Urine pH, urine saturation and feline uroliths:
What we know (and don't)

Joe Bartges
DVM, PhD, DACVIM (Small Animal), DACVN
Professor of Medicine and Nutrition
Acree Endowed Chair of Small Animal Research, University of Tennessee, Knoxville, TN, USA

Formation of uroliths is not a disease, but rather a complication of several disorders.[1] Some disorders can be identified and corrected (such as infection-induced struvite urolith formation), some can be identified but not corrected (such as hyperuricosuria that occurs in Dalmatians that form ammonium urate uroliths), while for others the underlying etiopathogenesis is not known (such as calcium oxalate urolith formation in many cats). A common denominator of these disorders is that they can from time to time create oversaturation of urine with one or more crystal precursors resulting in formation of crystals. In order to develop rational and effective approaches to treatment, abnormalities that promote urolith formation must be identified with the goal of eliminating or modifying them. It is important, therefore, to understand several basic concepts associated with urolithiasis.

What we know and what we don't

Medical dissolution of certain types of uroliths is achieved by inducing a state of undersaturation with respect to the calculogenic minerals. Medical prevention is achieved by inducing a state of undersaturation or low- to mid-metastability as long as there is no mechanism for heterogeneous nucleation present. Urinary supersaturation with calculogenic minerals represents an increased risk towards urolith formation and is required for urolith formation, but other factors are important.

Various factors involved with urolith formation may be evaluated by:

- Epidemiological studies performed at urolith centers and designed to identify risk and protective factors.
- Measuring urine concentrations of calculogenic substances.
- Evaluating the influence of urine pH on crystal formation.
- Measuring the degree of undersaturation, supersaturation, and/or oversaturation of urine with crystallogenic substances.

Determination of urinary biochemical parameters and urinary saturation can only be done in patients that are 'stone free' because active urolith disease results in depletion of calculogenic compounds in urine that alters results.[2]

Urinary mineral concentrations

Measurement of urinary mineral concentrations is helpful in identifying animals that excrete large quantities and/or abnormal types of calculogenic substances. However, the concentration of a mineral is influenced by many variables including dietary consumption, intestinal absorption, endogenous production, renal excretion, and urine volume. Because many variables are involved, measurement of urine concentration of minerals per se is not a reliable index of whether or not uroliths will form.

Urinary pH

The influence of urine pH on canine and feline urolith formation, particularly those composed of struvite and calcium oxalate, has received considerable attention.[3–9] Urine pH may have a profound effect on excretion and solubility of minerals.[10,11] Struvite solubility increases substantially in acidic urine and markedly decreases in alkaline urine.[12] In a study comparing a struvite dissolution diet and a struvite preventative diet, a lower urinary pH was associated with more rapid dissolution of sterile struvite uroliths in cats.[13] Urine pH has also been shown to influence calcium oxalate formation in cats with calcium oxalate being more soluble in alkaluria when compared with aciduria.[14] Potassium bicarbonate administration to cats induces alkaluria, increased urinary potassium excretion and decreased calcium excretion in cats.[15] Urine pH also influences inhibitors of uroliths, especially calcium oxalate formation. Feeding an alkaluria-inducing 'oxalate preventative diet' is associated with increased urinary glycosaminoglycan excretion in cats, which decreases risk of urolith formation.[16] Inducing a neutral to alkaline urine pH in cats is associated with increased excretion of citrate and potassium and decreased calcium excretion, and more importantly decreases urinary saturation for calcium oxalate.[14] While urine pH influences urolith formation it does not provide a reliable index of urolith formation, but indicates increased or decreased risk in combination with other factors.

Mineral supersaturation in urine

Although results of epidemiologic studies and measurement of urinary mineral concentrations and urine pH are often helpful in diagnosis and management of stone disease, they are insensitive tests. Because supersaturation of urine with stone-forming substances is necessary for stones to form, measurement of urine saturation with minerals is a more accurate means of assessing risk of stone formation. An emphasis of urolithiasis research is evaluation of crystallization methods as urolith formation is preceded and advanced with crystal formation.

'Crystallization is a physical chemical process involving a change of state from solution to solid. The supersaturation, which is a measure of the chemical energy available for this process, is a crucial factor and governs all aspects of crystallization such as nucleation, growth, and aggregation. As the reaction proceeds, the supersation will decline (unless replenished) and this in turn will impact upon the kinetic behavior of the crystallization process. While the physical chemistry and kinetics are always important, the process of stone formation takes place in a biological environment.' – JP Kavanagh, 2006[17]

In addition to various techniques developed to evaluate crystallization, several 'risk formulae' have been proposed to evaluate propensity for urolith recurrence (primarily for calcium oxalate) in human patients,[18] although debate exists as to the utility of these formulae.[19] These include: urinary calcium-to-magnesium ratio, urinary calcium-to-citrate ratio, saturation-inhibition index, 24-hour urine quotients [(calcium x oxalate/magnesium x creatinine) and (calcium x oxalate/magnesium x creatinine x inhibition of calcium oxalate crystal growth in dilute urine)], probability index and the ion-activity product index.[18]

Supersaturation

In solution chemistry, the difference in chemical potential of two states ($\Delta\mu$) is dependent on the activities of the crystallizing salt in the supersaturated solution (a) and in the solution when it has come to equilibrium (a_{eq}):

$$\Delta\mu = RT\ln(a/a_{eq})$$

where R is the universal gas constant and T is the absolute temperature (Kelvin). The activity of the crystallizing salt is represented by the activity product (AP) for that salt where the activities of the ions comprising that salt are multiplied. The term 'activity' of a mineral is an index of the likelihood that the mineral will combine with other substances in urine, and is determined by multiplying the concentration of the ion by the activity coefficient for similarly charged molecules. For example, the activity of calcium is determined by multiplying the concentration of calcium in solution (molarity) by the activity coefficient for a doubly charged molecule since calcium carries a '2+' charge. The 'activity' of a mineral is dependent on several factors including the:

- Urine concentration of that mineral
- Urine concentrations of other substances such as sodium, potassium, calcium, etc
- Quantity and functional state of non-mineral or non-measured mineral inhibitors and promoters of crystal formation, growth and aggregation
- Urine pH
- Temperature of urine

Growth of crystals may occur through enlargement of existing crystals by direct incorporation of solution species into the solid crystal lattice or by aggregation of crystals. Aggregation can also result in enlargement of the crystal mass, and occurs through the net result of crystals colliding and either dispersing or consolidating, with the outcome being dependent on an efficiency factor. As consolidation is achieved by crystal bridges that fuse the lattice structures of individual crystals, aggregation also is dependent on supersaturation.[20,21]

Relative supersaturation

Determining the relative supersaturation of a urolith-forming substance in a patient's urine is one technique used to assess risk of urolith formation.[22,23] Relative supersaturation (RSS) is determined by measuring urine concentrations of several analytes including ammonium, calcium, chloride, citrate, hydrogen (pH), magnesium, oxalate, phosphate, potassium, and sodium (and possibly cystine, sulfate, uric acid and other compounds), in urine. These values are then entered into a computer program (EQUIL or SUPERSAT), which calculates the activity coefficients for the various ions and combines the relevant ion concentrations and activity coefficients to produce the activity product (AP). For example, the AP of calcium oxalate is calculated as the mathematical product of the activity of calcium and activity of oxalic acid. The AP for each urolith-forming compound is divided by its known thermodynamic solubility product (SP) and the resultant RSS produced.

RSS = ion AP of the patient's urine / ion SP

Relative supersaturation is related to the energy available for crystal nucleation and growth; however, RSS values are limited by the fact that the

thermodynamic solubility products used for these calculations have not been measured in the patient's urine. It is probable that different macromolecules, including inhibitors and promoters of crystal formation, growth, and aggregation, in the patient's urine have a pronounced influence on free ion concentrations. By using calculations measured in urine from healthy human beings, RSS may overestimate SPs and APs of different minerals, and thus tend to underestimate the risk of urolith formation. Another technical problem in evaluating dogs and cats is that the computer program used to calculate RSS involves comparison of the pet's urine values to standardized values based on the composition of human urine.

Activity product ratios

Activity product ratios (APR) also are designed to express the degree of supersaturation of solutions with calculogenic minerals. APRs are obtained by calculating the ion AP in the patient's urine samples before and after equilibrium with various seed crystals such as calcium oxalate.

$$\text{APR} = \frac{\text{ion AP of patient's urine before incubation with seed crystals}}{\text{ion AP of patient's urine after incubation with seed crystals}}$$

In determining the APR, the patient's urine is incubated with preformed seed crystals composed of pure urolith-forming mineral of interest (for example, calcium oxalate). Following incubation for 48-hours with the seed crystals, the urine concentration of the same analytes are measured. The post-incubation concentrations of analytes are then used to calculate a 'post-incubation' AP. Dividing the 'pre-incubation' AP by the 'post-incubation' AP gives the APR for that patient's urine sample.

An exact measurement of supersaturation is not obtained by determining APR, but the method provides useful information about the relative increase or decrease of the ion AP in the patient's urine that result from seed crystal growth or seed crystal dissolution. An APR less than one represents undersaturation of urine with the mineral being evaluated. An APR equal to one represents saturation of the patient's urine sample. An APR value >1 indicates that the patient's urine sample is supersaturated.

Activity product ratios can be calculated for any calculogenic mineral as long as pure seed crystals for that type of mineral are available. Use of APR methodology will not eliminate errors associated with the effect of unknown factors such as crystallization inhibitors or promoters on ion activities; however, since the same urine sample obtained from the patient is analyzed before and then after equilibration with seed crystals (such as calcium oxalate), the same type of error occurs in evaluation of both analyses and therefore the errors cancel. Whereas calculation of RSS can overestimate supersaturation, saturation, and undersaturation, the APR method overestimates undersaturation, underestimates supersaturation, and correctly measures saturation, provided that a sufficient amount of seed crystals have been used. One limitation of APR determination is the assumption that urine has reached the SP for the salt following 48 hours of incubation, which has been shown to be a false assumption in some cases.[24] Urine may not reach true equilibrium saturation level, particularly when coming from a supersaturated level, presumably due to presence of various inhibitors of crystal growth that slow down the approach to equilibrium. In this instance, when the true RSS is measured following 48 hours of seed incubation, the AP achieved at that point may be two to three times higher than the thermodynamic solubility product. The APR calculated at this point, therefore, systematically underestimates the actual level of supersaturation since the denominator (AP/SP) is too large. The opposite may occur when the urine is undersaturated.

Other measures of urine saturation

There are other techniques for estimating urine saturation in addition to RSS and APR such as the Bonn-Risk Index and Joint Expert Speciation System (JESS). These are used in human beings and could have potential for veterinary patients.

Use of urinary saturation testing

Limited studies utilizing urine saturation testing have been performed in veterinary medicine, particularly in animals that have formed uroliths. Despite the number of studies, very few have been performed in dogs or cats that are urolith-formers and no studies exist that compares estimates of urinary saturation with recurrence rates of uroliths. In cats, calcium oxalate urolith formation typically occurs when urinary RSS for calcium oxalate is greater than 12; the metastable zone lies between an RSS value for calcium oxalate of 1 and approximately 12.[25] Sterile struvite urolith formation in cats typically occurs when urinary RSS for struvite is greater than 2.5; the metastable zone lies between a RSS value for struvite of 1 and approximately 2.5.[25] Urinary supersaturation represents a risk for urolith formation, but as in human beings, there is overlap in values between urolith-forming animals and healthy, non-urolith-forming animals;[26,27] therefore, other factors are important. Use of urinary saturation studies can provide further information as to mechanisms of urolith formation, screening of animals at risk for urolith formation, and monitoring efficacy of urolith management.

Clinical application to dogs and cats

So what does all of this mean? There are several factors to keep in mind:

- Urinary saturation is the most important, but not the only, driving force for crystallization and urolith formation.
- Several methods exist for estimating urinary saturation; however, none of them adequately describe what is occurring naturally in the biological system (urinary tract).
- Determination of RSS and APR values, while used to estimate urinary saturation, give different results and information. Determination of RSS is a valuable and reasonably reliable technique for estimating urinary saturation; however, it (a) is heavily influenced by concentration of analytes measured, which, in turn, is influenced by urine volume, and (b) it does not account for urinary constituents that are not measured including the influence of inhibitors. Because it is influenced by urine volume, methods designed to increase urine volume (eg, feeding canned foods, administration

of diuretics, and stimulating water consumption by increased levels of dietary sodium) would be expected to lower the RSS; however, clinical studies in urolith-forming dogs and cats are lacking.[28–33] Determining APR values do not give an exact estimation of the supersaturation; however, because a patient's urine is used pre- and post-incubation with seed crystals, this technique does account for unmeasured urinary constituents and the influence of inhibitors.

- Medical dissolution of uroliths is accomplished by inducing a state of undersaturation of urine (below the solubility product) with the minerals that formed the uroliths.
- Medical prevention of uroliths is accomplished by inducing a state of undersaturation of urine or at least a state of saturation at the lower end of the metastability limit.
- Despite use of estimates of urinary saturation, there are no published studies in urolith-forming dogs and cats that validate their prediction of urolith recurrence. Until that time, these techniques are useful for formulating diets, but await recurrence data for validation.
- Means to decrease urinary saturation include increasing urine volume ('dilution is the solution to pollution') thereby decreasing the concentrations of calculogenic substances and decreasing dietary intake of calculogenic substances. Despite these measures, they do not guarantee prevention of urolith recurrence in all patients demonstrating that urolith formation is a complex process and many questions remain unanswered.

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