Nephrolithiasis: Journey Through Animal Evolution and Human History

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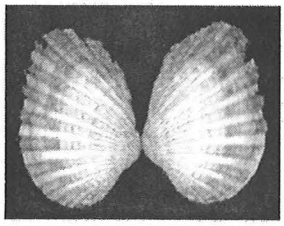

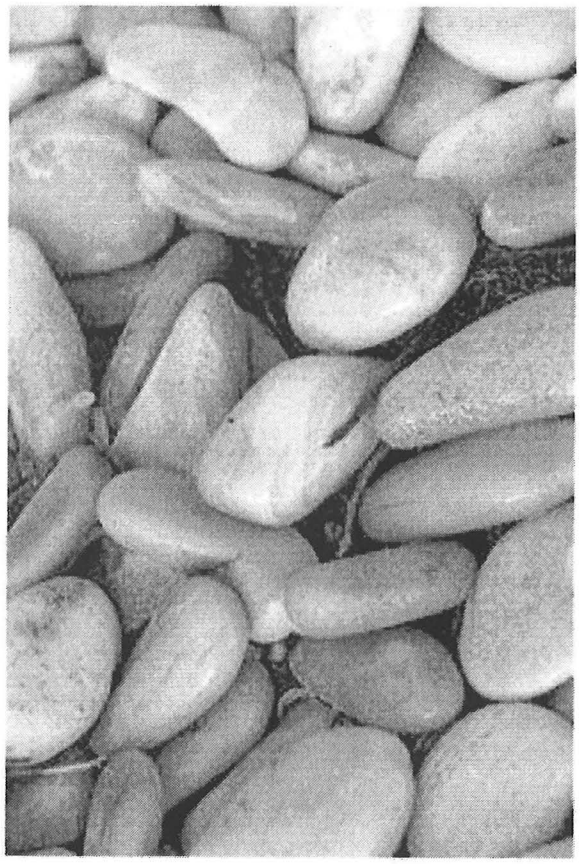

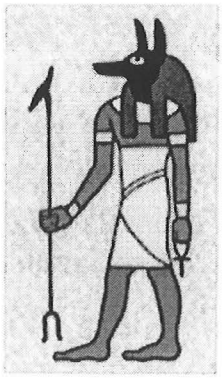

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Research: Renal physiology and metabolism. Pathophysiology of nephrolithiasis.

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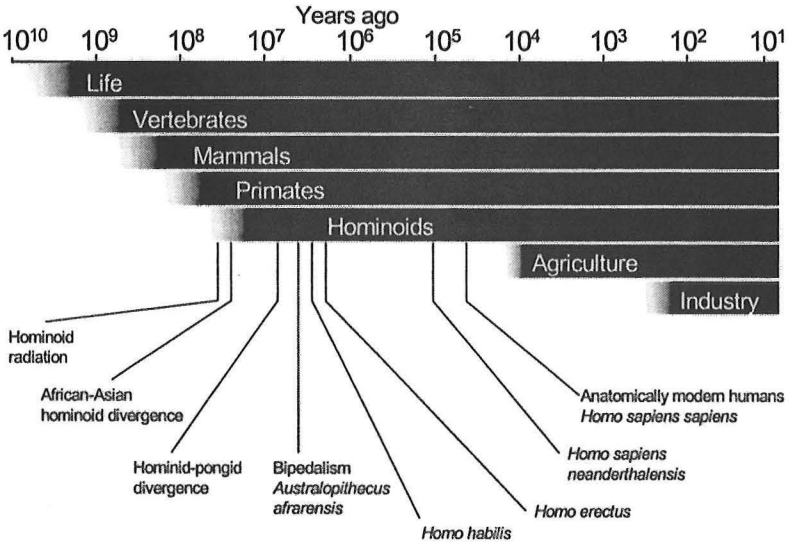
INTRODUCTION

LIFE ON THE PLANET

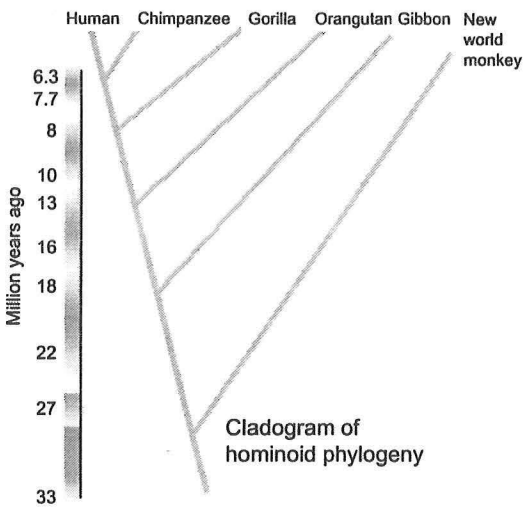
The earth is approximately 4.6 billion years old. It is believed that the formation of the moon a little over 4 billion years ago stabilized the earth atmospheric conditions and rendered it permissive for the birth of simple life forms. The following timetable puts the events of interest in perspective in log scale. Of the 4.6 million years of life, vertebrates have lived for about a billion.

The earliest mammals appeared a little more than 200 million years ago. Paleontologists believe they were small insectivores somewhat similar to today's tree shrew such as the tupaia of Borneo and Sumatra (Martin 1986). In part, the tree shrew taxonomically resembles marsupials, rodents, and primates but cannot be slotted into either one of these groups. After the mass extinction of dinosaurs and megareptiles, mammals underwent expansion and primates came into being around 70 million years ago. Primates with some resemblance to their modern day counterparts appeared about 50 million years ago. These are the hominoids which included hominids and apes. While bipedalism started with *Australopithecus afarensis* (better known to the public as "Lucy"), the first human species *Homo habilis* did not appear till about 2.2 million years ago with its successor *Homo erectus* evolving around 1.7 million years ago. Anatomically modern humans *H sapiens sapiens* are believed to have appeared around 90 thousand years ago. The agricultural and industrial revolution is merely 10,000 and 200 years old respectively.



Environment and lifestyle certainly can exert selection pressure over our genome. Major changes in lifestyle of *Homo sapiens* never really occurred before the end of the late Paleolithic period (some 20,000 yrs ago). Hominoids occupied less than 2% of the time span of life on the planet with humans being about 2% of the history of bipedal hominids, and agriculture and industry influenced 4% of the time span of humans.

The $3x10^9$ nucleotides harboring $3x10^4$ or so coding sequences in the human genome were fashioned over four billion years of evolution on the planet. Dorit and coworkers suggested that the minimal genome of the earliest simple organisms consisted of about 1000-7000 independent exons that formed the foundation for subsequent reshuffling, fusions, and duplications (Dorit 1990). If our 30,000 or so exons descended from the 1000-7000 initial precursors over four billion years, one can immediately appreciate that this is not a rapid process. James Neel studied the coding sequence of 11 well-characterized proteins in humans, chimpanzees and gorillas and found 0.42% difference between humans and chimps and 0.85% between humans and gorilla's; even the intron sequence of globin differs only 1.84% between humans and chimpanzees (Neel 1984). Although there are some disputes as to the exactly branch pattern of hominoid phylogeny, there is little doubt that humans and chimpanzees diverted about 7 million years ago (Sarich 1973, Sibley 1984). Some 7 million years have elapsed before our genome diverged by less than 2%. *Evolution at the molecular level proceeds at a remarkably slow pace.*



While accumulation of base changes is very slow, once polymorphism is established, changes in allelic frequency can proceed at a slightly faster pace. Some genes in humans have responded to short-term environmental selection pressure. The best-known group of genes is the ones evoked by the "malaria hypothesis" (Carter 2002). Frequency of certain alleles of globin and erythrocyte glycolytic enzyme and transporter were enriched due to their conference of infection resilience to the red cell and survival advantage to the host when *H. sapiens* co-existed with *Plasmodium.* The observation of high frequencies of sickle cell trait and thalassemia in areas endemic for malaria was made back in 1946 and 1948 respectively (Beet 1946, Haldane 1948). In addition to the globin chains, several other genes such as glucose-6-phosphate dehydrogenase, the anion exchanger-1, and the Duffy antigen have also been implicated in the malaria hypothesis. Genetic polymorphisms of lactase expression has been linked to selection by malaria. However, the mechanism of this protective is obscure and even the epidemiological data of the association is contradictory (Anderson 1994, Meloni 1998, Aurrichio 1998). Carter and Mendis analyzed the rate of allelic selection of these genes by *Plasmodium* (Carter 2002).

Glucose-6-phosphate dehydrogenase deficiency and thalassemia represent mutations that are moderately protective against malaria in their heterozygous states. At the same time, they carry a low balancing cost to a population because homozygosity does not impacts negatively on survival or reproductive fitness. These genes were the first to reach elevated frequency. This is observed in Europe along the Mediterranean coast where malaria arrived around 2,500 years ago. Hemoglobin S and mutations of the anion exchanger-1 (AE1) or red cell band-3 protein, are highly protective in its heterozygous state. However, at the same time, they carry a substantial balancing cost in terms of mortality of homozygotes. The estimate is that these genes have been estimated selected for more than 3,000-4,000 years of malaria exposure; in Africa for hemoglobin S and in Southeast Asia for AE1. The longest duration required for detectable changes are the alleles that are only protective only when homozygous. This will be hemoglobin C for *P. falciparum* and RBC Duffy for *P. vivax.* The fact that neither one of these are protective against all species of *Plasmodium* further reduces the selection. However, the fact that the homozygotic state *per se* does not decrease survival helps the selection process. These are examples of changes in gene frequency of (probably) existing alleles. One does not really know over what duration did the allelic divergence occurred.

Summary: Life on the Planet

- In general, the duration of environmental or lifestyle changes is remarkably short compared to the history of organisms and represents no more than a mere flicker in the time scale of evolution.
- Considering the 2.2 million years of the genus *Homo* and the 20,000 years since the agricultural revolution, one can state with considerable confidence that human physiology did not evolve to accommodate our current lifestyle.
- Change in allele frequency in response to selection pressure is detectable over thousands of years if the impact on survival and/or reproduction is strong enough and the balancing cost is not too high.

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BIOMINERALIZATION

"Stone formation" is mineral crystallization in tissue or body fluid. This is a universal process of deposition of inorganic mineralization, crystalline or non-crystalline, around biomolecules. A number of crystalline substances are normally present in the body. Examples are all over the animal kingdom where inorganic crystals are harnessed to become an integral part of organic tissue to provide hardness and strength. These inorganic substances are capable of reversible interaction with biomolecules so the crystalline structures can be remodeled for physiologic needs. Williams discussed the unique ability of calcium to interact with biomolecules from the point of view of its concentration, binding strength, rate constants, and molecular structure (Williams 1976). X ray diffraction studies on single crystals have shown that calcium salts have highly adaptable coordination spheres around the cation. The coordination geometry is highly irregular in both bond angle and bond length. These structures render calcium very adaptable in terms of cross-linking in its solid-state or solution chemistry. This greatly facilitates binding of calcium to the irregular geometry of proteins. While disulphide, phosphodiester, and sugar –peptide bonds are covalent and not easily reversible, calcium ion provides a reversible cross-link.

Good vs. bad mineralization

John Hunter (1728-1793) wrote about the similarity between stone formation and calcification (Hunter 1771). He articulated more than 200 years ago the equivalence of formation of enamel, eggshell, gallstones, and kidney stones. Mineralization can be arbitrarily divided into physiologic and pathologic although the partition is not always that distinct. The following is a few illustrative examples where physiologic and pathophysiologic is distinguished on the basis of favorable vs. undesired consequences to the organism.

☺ "Physiologic" mineralization

Exoskeleton Mineralization on a biomatrix is the basis for all organisms with exoskeletal structures such as corals, sea shell, turtle shells, insects. Some of the most sophisticated mechanisms of calcium transport and metabolism can be found in organisms with exoskeletons.

Pearl formation The much-prized mollusca concretion from oysters, mussels, and clams, is another example of physiologic mineralization. When a foreign substance such as grain of sand slips into the oyster between the mantle and the shell, it irritates the mantle not unlike a splinter in a finger. The oyster reacts by covering up the irritant to protect itself. The mantle covers the irritant with layers of the same nacre substance that is used to create the shell. A pearl though aesthetically pleasing, is none more than a leftover of mineralization in self-defense.

Endoskeleton Mineralization is of course critical to the process of chondrogenesis and osteogenesis. This is a continuous process in vertebrate development, initiated during fetal life and persists throughout adult life in the form of remodeling and repair. There is a myriad of regulatory molecules that direct chondrogenesis and osteogenesis in every skeletal element of the body in a highly coordinated fashion.

Dentition During development of the tooth crown, two matrices dentin and enamel are derived from the odontoblast and the ameloblast respectively and layered in opposition to each other at the dentino-enamel junction. Dentin is a collagen-based matrix while enamel contains various proteins including amelogenin. The ameloblast secretes the organic matrix along the dentino-enamel junction, which in turn regulates the biomineralization of enamel which is basically deposition of hydroxyapaptite (Fincham 2000).

☹ "Pathologic" mineralization

Synovial fluid Crystal acute arthropathy is primarily a disease of deposition of crystals (either uric acid or calcium pyrophosphate) in synovial fluid which can elicit an acute inflammatory arthritis. The culprits are monosodium urate and calcium pyrophosphate dihydrate primarily in the synovium and articular cartilaginous surface respectively. Crystals activates mononuclear leukocytes as well as generates a "broth" of cell-derived cytokine mediators of inflammation, which creates havoc and destruction in the joint.

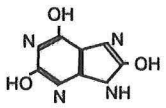
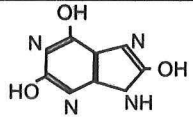
Gall bladder. A great majority of gall stones are crystalline organic material, usually cholesterol which is strictly speaking not mineralization. However pigmented gallstones contains carbonate, phosphate, and bilirubates crystals of calcium. In addition to supersaturation, promoters of nucleation and hypomotility also contribute to gall stone formation.

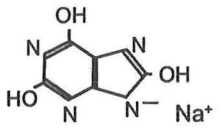
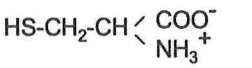
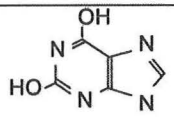
Blood vessel wall Cells that have morphologic resemblance to and express markers of osteoblasts and osteoclasts have been described and extracellular ligands associated with bone remodeling have been found in the vasculature. To date, true bone structure has not been documented. At present, there is a controversy of whether this process vascular calcification (Karsenty, 2000) vs. true vascular ossification (Demer, 2003) as in ectopic bone formation.

Urinary space While most physiologic fluids are relatively stable in chemical composition, urine is one fluid that is constantly changing over a wide range. Renal calculi are pathological crystalline build-ups of minerals identical with those found in nature (see table below). A few of the crystalline compounds that may form are purely organic chemicals such as uric acid, xanthine, or cystine. Stones of similar nature and composition can be found in nature. This table provides a reference for those who are confused with the frequently interchangeable usage of chemical and common names.

Chemical name	Chemical formula	Common name	Comments
Calcium oxalate monohydrate	$CaC_2O_4 \cdot H_2O$	Whewellite	Occurs in septarian nodules from marine shale near Havre, Montana, with golden calcite at Custer, South Dakota, and as a fault filling with celestite near Moab, Utah. It is found in hydrothermal veins with calcite and silver in Europe, and it often occurs in association with carbonaceous materials like coal, particularly in Saxony, Czechoslovakia, and Alsace.
Calcium oxalate dihydrate	$CaC_2O_4 \cdot 2H_2O$	Weddellite	Classic "envelope" crystalluria. Name derived from crystals found in bottom sediments of the Weddell Sea off Antarctica. The sharp yellow crystals in urine are much larger. Weddellite commonly forms upon the outer surface of a smooth whewellite stone. Occasionally, weddellite partially dehydrates to whewellite, forming excellent pseudomorphs of grainy whewellite after weddellite's short tetragonal dipyramids. Apatite, whewellite, and weddellite constitute the common urinary stones.
Calcium phosphate	$Ca_{10}(PO_4)_6(OH)_2$	Hydroxyapatite Apatite	Occurs naturally as a white powdery mineral deposit. Fundamental mineral component in bones and teeth, and when apatite has fluorine in its crystal structure (replacing some phosphate), it is stronger. This is why fluorine is added to water and toothpaste. In kidney stones, carbonate (CO_3) can substitute for some of the phosphate $Ca_5(PO_4,CO_3)_3$, making a mineral that is relatively poorly crystallized. Well-crystallized or not, apatite often forms the nucleus upon which other urinary minerals are deposited.
Calcium hydrogen phosphate	$CaHPO_4 \cdot 2H_2O$	Brushite	A soft, silky mineral, usually honey-brown and showing a fine radial fibrous structure. A common cave mineral in guano deposits, and in phosphorites, formed at low pH by reaction of phosphate-rich solutions with calcite and clay. Found naturally on Aves Island, Venezuela, west of Dominica, and in the Caribbean Sea. Name so to honor Prof. George Jarvis Brush (1831-1912), American mineralogist, Yale University.
Calcium pyrophosphate	$Ca_9(Mg,Fe^{2+})(PO_4)_6(PO_3OH)$ or $Ca_9(Mg,Fe)H(PO_4)_7$	Whitlockite	Rarely found in the urinary system but it is the most common mineral found in prostate stones. It is a calcium phosphate with small amounts of magnesium, and its occurrence may be stabilized by trace amounts of zinc. Prostate fluid has a very high zinc content. The mineral is a resinous, brown, hackly-fracturing material, and it commonly forms multiple small stones in the prostate.
Magnesium ammonium phosphate hexahydrate	$MgNH_4PO_4 \cdot 6H_2O$	Struvite,	Bright shiny crystals. Forms the classic "coffin-shape" crystals. Found naturally in exotic organic debri such as bat guano. Masses of struvite can grow togther with apatie to form huge calculi called staghorns which can fill up the entire urinary system. Forms in alkaline with high NH_4 content in infected urine.
Magnesium acid phosphate trihydrate	$MgHPO_4 \cdot 3H_2O$	Newberryite	An acid Mg phosphate, (unlike struvite, which contains ammonium). Rare in kidney stones but when it does occur, it does so as tiny isolated globular crystals on the surfaces of apatite-struvite stones. Newberyite is associated with *Proteus* infections.

Crystallization is not restricted to inorganic chemicals. There are stones comprised primarily of organic crystals. Because of the rarity of these crystals existing outside a living organism thereby obviating cross-reference to the geology literature, the nomenclature is much simpler.

Chemical name	Chemical fornula	
Uric acid monohydrate	 H_20	Uric acid is sparingly soluble in water (97 mg/L). It has a pK of 5.6 in N at position 9. The other protons dissociate a pH's way beyond physiologic range. The single most important determinant of how much total uric acid is tolerated in the urine is the pH
Uric acid dihydrate	 $2H_2O$	Uric acid was first isolated from urine by Scheele in 1776 and later identified in voided urinary concretions by Wollaston in 1810.

Monosodium urate		Although urate is more soluble than uric acid, it is not infinitely soluble. In the absence of unduly acid urine, hyperuricosuria tends to saturate the urine with monosodium urate, which precipitates calcium oxalate.
Cystine		Defects in b^{+0} amino acid transport system leads to wasting of several amino acids. Only cystine is of limited solubility resulting in precipitation constituting the common and frequently only phenotype.
Xanthine		

Summary: Biomineralization

- Calcium is a cation with unique properties to interact with organic molecules. Biomineralization is pervasive in nature and in biology
- Mineralization is a process used extensively in many physiologic processes. When crystal appear when they should not, a disease process may result

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RENAL CALCULI IN ANIMALS AND HUMANS

NEPHROLITHIASIS IN THE ANIMAL KINGDOM

Complex multicellular organisms have evolved its physiology in the cradle of a constant *milieu interne.* To preserve this constancy while existing in and incessantly assaulted by a fluctuating environment, homeostasis is achieved by the concerted efforts of multiple organs. The kidney assumes a pivotal role in negating the extensively varying input into the organism by constantly excreting the invading substances and/or their metabolites. This task mandates that urinary biochemical composition be perpetually variable. On major constraint of terrestrial existence is low urinary aqueous volume which significantly amplifies the variation in the concentration of urinary solutes. While these physiologic needs are met most of the time, there are situations where these limits are exceeded and crystallization will occur. As one peruses through the various renal calculi in the animal kingdom, one finds either genetic disorders or more commonly an environmental condition that has wandered off the limits of normalcy and precipitated stone formation.

Kidney stones are ubiquitous in the animal kingdom. Examples are found in amphibians, reptiles, birds, and various orders in mammals including horses, cows, seals, wolf, dogs, and cats. The omnipresence of nephrolithiasis includes any organism that has kidneys. The literature does not authentically reflect the relative incidence in the various species because it tends to be biased by

occurrence of urolithiasis in species that come to veterinarian attentions such as dogs, cats, horses, and cows. Even with this caveat, there is a true paucity of reports of renal calculi in aquatic species; a fact that can be explained by their very high urinary volume. Rare cases of genito-urinary calculi reported in aquatic animals are usually in the background of infection (Walsh 1987).

Mammals

Although there is some preponderance for certain species to get certain stones, the composition of kidney stones in the animal kingdom by and large mirrors that of humans. Canine stones are almost identical to that of humans except for a higher prevalence of struvite and lower uric acid (Ross 1999). This is explained by the high incidence of urinary tract infections in dogs and the presence of uricase in most canine species. The chemical profile of aminoacidiuria in a cystinuric wolf is very similar to humans with congenital cystinuria (Bush 1978) with cystine, arginine, and lysine wasting. In horses, nephrolithiasis is a documented cause of chronic obstruction and renal failure (Divers 1983, Ehnen 1990). Compared to humans, horses have high calcium carbonate in their calculi in addition to calcium oxalate and phosphate (Divers 1983, Holt 1984, Hope 1989, Ehnen 1990).

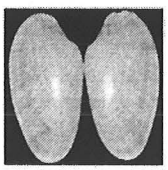
Environmental factors likely play an important role in the pathogenesis of renal calculi in animals. The true prevalence of nephrolithiasis for the Asian small-clawed otter, *Aonyx cinerea*, in their natural habitat is not known but one study suggests that it is very low (Dacngsvang 1973) and occurs only secondary to infection by the renal parasite *Gnathostoma vietnamicum*. In sharp contrast, the exact same species reared in captivity has a 66-90% prevalence of nephrolithiasis (Calle 1988, Petrini 1999). The stones are primarily calcium oxalate and one study suggested hyperoxaluria as the factor but due to lack of normal values for urinary electrolytes in otters, it is difficult to substantiate that conclusion (Petrini 1999). One cannot avoid suspecting that this is due to the fact that what they are fed in the zoo is not what they forage in their natural habitats.

Fish and aquatic amphibians

Reports of renal calculi in fish and aquatic amphibians are exceedingly rare. They are usually associated with either massive dietary changes or genitourinary infections. An example of diet-induced stone is seen in frogs (Smith 1972). The spinach-supplemented diet is used to bolster growth in tadpoles of *Rana pipiens*. One complication of this rearing method is death from renal failure caused by calcium oxalate nephrolithiasis (Berns 1965). Even aquatic organisms can be overwhelmed by excessive loading from a dietary source.

Reptiles and Birds

While kidney stones in aquatic reptiles are extremely rare, renal calculi in dessert reptiles are very common (Frye 1972, Blahak 1994, McKown RD 1998, Homer 1998). The same is true for birds. In these species, there is a switch in composition from calcium oxalate to primarily uric acid. These uricotelic (excretes nitrogen as uric acid) organisms intend to have uric acid precipitate in their urine and it is very hard to distinguish physiology from pathophysiology. The journey gets more interesting as one goes to invertebrates.



Molluscs

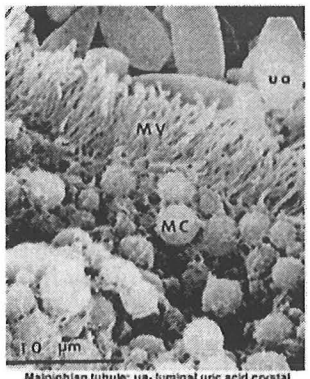
Molluscs are one of the most diverse in form and habit of all the phyla. Nephrolithiasis has been described in mollusks. Such articles are more frequently found in biologic or zoologic rather biomedical journals. However William Tiffany did published two articles about the Sunray Venus clam, *Macrocallista nimbosa* in

urology journals. Excretory concretions have been observed in many species of bivalves and their composition was first analyzed by X-ray diffraction by Doyle and coworkers (Doyle 1978). For years, these hard dark granular masses were not considered as renal calculi until the work of Tiffany and colleagues in 1980. The presence of renal calculi is extremely common in the species *M. nimbosa* that resides off the Atlantic shoreline and the Gulf of Mexico.

The renal tubules of *M. nimbosa* resembles the mammalian proximal tubule. The calculus is primarily calcium phosphate with concentric laminar morphology. Tiffany proposed in 1980 that molluscan renal calculi start with intracellular "precursor". They grow by concentric deposition follow quickly by calcification. The compression of the surrounding cells causes disruption of membrane and protrusion or extrusion of the mural stone into the lumen where they grow by aggregation and epitaxy. This is not very different from the paradigm for stone growth in the human kidney as proposed by Malek et al. and Evan et al (Malek 1977, Evan 2003). One should take caution in the interpretation of these findings. It is unknown whether these concretions in *M. nimbosa* are truly "pathologic" because biologists do not know whether they have a negative impact on the clam's health. As one ventures lower in the animal kingdom, the search for renal calculi gets harder. This is especially true when there are no such structures as kidneys. But mineralized inorganic materials in nephridia are common such as the calcium concretions found in the Malpighian tubules of insects.

Arthropods

The insect Malpighian tubules are equivalent to "kidneys". In the glow-worm, *Arachnocampa luminosa*, Green 1979), crystalline concretions called "spherites" of calcium phosphate and uric acid and accumulate in the tubules. When calcium is ingested by or injected into the hematophagous *Rhodnius prolixus,* accumulation can be detected in tubule cells (Maddrell 1991). These appear to be storage granules for calcium. Mineralized concretions are found commonly in the common household cockcroach *Periplaneta americana* (Wall 1975). In the skipper butterfly, *Calpodes ethlius,* mineral concretions of calcium phosphate accumulate in larval tubules, persists throughout metamophosis and decline in number in adults (Ryerse 1979). Mineralized concretions may serve a storage function of calcium later used for exoskeleton formation.



Malpighian tubule: ua- luminal uric acid crystal
MV- Microvilli, MC-microspheric concretions

Summary: Nephrolithiasis in the Animal Kingdom

- Renal calculi are found in many vertebrates. The composition of kidney stones in mammals is not that different from humans.
- Mineral concretions of the "urinary system" are also found in invertebrates that have no true kidneys.
- Presence of mineralized concretions in the urinary space os pervasive. It may be difficult to discern with any clarity whether this is physiology or pathophysiology.

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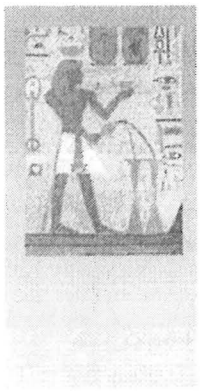
History of Human Kidney Stones

Nephrolithiasis is as old as humankind. Like geologic minerals, human calculi can be very durable and frequently survive the environmental vicissitudes long after those whom they ailed have perished. Therefore, kidney stones are valuable medico-archeological records. Because calculi are crystals, they lend themselves to study by X ray diffraction and their composition can be accurately documented even after several thousand years. The earliest discovery of stones in the genitourinary tract originated primarily from paleontologic studies during excavations in Egypt (Wood-Jones 1910, Cockburn 1983), North America (Beck 1966), South America (Gersten 1983), New Zealand (Houghton 1975), Europe (Boross 1963, Szalai 1987), and Africa (Morris 1989). Scholars have written about the "scarcity" of stones in archeological records. For example, of the 30,000 mummies excavated by Prof. Elliot Smith, 2 cases of bladder stones and 3 cases of kidneys stones were noted. However, this is most likely due to the failure to recognize them because unless the archeologists dedicate special attention to seeking calculi, it will likely be missed.

4,800 BC

The oldest documented human genitourinary calculus was a bladder stone in the pelvis of a 16 yr old boy mummy discovered by British Egyptologist G. Elliot Smith in at El Amrah in Upper Eqypt in 1901.



The mummy is believed to have lived close to 7,000 year ago in pre-dynastic Egypt. Prof. Smith presented the stone to the Museum of royal College of surgeons of England and Professor S.G. Shattock studied it and presented the findings in a treatise in 1905 (Shattock 1905). X-ray diffraction revealed it to be calcim oxlalte (75%), magnesium oxalate (9%), magnesium ammonium phosphate hexahydrate (6%), carbonate apatite (4%), uric acid (3%),

4,400 BC

There were several tombs explored from the Second Dynasty on the Nubia plain. Studies of other mummies have revealed lithoid bodies directly beside the first lumber vertebra and presumed to be renal in origin. This stone was 1.5 cm in longest diameter and composed of calcium carbonate, phosphate, and oxalate. Gray also studied stones in mummies *in situ* using radiography (Gray 1967).

3500 BC

Kidney stones were found in the remains of a Bronze age burial from Yorkshire, England (Mortimer 1905).

1000BC

Persian literature described symptoms of stone disease and the various treatment methods. Medical therapy included avoidance of eggs, meat and fish.

500 BC

The procedure which later will be referred to as "lithotomy" was mentioned in Sanskrit and Greek and was practiced by Hindu and Greek surgeons.

400 BC

In Cos, the small island in the Aegean resides the temple of Asclepius, the son of Apollo and also for one of the most famous physician Hippocratus (born 460 BC). Hippocrates speculated on the possible cause of stones and while recognizing the high complication rate of lithotomy, warned physicians against the practice of this procedure.

"*I will not cut persons laboring under the stone but will leave this to be done by men who are practitioners of this work*"

It is unclear why Hippocrates so adamantly condemned lithitomy. He further stated that suprapubic incisions are universally lethal. This admonition to the medical profession was to be held for centuries. Hippocrates did describe in detail the different maladies of the kidney. He wrote of four diseases of the kidney; the first of which is renal colic. "In the first disease of the kidney, an acute pain is felt in the flank, the loins, and the testes of the affected side. The patient passes urine frequently and gradually the urine is suppressed. With the urine, sand is passed. As the sand passes the urethra, , it causes severe local pain which is relieved when it is expelled. "

10 AD

Houghton described a renal stone at the ventral side of the spine of a 2,000 year old female belonging to the Maori tribe in New Zealand.

100-200 AD

Rufus of Epheseus wrote probably the first monograph on urinary disease which he titled "*De vesicae rerumque affectibus*". In this manuscript, there was a protracted description of "nephritis". Many consider Galen to be second only to Hippocrates in fame in medicine. Galen described hematuria, renal abscesses, and suppurative ulceration can all be secondary to renal stone diseases. Galen also described the condition "nephritis" which bears a very different meaning than what we know today. Galen referred to nephritis as "*lithiasis, id est calculi morbus nephritis appelatus*." Galen postulated that dietary factors are the most important determinant in kidney stone formation.

500 AD

In North America, genitourinary stones were discovered in North American Indian mummies (Williams 1926). The earliest stone in North America was retrieved by an archeological team in 1928 at the

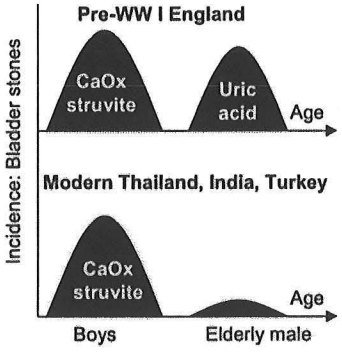
Vandal Cave in Northeastern Arizona from an Indian mummy that was dated to be 1,500 years old (Streitz 1981). "Natural" mummification without embalming was common in the extremely dehydrating environment of Arizona. The bladder was anatomically normal but a single bladder calculus was identified to be calcium oxalate monohydrate (75%), magnesium oxalate (9%), magnesium ammonium phosphate hexahydrate (6%), carbonate apatite (4%), uric acid (3%), ammonium hydrogen urate (2%), silicon dioxide.

1000 AD

In medieval Europe, bladder stones were so common that lithotomy became a profession. The lithotomist was distinguished from the physician and surgeon partly because there were more than enough patients to sustain this single profession and partly because the medical profession avoided this procedure. Pierre Franco (1505-1570) from Provence was an expert lithotomists. Franco once wrote, "Physicians and surgeons can defend themselves when unfortunate, but if we lithotomists have a mishap, we must run for our lives." An extensive body of literature is available in the urology journal dedicated to the procedure of lithotomy.

Bladder stones vs. kidney stones

Clearly, bladder stones dominated over kidney in the early literature. The explanation is likely manifold. One is that kidney stones were difficult to diagnose before the advent of radiography by Roentgen in 1895. Even if upper tract stones were missed in the pre-roentgen days, the virtual disappearance of bladder stones in the 20th century in industrialized countries is indisputable. Another reason is that bladder stones received disproportionately more attention because of the drama associated with its treatment. Lithotomy was one of the first and few operations that was feasible and had a finite rate of success. Because of the absence of quality control, in the hands of unqualified individuals, this procedure also resulted in the highest surgical mortalities ever observed. Hippocrates himself cautioned the physicians against the practice of this procedure. As recent as the early 18th century, the great William Cheselden (1688-1752), an attending surgeon at St. Thomas hospital, achieved great fame because he perfected and revolutionized the technique of lithotomy and dramatically reduced the surgical mortality down to 16%. It is no surprise that lithotomy and bladder stones garnered fame and notoriety.



Finally, there is probably a true shift from bladder stones to kidney stones over human history. While bladders stones are very rare in England today for all ages. Cystic calculi are still endemic in parts of Northern Thailand, India, and Turkey. High prevalence has also been reported in Syria, Bulgaria, Iceland, Madagascar, and parts of China. In modern Thailand, India and Turkey, these stones affect mainly prepubertal boys, compose of ammonium acid urate and calcium oxalate, and are identical to the condition suffered by boys in pre-World War I Britain. The uric acid bladder stones in elderly men were probably dietary in origin. With wartime rationing followed by improvement of nutritional knowledge, the extremely high protein consumption was abandoned.

Summary: History of human kidney stones

- Renal calculi are as old as humankind and are omnipresent in every cultural and geographic niche. It is definitely not a disease of modern lifestyle
- Due to the stability of calculi, even archeologic samples are subjectable to physical and chemical analyses thousands of years "post mortem".
- There is a dramatic decline of bladder stones in industrialized countries in the last century. Bladder stones are still common in parts of the developing world.

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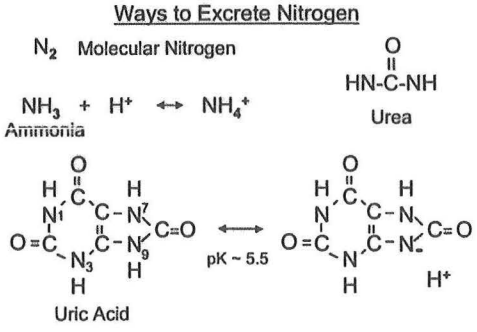
URIC ACID NEPHROLITHIASIS

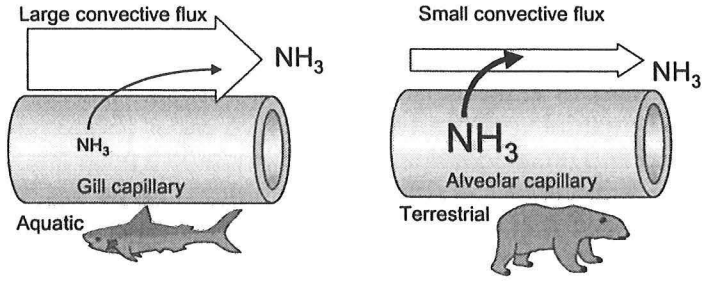
Nephrolithiasis can be traced back to as far as archeological methods can reach. It is definitely not a disease of modern civilization. That is not to say that the incidence and composition of urolithiasis has not changed as a result of modern lifestyle. Although uric acid stones are much less common than calcium stones in humans, like calcareous stones, uric acid stones are found extensively throughout the animal kingdom. Uric acid stones are caused by one of two mechanisms; hyperuricosuria or unduly acid urine. Uric acid calculi in non-humans are usually caused by overwhelming hyperuricosuria. In humans, uric acid stones are due to acidic urine.

NITROGEN METABOLISM IN VERTEBRATES

As organisms on this planet are primarily carbon (C)- and nitrogen (N)-based, one is confronted with the task of excretion of C and N atoms that cannot be recycled. There are two fundamental differences between excretion of C vs. N. There is a virtually infinite pool of storage for C skeletons in adipocytes. N-containing compounds such as amino or nucleic acids are either utilized or destroyed. There is really no N storage pool. The principal end product of C, CO_2, is volatile and can be easily be eliminated by diffusion and convention at a specialized organism-exterior interface (alveolus). On the contrary, N end products in terrestrial vertebrates cannot be eliminated easily via convection to the exterior so the gastrointestinal tract and the kidney are charged with that task. *N excretion by the kidney is intimately intertwined with and inseparable from acid-base and water homeostasis*; hence these topics will be

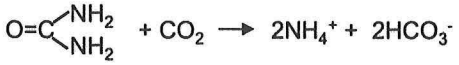


reviewed together. Denitrifying microbes (reversal of atmospheric N fixation) are capable of forming molecular N_2 from organic compounds and can simply "blow" it into our atmosphere much like we do with CO_2 (Mancinelli 1988). However, most organisms excrete N in an organic form; the simplest mode being ammonia as it is the direct product of deamination. Unfortunately, ammonia is highly toxic to most organisms (with some rare exceptions) so only very low plasma levels are tolerated.

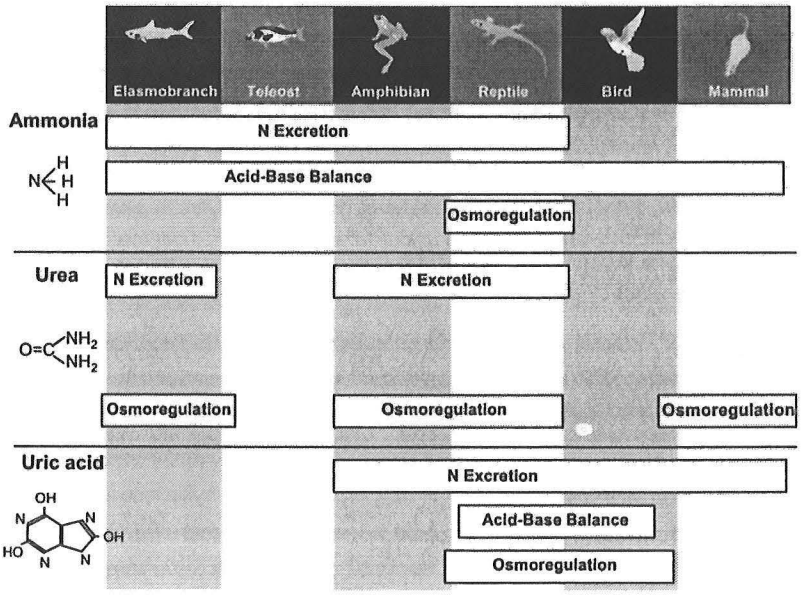


In unicellular organisms and aquatic vertebrates, this mode of N excretion is feasible due to the high aqueous diffusibility and high solubility of ammonia and high convective volumes of fluid bathing the organism; so very low level of ammonia in the body fluid is sufficient to sustain diffusive elimination. With terrestrial migration, the ability to excrete directly into the environment is lost. Although all vertebrates still excrete ammonia, changes have transpired. 1. Ammonia is produced locally in an organ such as the kidney and is then trapped and dispensed directly into the urine without traversing the systemic circulation (Tannen 1990). 2. A majority of N excretion is delegated to relatively non-toxic compounds such as urea and uric acid (King 1985, Wright 1995). An organism that uses one mode predominantly to excrete N is categorized as either *ammoniotelic*, *ureotelic*, or *uricotelic* respectively (Wright 1995).

Ammonia Direct diffusion of NH_3 into the water rushing through the gills is feasible for all aquatic species as a mean of N excretion. For all species, the excretion of NH_3 always carries a H^+ because the excreted species in solution is really NH_4^+. If NH_4^+ is disposed with a Cl^-, net acid excretion would be achieved along with N excretion. Ammonia in the urine still serves to eliminate excess nitrogen but its primary role in higher vertebrates is to serve as a carrier to excrete H^+ and hence is pivotal in determining urinary pH. If NH_4^+ is excreted with a base such as HCO_3^-, then N excretion can be attained without acid excretion as in the alligator. A lot of reptiles that are air breathing actually use NH_4HCO_3 in the urine to excrete N. As will be discussed later, alligators are ureotelic. However, when challenged with a large water load (which happens frequently in their freshwater habitat), alligators can switch from urea into ammonium excretion by splitting urea.



This allows the same N to be excreted with 3 more osmoles thereby enhancing water excretion with solute other than Na^+ or K^+ thus augmenting electrolyte-free water excretion.



Urea Urea evolved as a non-toxic alternative to ammonia. In marine vertebrates who are primarily ammoneotelic, urea serves as a balancing osmolyte in cartilaginous fish, coelacanths, some amphibians, and the aestivating lungfish (Yancy 1982, Griffith 1991, Abe 1991). Urea is tissue serves to balance the very high osmolarity of seawater. In

addition to osmotic balance, urea also increases the positive buoyancy of organisms by the molal volume effect (Withers 1994). Since the animal uses ureagenesis for osmoregulation, some of the urea do get excreted and hence does contribute to nitrogen excretion. In terrestrial ureotelics, urea is an indispensable solute for urinary concentration (Sands 1996). The ureotelic Australian hopping mouse has a fractional excretion of urea less than 2%, which illustrates the osmoregulating function of urea in desert mammals (Hewitt 1981). The uricotelic avian kidney uses osmotic gradient to absorb water but it is achieved without concentrating urea (Lien 1993).

Uric acid Uricotelism is a quintessential example of adaptation to arid environment. The current theory is that uric acid excretion evolved in terrestrial vertebrates to conserve water (Shoemaker 1972). Urea is excreted in an aqueous solution and urea-containing urine mandates a finite amount of water as solvent. Uric acid was adopted because of its low solubility and hence can be excreted as a solid (Shoemaker 1972). Insects, birds, and lots of reptilian species with minimal urinary volumes are largely uricotelic and the crystal-adorned semi-solid urine is either discharged into the gastrointestinal tract or deposited in inert body compartments. Uric acid is a metabolic cul-de-sac in uricotelic organisms as its sole fate is excretion. Uric acid removal from the urine contributes significantly to the kidney's ability to conserve water. Because of the large amount of uric acid in the urine, it actually constitutes a significantly part of titratable acid (Long 1983). Uric acid is also produced in non-uricotelics as an end product of purine metabolism.

Summary: Nitrogen Metabolism in Vertebrates

- An organism can be ammoniotelic, ureotelic or uricotelic depending on which is the major mode of N excretion. These modes are not mutually exclusive. Some animals can ultilize more than one pathway and can switch from one to another in response to physiologic demands.

- Nitrogen excretion is connected with acid-base homeostasis and osmoregulation.

	N excretion	Acid-base balance	Osmoregualtion
Ammmonia	✓	✓	✓
Urea	✓		✓
Uric acid	✓	✓	✓

- Inherent to a highly multitasking system is some degree of compromise

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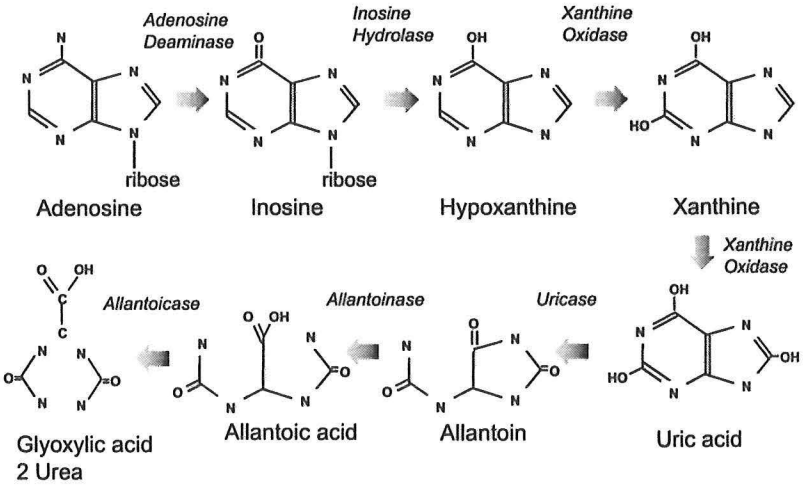
URIC ACID METABOLISM IN VERTEBRATES

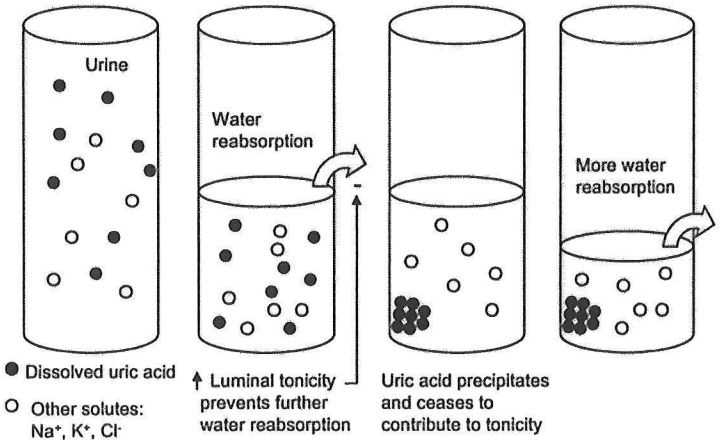
Uric acid is generated from both purine and amino acid. Its role in N excretion was discussed in the preceding section. In most ureotelic animals, uric acid is converted to allantoin by uricase. In organisms with uricase, urea can further be metabolized to ammonium and bicarbonate. Therefore, in ammoneotelic and ureotelic organisms, the end product of purine metabolism can be urea and/or ammonium. Different vertebrates set their metabolic endpoints at different sites on this cascade.



Organism	*Last enzyme*	*Final product*
Uricotelics		
Birds and some reptile	Xanthine oxidase	Uric acid
Ureotelic		
Dalmatian dogs*	Xanthine oxidase	Uric acid
Most primates*		
Most mammals	Uricase	Allantoin
Some reptiles		
Mollusks		
Ammoneotelic/ureotelic		
Some fish	Allantoinase	Allantoic acid
Most fish	Allantoicase	Urea
Amphibians		
Ammoneotelic/ureotelic	Urease	Ammonia
Some fish		
Some amphibians		
Aquatic reptiles		

(Moyle 1949, Gutman 1965, Minnich 1972, McNabb 1975) * unusual ureotelics

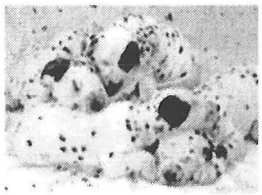
Uric acid is one compound on this cascade that is sparingly soluble in water. Evolution of uricotelism marks the migration of vertebrate to land dwelling. Because of the scarcity of water, many terrestrial reptiles and most birds arrest the cascade prior to uricase and uses uric acid as the primary mode of nitrogen excretion. Water conservation requires a highly concentrated urine. This is limited by the how high can the kidney mount the medullary tonicity. Some rare dessert



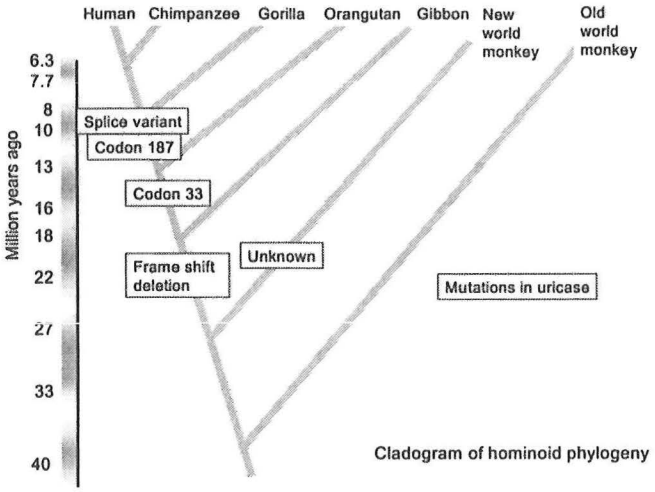
rodents can raise the tonicity of their medullary interstitium close to 10,000 mOsm but most land-dwellers cannot achieve this. Precipitation of uric acid removes a major solute from the urine and lowers the lumimal tonicity to allow more water to be reabsorbed from the urine. The fundamental purpose of uricotelism is for uric acid to precipitate in the urine. One can say that, *"Uric acid stones are meant to be !"*. While this seems to make perfect sense for the avian world, there is incongruence to this argument. Why do certain ureotelics such as the Dalmatian and most primates including humans, arrest uric acid metabolism before uricase and excrete this highly insoluble substance rather than the harmless inert and soluble allantoin?

The Dalmatian dog is a good model. Dalmatians have high urinary uric acid excretion rate (Benedict 1916). Friedman noted that the sum of uric acid + allantoin is practically equivalent in Dalmatians vs. non-Dalmatian dogs suggesting an inability to convert uric acid to allantoin (Friedman 1948). However, uricase activity in hepatic homogenate is normal in Dalmatians (Klemperer 1938). In addition, Dalmatians have very high fractional excretion of uric acid (>100%) (Wolfson 1950). This led some to postulate that the defect is "renal leak" of uric acid leading to escape of uric acid from hepatic uricase. The allogeneic transplantation experiments definitely refuted this conjecture. Both liver (Kuster 1972) and kidney (Appleman 1966) cross-transplantations were performed and the results are summarized in a simplified format.

Donor	*Organ*	*Recipient*	*Urinary uric acid*
Dalmatian	Kidney	Mongrel	High
Mongrel	Kidney	Dalmatian	Low
Dalmatian	Liver	Mongrel	High
Dalmatian	Liver	Dalmatian	High
Mongrel	Liver	Dalmatian	Low
Mongrel	Liver	Mongrel	Low



These experiments are definitive. The phenotype tracks with the liver, and the hepatic defect appears to be is the lack of adequate uricase activity. However, both enzymatic (Klemperer 1938) and morphologic studies (Hruban 1964, Afzelius 1965) showed that this may be a quantitative or functional defect in the sense that active enzyme for some reason is not acting on uric acid. The Dalmatian phenotype can be reproduced in mongrels using the uricase inhibitor oxonic acid (Yu 1971). To date, there have not been reports of genotyping of uricase in Dalmatians. Functional studies however suggest that the defect is not in Dalmation uricase but rather impaired uptake of uric acid uptake into the liver (Giesecke 1984, 1985). The answer will await the cloning of uric acid transporters and uricase in dalmatians. A phenotype similar to that of Dalmatian is found in the anthropoid group of primates including gorilla, orangutan, chimpanzee, and humans. In humans, there is no significant measurable uricase activity.



Cladogram of hominoid phylogeny

Inactivation of uricase in primates

The lack of uricase activity has been studied in more detail in primates. This is a most remarkable feat of gene silencing. Several mutations have been identified (Lee 1988, Wu 1989, 1992).:

Codon 33 Nonsense
Codon 187 Nonsense
Variant splice mutation exon 3,
13 bp frame-shifting deletion

In all cases, a uricase null phenotype results. Wu and coworkers placed these mutations in a hominoid cladogram and proposed the

timing of these base changes in reference to the divergence nodes. Uricase is also altered in New World monkeys resulting in loss of uricase activity although the corresponding genotypic information has not been obtain. Moving back further into the Old World monkeys (Christen 1970), uricase activity appears to be present but not stable. Uricase activity is fully expressed in more primitive primates and all mammals with the exception of the Dalmatian dog. *Is this an accident or is this an adaptive evolutionary change?* The answer is unknown but two theories have been put forth.

Antioxidant hypothesis

The most prevailing explanation is that the "antioxidant" activity of uric acid provided positive selection pressure for lack of uricase, which resulted in the current dominance of the null mutant. This hypothesis is founded on observations accumulated since the 1980's.

1. Uric acid has antioxidant activity by virtue of its ability to scavenge singlet oxygen and other reactive free radicals (Ames1981).
2. In the joint, uric acid has been proposed to counteract the inflammation of rheumatoid arthritis (Agudelo 1984).
3. Uric acid ameliorate shock liver (Tsukada 2000).
4. In the central nervous system, uric acid interacts with free radical formed from peroxynitrite and carbon dioxide, and alleviates experimental sciatic nerve injury (Liu 2000, Squadrito 2000).
5. High uric acid levels were found in preterm infants on mechanical ventilation- a corelative observation (Vento 2000)

Currently, it is still controversial whether uric acid actually acts as an endogenous antioxidant in the various human conditions. Even more controversial is whether this is responsible for the global elimination of uricase expression in humans and apes. It is difficult to fathom how an antioxidant advantage is powerful enough to extinguish all uricase expressors (reducing wild type gene frequency to zero) and why similar selection does not this does not happen to other mammals.

Blood pressure hypothesis

Rick Johnson and colleague at Baylor University came forth with an alternative hypothesis. This stemmed from two observations from the Johnson laboratory and some older epidemiological studies. When mild hyperuricemia was induced in rats, they became hypertensive when placed on a low salt diet while normouricemic rats remained normotensive (Mazzali 2001, 2002). This effect was reversible by reversing the hyperuricemia and partially blocked by inhibition of the rennin-angiotensin system (Mazzali 2001, 2002). The hypertension was accompanied by rena; arteriolosclerosis and interstitial inflammation. When rats with a previous history of hyperuricemia was subjected to a high salt diet. They became markedly hypertensive. The model is that uric acid directly cause glomeruloarteriolopathy and induced salt-sensitivity. Johnson and coworkers postulated that the hyperuricemia-induced hypertension was favorable in times of low salt intake in terrestrial primates and conferred a survial advantage. Again, this theory is presently unproven.

Summary: Uric acid metabolism

- Uric acid is generated from purine or amino acid metabolism and can be further metabolized to allantoin, urea, and even ammonia.
- In uricotelic organisms, uric acid is the end product of metabolism because they lack uricase and the downstream enzymes.
- Uricotelics take advantage of the extremely low solubility of uric acid so it will crystallize in urine and not contribute to urinary osmolarity. This maximizes water conservation.
- Some canines and primates including human are deficient in uricase and as a result excretes large amounts of uric acid. The reason for loss of uricase in ureotelics is unclear but some have postulated possible survival advantage.

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URIC ACID NEPHROLITHIASIS IN ANIMALS AND HUMANS

Uric acid nephrolithiasis affects 8% of human kidney stone formers in the United States (Moe 2002). Uricotelics and a few ureotelics (Dalmatians, primates including humans) do not possess uricase and hence sets the stage for uric acid precipitation in the urine. Uric acid is a weak acid with a dissociation constant of 5.35 in urine at 37°C (Coe 80) with sparingly low solubility of 97 mg/L which is problematic for humans who normally excretes 300-500 mg/day. Three independent physiologic factors cause uric acid precipitation: (a) hyperuricosuria, (b) persistent urine acidity and (c) decreased urinary volume. A number of diseases can cause uric acid nephrolithiasis via a

combination of these three factors (Pak 90, Reise 92, Asplin 96). There is a distinct difference between animal uric acid stones and modern day human stones.

Uric acid stones in animals

In the mink (Tomlinson 1982), this appears to be very similar to the monogenetic uric acid hyperproduction syndrome in humans (Nyhan 1997). Serum uric acid concentration of the affected minks was 4-5 times that of unaffected animals. The urinary pH was between 6-6.2. The renal pelvis was dilated and completely filled with urate calculi.

Uric acid nephrolithiasis from hyperproduction is also well described in uricotelic vertebrates such as birds (Sonmez G). A dietary component can be identified. Uric acid is both an endproduct of amino acid and purine metabolism. When chicken were accidentally fed a high purine diet, endemic uric acid nephrolithiasis can occur due to hyperproduction (Kozhevnikov 1978).

Diet	*Chicken*	*Uric acid (mg/100 ml)*
Normal chow	Healthy	5.8
Fed "high purine"	Few stones per kidney	15.0
	Many stones per kidney (ESRD)	24.1

Interestingly, there is no report in the literature on normouricosuric uric acid nephrolithiasis in animals. *Can this be unique to humans?*

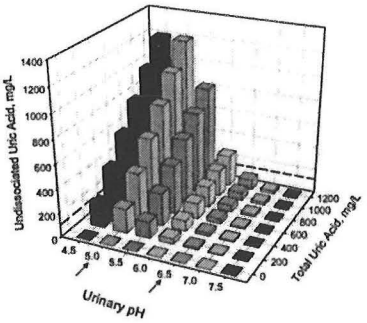
Uric acid stones in humans from pre-World War I United Kingdom.

One cannot help but suspect that the high incidence of renal and bladder uric acid stones from this period were due to mostly dietary causes. This ailment was particularly common in affluent elderly gentlemen. Both bladder and kidney uric acid stones were associated with tophaceous gout. Although still exist, but uric acid nephrolithiasis from sheer gluttony is not as common today.

The culprit: Unduly acid urine

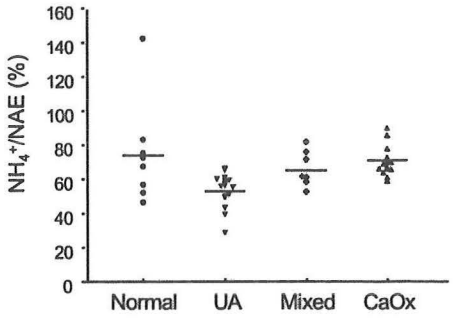
Only a small fraction of human patients form uric acid stones from hyperuricosuria. The commonest finding in uric acid nephrolithiasis is acidic urinary pH titrating uric acid exclusively to its non-ionic insoluble form. The most important factor in determining urinary uric acid solubility is urinary pH.

At a pH of 5.5, half of the uric acid is in its sparingly soluble undissociated form. With a solubility of undissociated uric acid of 97 mg/L, to exceed solubility at urinary pH's of 4.5, 5.5, and 6.5 would require total uric acid (undissociated + urate) concentrations of approximately 110, 200, and 1100 mg/L respectively. With a urinary volume of 1 L/per day, it is unlikely that physiologic variations in total uric acid excretion would increase from 110 to 1100 mg/day. However, variations in urinary pH between 4.6 and 6.6 are not unusual for any given individual. This is illustrated in the figure taken from Maalouf et al. (Maalouf In press).



Acidic urine pH and low urinary NH_4^+ has been described in patients with uric acid nephrolithiasis (Henneman 1962, Metcalf-Gibson 1965, Gutman 1965, Rappoprt 1967, Yu 1967) although the finding is not always universal (Rappoport 1967, Barzel 1964, Plante 1968, Falls 1972) and sometimes dependent on the underlying conditions (Plante 1968, Fallas 1972). The reason for the low urinary pH was unknown until recently.

Sakhaee and coworkers studied steady-state urinary acidification parameters on controlled metabolic diets and demonstrated lower 24-hour urinary pH and lower fraction of urinary net acid excretion as ammonium in uric acid stone formers (Sakhaee 2002). In addition to steady state changes, uric acid stone-formers had a much blunted rise in NH_4^+/NH_3 excretion when compared with controls when challenged with an acid load (Sakhaee 2002).



Thus a "uric acid precipitation disease" can be reduced to an "ammonium excretion disease". This decrease is not severe enough to cause systemic acid-base disturbance because net acid excretion is probably normal and these individuals are in acid-base balanceHowever, the normal net acid balance is achieved at the expense of higher titratable acid and lower urinary citrate. The reason for lower urinary citrate is not clear at present but the price of a low urinary pH can be uric acid precipitation. This begs the question- "why is urinary pH low?"

Evidence linking Uric acid Nephrolithiasis and Insulin Resistance

Epidemiologic

Over 50% of uric acid stone-formers have glucose intolerance or diabetes (Sakhaee 2002)
Over 33% of diabetic stone-formers form uric acid stones (Pak 2003)

Metabolic

Urine pH is inversely correlated to body mass (Maalouf In press)
Urine pH is positively correlated to peripheral insulin sensitivity (Abate In press)
Acute hyperinsulinemia increases urinary pH (Abate In press)

Laboratory

Insulin stimulates proximal tubule ammonium excretion (Chobanian 1987)
Insulin stimulates proximal tubule Na/H exchange (Klisic 2002)

Link of uric acid nephrolithiasis to insulin resistance

Several studies have suggested a link between insulin resistance and low urinary pH in gouty diathesis. This association is supported by epidemiologic, metabolic and physiological studies. Subjects with normouricosuric nephrolithiasis exhibit many features of the metabolic syndrome (Sakhaee 2002, Pak 2001) A retrospective analysis in nephrolithiasis patients found a much higher incidence of UA stones among stone-forming patients with type 2 diabetes mellitus, compared to the non-diabetic stone formers (34% vs 6%) (Pak 2003). In nearly 5,000 nephrolithiasis patients, a strong inverse relationship was found between urinary pH and body weight (Maalouf in press). Conversely, the prevalence of diabetes or impaired glucose tolerance in UA stone-formers is over 50% (Sakhaee 2002). Type 2 diabetic stone formers have unduly low urinary pH (Pak 2003). Taken together, the data suggest that uric acid stones and insulin resistance are both associated with a low urinary pH, although a causal relationship cannot be determined from epidemiology. Abate et al. which showed a strong inverse correlation between insulin resistance and urinary pH (Abate In press). Over a wide range of insulin sensitivity, the lowest urinary pH was associated with the most severe peripheral insulin resistance (Abate in press) UA stone-formers clustered in the region with the lowest urinary pH and insulin sensitivity. Acute infusion of insulin also

Summary: Uric acid nephrolithiasis

❒ There are basically two ways to get uric crystals in the urine. Uric acid concentration can exceed supersaturation limits if

1. Urine pH is very acid
2. there is hyperuricouria.

In the latter, sodium urate can also precipitate.

❒ Uric acid stones are pervasive in the animal kingdom.
There is a difference between animals and humans. Most uric acid stones in animals can be accounted for by hyperuricosuria. Unduly acidic urine appears to be a modern disease.

❒ There is mounting evidence that unduly acid urine in so patients may be linked to renal insulin resistance.

causes increase in urinary ammonium excretion (Abate in press).

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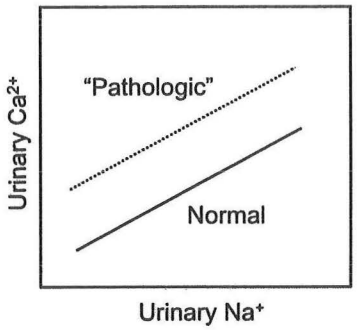
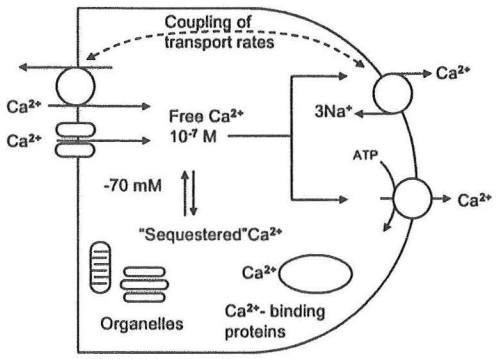
CALCIUM NEPHROLITHIASIS

Calcium stones are by far more common than uric acid stones. The etiology is diverse, not totally understood, and cannot be cover in this forum. We will draw a few lessons from vertebrate evolution and changes in human history.

CALCIUM AND SODIUM TRANSPORT IN EPITHELIA



At the level of the whole organism, there is an inverse relationship between urinary calcium and urinary sodium (Sakhaee 1993). This is observed in all normal individuals and can be viewed as physiologic. However, in patients with hypercalciuric calcium nephrolothiasis, the same relationship (slope) is preserved but the line is shifted upward (Timio 2003) so for any given Na excretion (=Na intake at steady state), there is higher calcium excretion in the urine. First we will consider the physiologic hypercalciuria.



Cellular calcium transport in and out of a cell and across epithelia is part of life on this planet. Transcellular calcium transport is a complex process but is universallypresent in all multicellular organisms (Hoenderop 2002, Friedman 2000). It is designed to take calcium form one mM compartment to another through a compartment (the cell) with 100 nM free calcium concentration. The following mechanisms are in place. 1. In the entry membrane (for the kidney, it is the urinary lumen), there are calcium channels to allow calcium to flow down its concentration gradient. 2. In the cell are mechanisms to protect the cell from a calcium holocaust. These consists of calcium binding proteins as buffers and organelles (ER and mitochondria) to sequester the calcium. Part of the buffers are also diffusible towards the opposite membrane. 3. In the exit membrane (in the kidney it is the plasma side), there are active transport mechanisms such as the Na^+/Ca^{2+} exchanger and/or the plasma membrane calcium pump. 4. There are "cross-talk" mechanisms to ensure equivalence of fluxes on the two membranes. This design is pervasive in all calcium transporting epithelia. In lower vertebrates, the flux rates can be enormous.

	Flux rate (nmol/cm^2/h)	*Function*
Crustacean (crab caraspcae)	3,000	To sustain molting cycles
Avian (Hen Oviduct)	20,000	To calcify the egg shell
Mammalian (Rat distal tubule)	200	To reabsorb filtered calcium

Although all three calcium transport pathways utilize the same design, the capacity differs widely. On the surface this makes sense because mammals do not molt or lay eggs, so the flux rates are relatively low. However, there is a problem. Mammlian nephrons are primarily of a filtration-reabsorption design and the high metabolic rate mandates a high glomerular filtration rate. Although the daily excretion of calcium in a 70 kg human may be 507 mmoles, the fluc rates in the kidney are much larger.

	Flux/day	*Mechanism*
Filtered load	250 mmol	Ultrafiltration
Reabsorption in the proximal nephron	220 mmol	Passive paracellular
Reabsorption at the distal nephron	25 mmol	Passive paracellular
Excreted	5 mmol	Active transcellular

Reabsorption in the proximal nephron is enormous but it is achieved by coupling calcium to sodium transport. It is passive transcellular and energetically and genetically economical. This is an

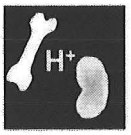
ingenious mechanism of calcium absorption that is characteristic of higher vertebrates. However, there is one caveat with this design. *The high calcium absorption in the proximal nephron relies on high proximal sodium absorption.*

This is only true of the organism is on a low salt diet. Such was true throughout most of evolution of all terrestrial vertebrates. Even in the *Homo* genus, Boyd Eaton has published several seminal monographs (Eaton 1985, 1991, 1997, 2000) describing the considerably lower salt intake during Paleolithic times compared to modern humans.

The end result is that high salt intake mandates suppressed proximal tubule sodium reabsorption and the reabsorption of its accompanying calcium ion, the "hitchhiker". Although the distal nephron can pick up the calcium in a sodium –independent fashion., the huge excess of calcium exiting the proximal nephron overwhelms the capacity of the distal nephron and hypercalciuria ensues.

Besides physiologic hypercalciuria, hypercalciuric patients also have enhanced gut calcium absorption (Pak 1980) but impaired incorporation of calcium into bone has also been postulated (Pietchmann 1992). There is familial clustering of these defects and a locus as well as a candidate gene has been linked to this phenotype (Reed 1999, 2002). In stone formers, urinary calcium is still relative to urinary sodium but the hypercalciuria is frequently above and beyond that expected from high sodium intake alone.

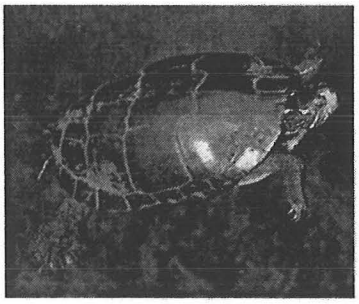
ACID-BASE AND CALCIUM HOMEOSTASIS



Although the kidneys can excrete invading protons with reasonable high capacity and precision to meet daily needs, it does not excrete the protons instantaneously. Before the kidneys can rid the protin, the body deals with it temporarily by buffer. The largest body of buffer in the body is the skeleton. So every time there is an acid load, the bicarbonate in the plasma, the proteins everywhere, and the carbonate in the bone take action to protect plasma pH. This is universally true in all species. In addition to bone effects, an acid load directly affects the kidney.

Lactic acidosis in a diving turtle The table shows plasma values in a painted turtle before a dive and 150 days later in an anoxic aquatic environment that is completely physiologic for this species (Reese 2000). The resultant prolonged anaerobic metabolism generates a colossal amount of lactic acid. The usual analysis by a physician would take the increase in anion gap ($\Delta = 34$) which roughly approximates the fall in bicarbonate ($\Delta = 45$) and assumes these changes represent the footprint of the lactate added which is equivalent to the amount of decomposed bicarbonate. A perusal of the actual measurements clearly demonstrates that this analysis is very far from the truth. Lactate has actually gone up by 181 mM instead of the 34-45 mM predicted from the Δ anion gap. The decrement in plasma bicarbonate is much lower than the addition of lactate because most of the H^+ from lactic acid is buffered by the carbonate/bicarbonate in the shell rather than plasma buffer. In addition, the cations released from the shell into the plasma in exchange for the H^+ greatly elevated the unmeasured cations. For those who share the belief that physicians have much to learn from non-mammalian biology, several excellent monographs are available for further reading about physiologic lactic acidosis and shell buffering in turtles (Reese 2000, Jackson 2000, 2000).
Plasma values of *Chrysemys picta marginate* (diving turtle)

	Before diving	Anoxic water x 150 days	
Concentration in mM			
Sodium	117	99	
Chloride	73	44	
Bicarbonate	39	5	Δ = 34
Anion Gap	5	50	Δ = 45
Unmeasured anions			
Lactate	4	185	Δ = 181
Unmeasured cations			
Potassium	2	10	
Total Magnesium*	2.6	12	
Total Calcium*	3.7	59	



*Not all in ionized form. Some exist in charged or uncharged soluble complexes so a valence of 2 should not be used for conversion from mmole to mEq. Values taken from Reese 2000

Lactating Cow. Cows are bred for their high yielding milk production and not necessarily for their ability to cope with this production rate. The condition *hypocalcemic postparturient paresis*, also called "milk fever" in lactating cows imparts a significant negative impact on the diary industry (Riond 2001). The syndrome affects 3-8% of cows worldwide and typically occurs days after calving when the cows presents with inappetence, extreme paresis, cessation of urination and defecation, lateral recombency, and eventually coma and death. If left untreated, there is approximately 70% mortality. The syndrome occurs as a result of massive calcium output into the colostrums at the time of parturition. For the high yielding animals, this can amount to 2.3 g calcium per L so a cow can lose 10L or 23 g of calcium in the first single milking. The physiologic adaptation is that of a tripartite effort of increased gut absorption, hypocalciuria, and increased bone resorption. In addition to treating the cows with pharmacologic doses of calcium and vitamin D, a very effective treatment is to provide a diet that is high in acid ash content. Cows, in general consume a highly alkali diet which is expected for a herbivore. That is why the normal urinary pH for a cow is about 8. When lactating cows were given a net acid diet, the mobilization of calcium from the bone is greatly enhanced and the incidence of hypocalcemic postparturient paresis is drastically reduced. In a meta-analysis of 75 published trials totaling 1165 cows, a direct correlation can be made between the incidence of the disease against the amount of sulphur (a surrogate marker for acid) in the diet (Oetzel 1991, 1993).

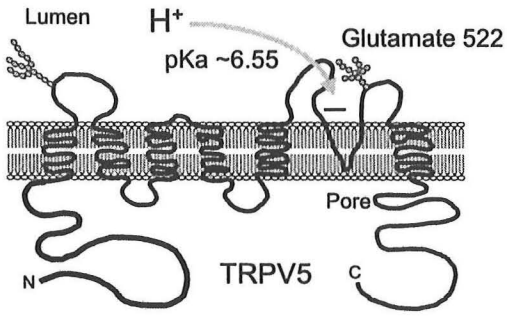
High performance athletic horses. This is another example where animals are selectively bred for excellence in very restricted traits. These high performance horses may have outstanding cardiopulmonary power because these were the traits of interest to the breeders. However, they do not necessarily exhibit skeletal superiority. One may expect that the innate and acquired athletic excellence should beget higher bone mass and quality, this should not be taken for granted. In fact, the repeated high impact on bone from sprinting causes microfractures in these animals.

The above examples shows bone buffering and calcium release in large magnitude. There are numerous studies in rodents and humans (Breslau 1988) showing acid-induced bone loss and hypercalciuria. A recent animal study by Amanzadeh and coworkers showed considerable bone loss and hypercalciuria in rats fed a high protein diet (Amanzadeh 2003).

Renal effects of acid

In addition to bone dissolution, acid load also has a direct effect on the kidney. Two of the these are particularly detrimental to calcium nephrolithiasis; renal calcium leak and hypocitraturia. The direct effect of acid on distal renal calcium absorption was described by Sutton and Dirks over 20 years ago in a seminal study (Sutton 1979). Although the phenomenon was well described, mechanism of

was not elucidated until recently by Yeh and coworkers (Yeh 2003).



The critical apical calcium entry step in the distal convoluted tubule is mediated by the calcium channel TRPV5 (ECaC1). Yeh and coworkers showed that this channel is directly gated by luminal acidic pH with a pK of 6.55 and involves at least in part an externally exposed glutamate residue around the pore. This provides a molecular mechanism by which acid can directly lead to hypercalciuria. There is likely a myriad of mechanisms by which acid load can lead to renal calcium leak.

In addition to renal calcium leak, another important effect of acid on the kidney is hypocitraturia. Citrate is a major base in the urine. The metabolism of a trivalent citrate to CO_2 consumes 3 H^+ thus is equivalent to production of 3 bicarbonates. Citraturia probably evolved to dissociate the acid excretory vs. N excretory role of ammonium without having to raise urinary pH.

Urine		*Nitrogen excretion*	*Acid excretion*	*Urinary pH*
NH_4^+	Cl^-	yes	yes	low
NH_4^+	HCO_3^-	yes	no	high
NH_4^+	citrate$^-$	yes	no	low

The alligator merrily excretes $NH_4^+HCO_3^-$ and not worry about the high urine pH because the high urine volume precludes calcium phosphate precipitation. When urine volume falls, the only way to excrete N without excreting acid is to send out ammonium citrate. However, as s rule rather than an exception, biomolecules in nature often have multiple roles. Citrate is also the most important chelator of calcium in urine. When faced with an acid load, the physiologic response is to conserve citrate in the urine. This is achieved by multiple mechanisms of increase apical citrate uptake, icytoplasmic, and mitochondrial metabolism of citrate in the proximal tubule (Aruga 2000, Melnick 1996, Melnick 1998). One detrimental consequence of this appropriate acid-base response is lack of chelation of urinary calcium. The combination of hypcalciuria and hypocitraturia is extremely undesirable.

Summary: Acid-base and calcium balance

- Obligatory bone calcium release is physiologic every time an acid load is sustained even in the presence of completely normal renal function.
- Independent of the calcium release from the bone, acid load directly leads to renal leak of calcium.
- Hypocitraturia is a physiologic response to acid load which is appropriate for acid-base balance. Because of citrate's dual role in the urine as a base and as a calcium chelator. acid-induced hypocitraturia further compounds the stone forming propensity of hypercalciuria.

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