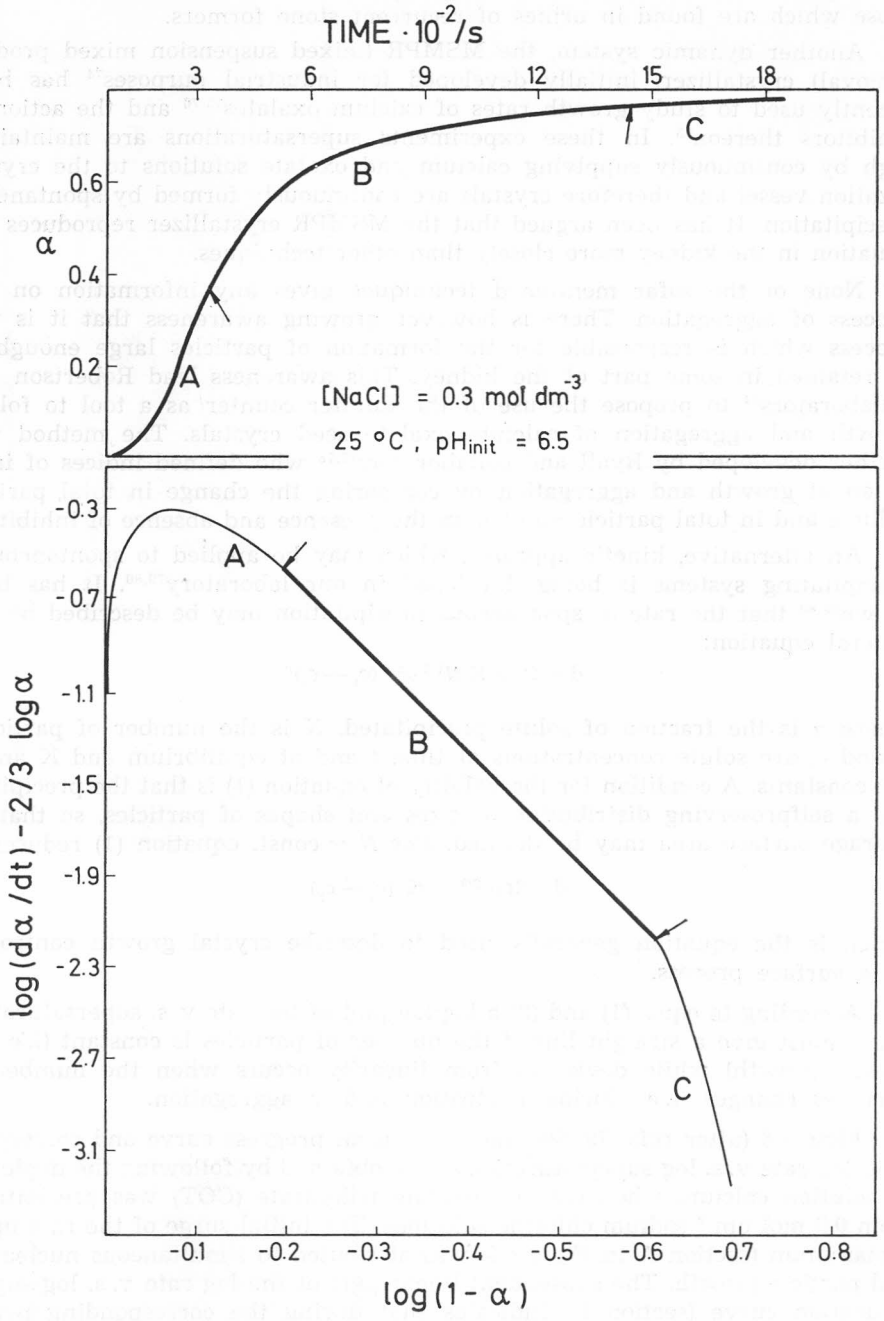

Figure 6. Typical progress curve (upper diagram) obtained by calcium selectrode and the corresponding log rate v. s. log supersaturation curve (lower diagram) showing the rate of precipitation of calcium oxalate from 0.3 mol dm^{-3} sodium chloride solutions. Section A, B and C are corresponding in time. (After refs. 79, 80).

solution/recrystallization or aggregation) has become significant, causing the precipitation rate to decrease faster than expected from eqn.(2).

Kinetic particle size analysis[80] of section C gave results which were consistent with the orthokinetic theory of aggregation of polydispersed systems[81].

The above described model system is promising because it enables the investigator to follow the kinetics of crystal growth and aggregation independently in spontaneously precipitating systems in which both processes proceed subsequently or simultaneously. The action of inhibitors on the described model system is being tested.

5. CONCLUSIONS

Biological mineralization and demineralization involves dynamic, non-equilibrium systems in which heterogeneous mineral phases are deposited or dissolved within an organic matrix. In the examples used (mineralization of bone and teeth, dental calculus formation, the formation of urinary calculi) the environments in contact with the solid phases (blood serum, saliva, urine) are complex, high ionic strength solutions which contain a large number of ions, small and macromolecules.

Although from the point of view of a biologist there are a variety of biological mineralizations, it is still possible to define a number of common physico chemical problems which ought to be considered:

— determination of the solubility and relative supersaturation of biological mineral in the surrounding body fluids.
— surface properties of the minerals and interactions at the solid/solution interfaces
— adsorption of biological macromolecules
— rates and mechanisms of nucleation, crystal growth and phase transformation and the effect of inhibitors and promoters thereon.

Another group of problems is specific for pathological conditions where the solids are formed or dissolved into fluids of widely variing composition (saliva, urine). These are:

— the dependence of the properties of precipitated solids (composition, structure, morphology) on the composition of the respective environment (dental and urinary calculi)
— the formation of mixed solid phases from solutions supersaturated to more than one salt (urinary calculi)
— rates and mechanisms of dissolution in the presence and absence of inhibitors (dental caries and urinary calculi)
— colloid stability and instability and rates of aggregation in the presence and absence of inhibitors (urinary calculi)

The above listed problems have been studied by a variety of methods which are based on thermodynamic and kinetic considerations. Some of these methods have been discussed.

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SAŽETAK

Taloženje i međufazne pojave u biološkoj mineralizaciji. Uvodne napomene

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U ovom pregledu diskutirali smo o fizičko-kemijskim aspektima biološke mineralizacije. Kao primjere obradili smo normalnu mineralizaciju kostiju i zubi, stvaranje zubnog kamenca i karijesa te urolitijazu. Ti procesi uključuju taloženje, odn. otapanje kalcij-fosfata (u svima mineraliziranim tkivima), kalcij-oksalata, magnezij-fosfata, mokraćne kiseline i natrij-urata (u mokraćnim kamencima) unutar organskih makromolekulskih matrica. Tjelesne tekućine iz kojih se čvrsta faza taloži (odn. u koje se otapa), tj. krvni serum, pljuvačka i urin vrlo su kompleksne otopine visoke ionske jakosti, koje sadržavaju mnoštvo različitih iona, malih molekula i makromolekula. Problemi mehanizma depozicije, odn. otapanja biološkog materijala privukli su pažnju mnogih istraživača. Mnogi fizičko-kemijski problemi zajednički su za sve vrste biološke mineralizacije, dok su drugi specifični za mineralizaciju u patološkim uvjetima. U ovom radu ukazali smo na bitne fizičko-kemijske probleme i diskutirali o metodama za njihovo rješavanje. Nadalje smo usporedili rezultate fizičko-kemijskih istraživanja s nalazima i teorijama koji su zasnovani na ispitivanjima biološkog materijala i pokazali kako je moguće tim postupkom potvrditi odn. eliminirati pojedine teorije.