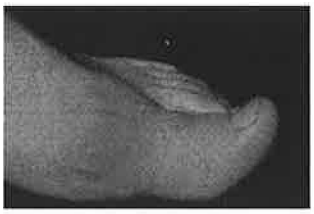


Nephrolithiasis and Arthritis: Are There Other Reasons To Have Uric Acid?

尿酸

Grand Rounds
Department of Internal Medicine
April 2006

13

Orson W. Moe, M.D.
Department of Internal Medicine
University of Texas Southwestern Medical Center

This is to acknowledge that Orson Moe has not disclosed any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Moe will not be discussing off-label uses in his presentation.

Professor of Internal Medicine
Division of Nephrology
and
Charles and Jane Pak Center of Mineral Metabolism and Clinical Research

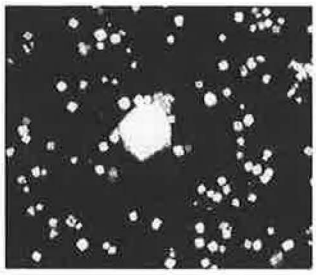
Research: Renal physiology and metabolism.

The speaker wishes to acknowledge and thank the assistance of Ms. Vicki Perkins for her help in the preparation of this Medical Grand Round.

INTRODUCTION

Clinicians know of uric acid as the culprit behind gouty arthritis and uric acid stones. Less commonly, one encounters acute uric acid nephropathy usually after tumor lysis syndrome and more recently, uric acid as an independent cardiovascular risk factor was resurrected. Thus far, we have not heard anything good about this compound. It seems to bring nothing but trouble. I will submit the question- "Why do we need this?" How come evolution has not eliminated this awful compound?

In 1965, Dr. Alex Guttman hypothesized on an intrinsic defect in ammoniagenesis leads to spill-over of nitrogen to the uric acid synthetic pathway in an attempt to unify the findings of low urinary ammonium and high uric production. In the audience were many illustrious experts in physiology and metabolism. Dr. Robert Berliner commented "Teleological argument is always dangerous and distasteful. There is not much sense in making uric acid in the first place, and if it is made, there is no sense in holding onto it, as man appears to do so." This is an irony as stated eloquently by Dr. Berliner. We will try to analyze this paradox. In particular, we will focus on the statement "*there is not much sense in making uric acid in the first place*."



Outline for Grand Rounds

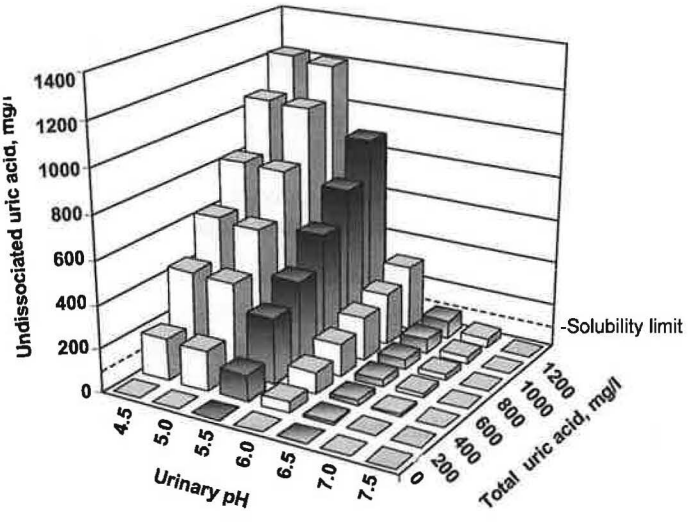
Uric acid nephrolithiasis
Nitrogen metabolism
Evolution of uricotylism
As an antioxidant
Effects on central nervous system
Immune signaling molecule
Cardiovascular effects

Reference

Gutman AB. Significance of uric acid as nitrogenous waste in vertebrate evolution. Arthritis and Rheumatism 1965; 8: 614-626.

URIC ACID NEPHROLITHIASIS

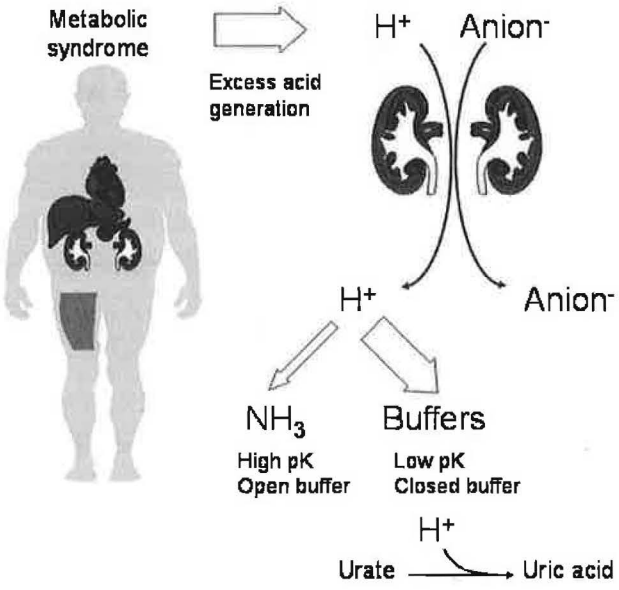
Formation and growth of stones in the urinary space is influenced by both urinary biochemical and epithelial factors. Pathogenesis of uric acid stones is attributed principally to disturbance of urinary chemical composition due to paucity of evidence for predisposition by epithelial factors. The singular most important urinary factor is high activity of free H^+ which titrates urate to the highly insoluble uric acid. Unduly acidic urine is by far much more important than hyperuricosuria in causing uric acid precipitation. A quantitative analysis reveals that with a urinary pH of 6.5, it takes a massive



Amount of total uric acid allowed in urine based on pH. Taken from Maalouf et al 2004

amount of total uric acid to exceed its saturation, while a fall of urinary pH to 5.5 or below will readily crystallize what is considered a physiologic concentration of uric acid in urine. Stated simply, uric acid nephrolithiasis is a disease of urinary pH. A significant amount of effort has been invested in all fronts and some modest progress has been achieved recently in understanding the pathogenesis of excessively low urine pH in uric acid stone formers.

Highly acidic urine maybe genetically and energetically costly to maintain (direct coupling to H^+-pumping to hydrolysis of high energy bonds and tight epithelia), but acidic urine *per se* does not necessarily confer negative effects on the urine or uroepithelium. The "problem" emerges when one harbors a substance such as urate (pK= 5.6) into the urine where its protonated form (uric acid) comes out of solution. This low solubility of uric acid is exploited in many avian and some reptilian species to conserve water and hence represents physiologic uric acid crystalluria. Humans do not belong under this canopy. When uric acid precipitates in human urine, it is considered a "disease". The current model is far from complete. In brief, the collective data suggests a multisystemic defect of excessive generation of acid equivalents coupled with a mild impairment in provision of high pK open urinary buffer, namely ammonia. The employment of low pK closed buffers (titratable acidity) to excrete this acid load mandates low urinary pH and uric acid precipitates as an innocent (or not so innocent) bystander. There are other possible contributing factors such as promoters and inhibitors of stone formation, as well as epithelial factors that are being studied. Urinary pH has received the due attention it deserves because of its critical role in the human syndrome of uric acid nephrolithiasis and the physician's ability to effectively manipulate this risk factor.



Working model: Pathogenesis of uric acid nephrolithiasis

References for Uric Acid Nephrolithiasis

Abate N, Chandalia M, Cabo-Chan AV, Moe OW, Sakhaee K. The metabolic syndrome and uric acid nephrolithiasis: Novel features of renal manifestation of insulin resistance. Kidney Intern 2004; 65: 386-395.

Cameron MA, Maalouf N, Adams-Huet B, Moe OW, and Sakhaee K. Risk factors for uric acid nephrolithiasis in Type 2 diabetes mellitus. Clin J Am Soc Nephrology In Press

Curhan GC, Willett WC, Rimm EB, Speizer FE, Stampfer MJ. Body size and risk of kidney stones. J Am Soc Nephrol 1998; 9: 1645-1652.

Daudon M, Lacour B, Jungers P. Influence of body size on urinary stone composition in men and women. Urol Res 2006; 11: 1-7.

Daudon M, Lacour B, Jungers P. High prevalence of uric acid calculi in diabetic stone formers. Nephrol Dial Transplant 2005; 20: 468-469.

Maalouf N, Gaska MA, Abate N, Sakhaee K, Moe OW. Novel insights in the pathogenesis of uric acid nephrolithiasis. Curr Opin Nephrol Hypertens 2004; 13: 181-189.

Maalouf N, Sakhaee K, Parks JH, Coe FL, Adams-Huet B, Pak CY. Association of urinary pH with body weight in nephrolithiasis. Kidney Intern 2004; 65: 1422-1425.

Moe OW, Abate N, Sakhaee K. Normouricosuric uric acid urolithiasis: A systemic disease with defective renal acidification. Endo Clin N America 2002; 31: 895-914.

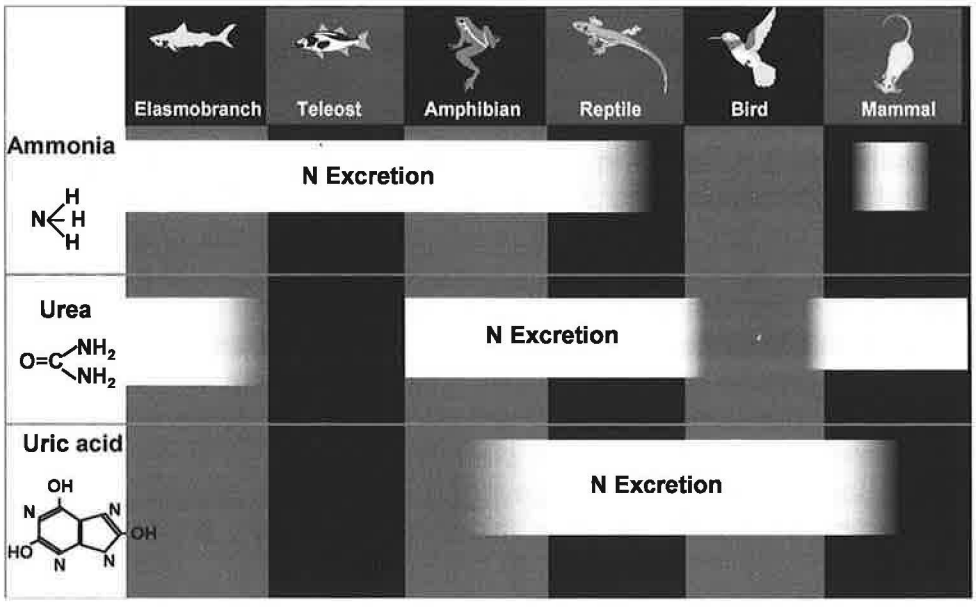
Pak CY, Sakhaee K, Moe OW, Preminger GM, Poindexter JR, Peterson RD, Pietrow P, Ekeruo W. Biochemical profile of stone forming patients with diabetes mellitus. Urology 2003; 61: 523-527.

Pak CY, Moe OW, Sakhaee K, Peterson RD, Poindexter JR. Physicochemical metabolic characteristics for calcium oxalate stone formation in patients with gouty diathesis. J Urol 2005; 173: 1606-1609.

Sakhaee K, Adams-Huet B, Moe OW, Pak CYC. Pathophysiologic basis for normouricosuric acid nephrolithiasis. Kidney Intern 2002; 62: 971-979.

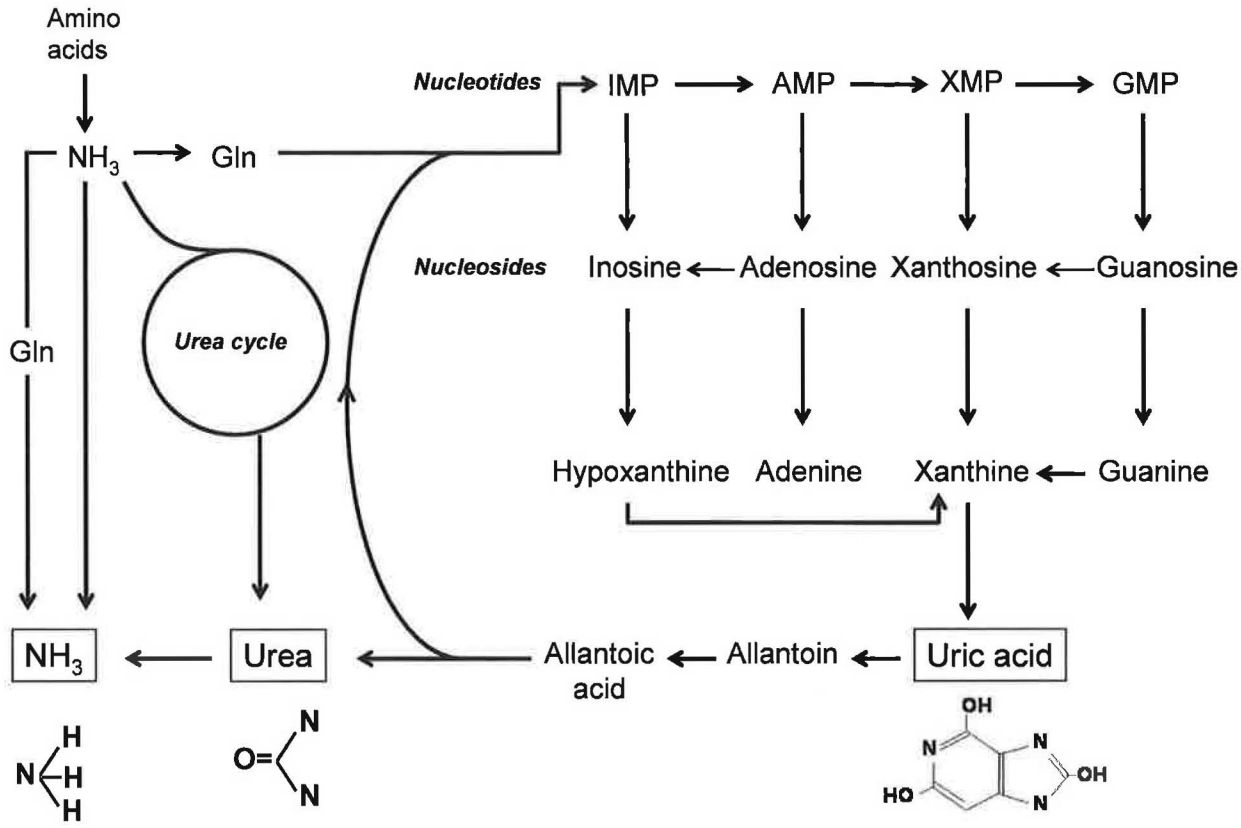
NITROGEN METABOLISM

Uric acid is a carrier of excess unassimilated nitrogen atoms fed in from the dual source of amino acids and purines. Amino acids cannot be stored and has to be used for building proteins or metabolized. Simple denitrifying microbes can simply blow the molecular dinitrogen into the atmosphere which will be the most convenient and cleanest way of disposing nitrogen. This process does not exist in higher eukaryotes. While the carbon skeleton of amino acids can be salvaged (e.g. gluconeogenesis), the nitrogen has to be excreted. The use of ammonia, urea, or uric acid as the predominant species of nitrogen excretion is termed ammonotelism, ureotelism, or uricotelism respectively.



Ammonotelism, ureotelism, and uricotelism in vertebrates

This figure summarizes how a broad range of animal species uses ammonia, urea and uric acid to excrete nitrogen from amino acids and purines. Note that this refers to the predominant but not necessarily the exclusive compound being used. There are also numerous exceptions to this rule.



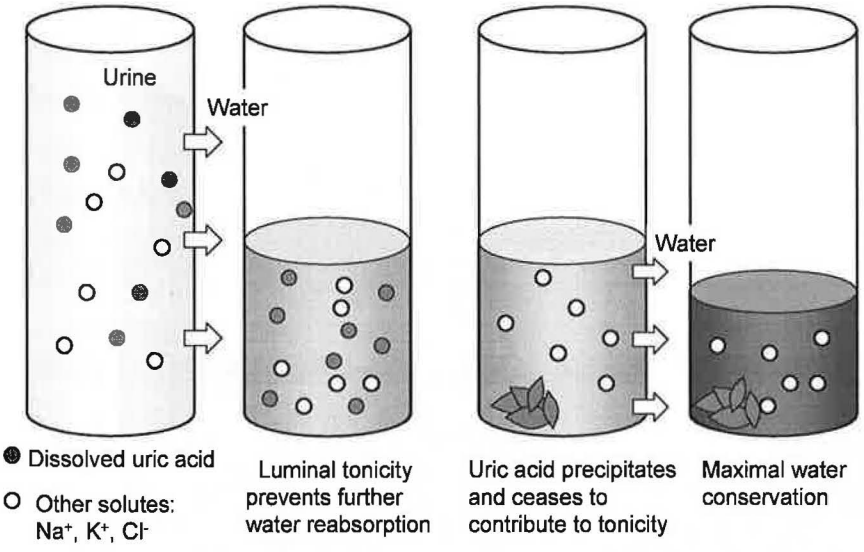
Inter-relationship of the three pathways of nitrogen metabolism.

A unifying scheme connecting the three pathways is presented which is modified from Balinsky. The three metabolic pathways are drawn in an interacting fashion where flow from one to the other is depicted and emphasized. The ability for precursors to flow from one pathway to another is compatible with the fact that most animals possess the ability to utilize ammonium, urea, and uric acid to some degree for nitrogen excretion. The three end products are shown in the bottom in boxed text. This summary does not necessarily imply presence and equivalent utilization of all pathways in the same organism. The far left depicts ammonotelism. Ammonia from amino acid breakdown can be excreted directly such as in elasmobranchs and teleosts, or it can be converted for to glutamine (Gln) for detoxification, and delivered to the kidney to be converted by to ammonium. The middle pathway depicts ureotelism. The ammonia generated from deamination of amino acids is converted to carbamyl phosphate and converted to citrulline and converted eventually to urea (biochemical details omitted) and excreted. The presence of urease can convert urea back to ammonia. The right panel show purine metabolism. However, the ammonia released from amino acid breakdown can be channeled to purine via glutamine and salvage of carbon skeletons from allantoic acid breakdown. The rings of uric acid can be traced back (N: glutamine, aspartate, glycine; C: serine, glycine, folate, bicarbonate).

The observation that protein loading simultaneously increases urinary ammonium, urea, and uric acid nitrogen in humans is probably not a good illustrative example of humans utilizing all

three pathways for nitrogen excretion because protein delivers acid and purine in addition to nitrogen load. The switch from ureotelism to and from uricotelism based on the aridity of the ambient habitat is well shown in numerous species. For example, the tree frog *Chiromantis xerampelina* alters its enzymes of urea and uric acid synthesis to operate on either ureotelism or uricotelism depending on whether the environment is damp or dry respectively. Whether H. sapiens can toggle from one form of nitrogen excretion to another at the beckoned call of physiology is not known but is rather unlikely. Regardless of whether we possess this metabolic plasticity, there is no doubt that humans are not strictly obligatory ureotelics.

Before one embarks on the discussion and speculation on the panoply of biologic functions of uric acid, it appears that one prime purpose of using uric acid to excrete nitrogen is to allow the organism to excrete nitrogen in a solid state. Uric acid is a metabolic cul-de-sac in uricotelic organisms as its sole fate is excretion. As previously stated, the very purpose of having uric acid in urine is so that it will precipitate.



Uric acid precipitation allows maximal water conservation

In the kingdom *Animalia*, uricotelism for the purpose of water conservation is prevalent in the phyla *Arthropoda* and *Chordata*. Within *Chordata*, it is observed in many species in the class of *Reptilia*, *Aves*, and *Amphibia*. Crystalluria and frank uric acid concretions do not pose a problem due to multiple reasons including the specialized anatomy of the urinary tract where urine is excreted directly into the cloaca and discharged with feces, deposition onto exoskeletons, and others.

References for Nitrogen Metabolism

Balinsky JB, Chemaly SM, Currin AE, Lee AR, Thompson RL, Van Der Westhuizen DR. A comparative study of enzymes of urea and uric acid metabolism in different species of amphibia, and the adaptation to the environment of the tree frog *chiromantis xerampelina*. Comp Biochem Physiol B 1976; 54: 549-555.

Balinsky JB. Phylogenetic aspects of purine metabolism. S Afr Med J 1972; 46: 993-997.

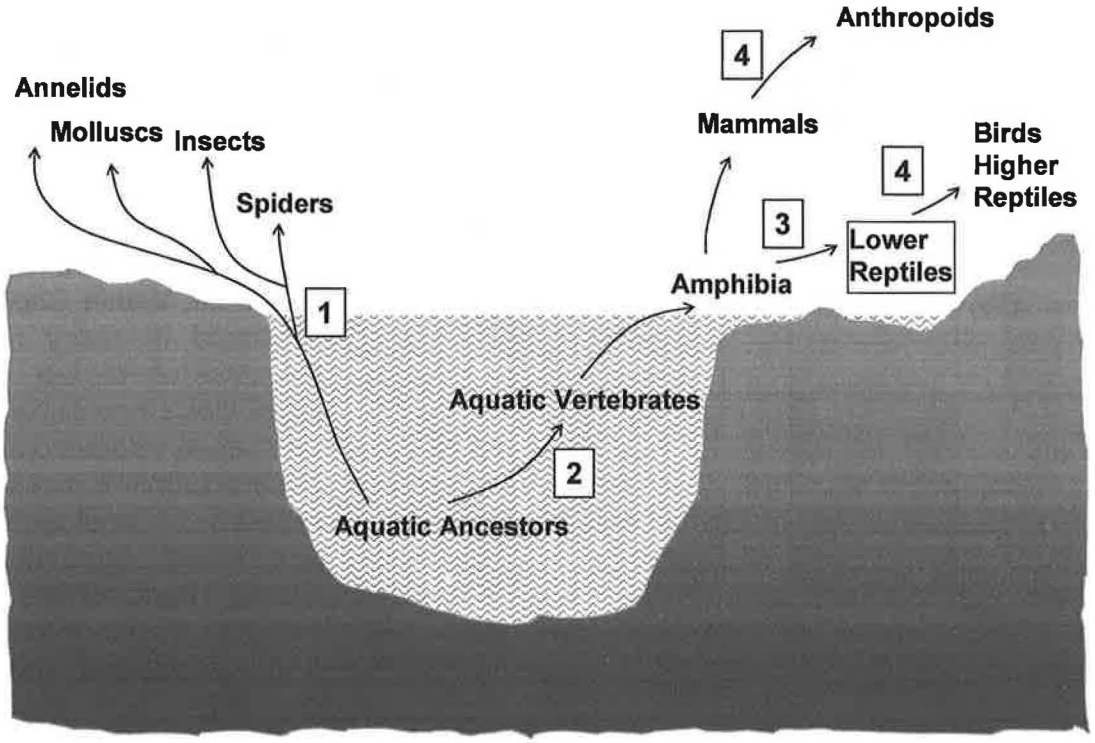
Bodansky M. Introduction to Physiologic Chemistry. 4th. New York, NY: John Wiley and Sons; 1938. pp. Campbell JW. Comparative Biochemistry of Nitrogen Metabolism. New York: Academic Press; 1970. 454-459.

Campbell JW. Comparative Biochemistry of Nitrogen Metabolism. New York: Academic Press; 1970.

Shoemaker VH, Baldin D, Ruibal R. Uricotelism and low evaporative water loss in a South American frog. Science 1972; 175: 1018-1020.

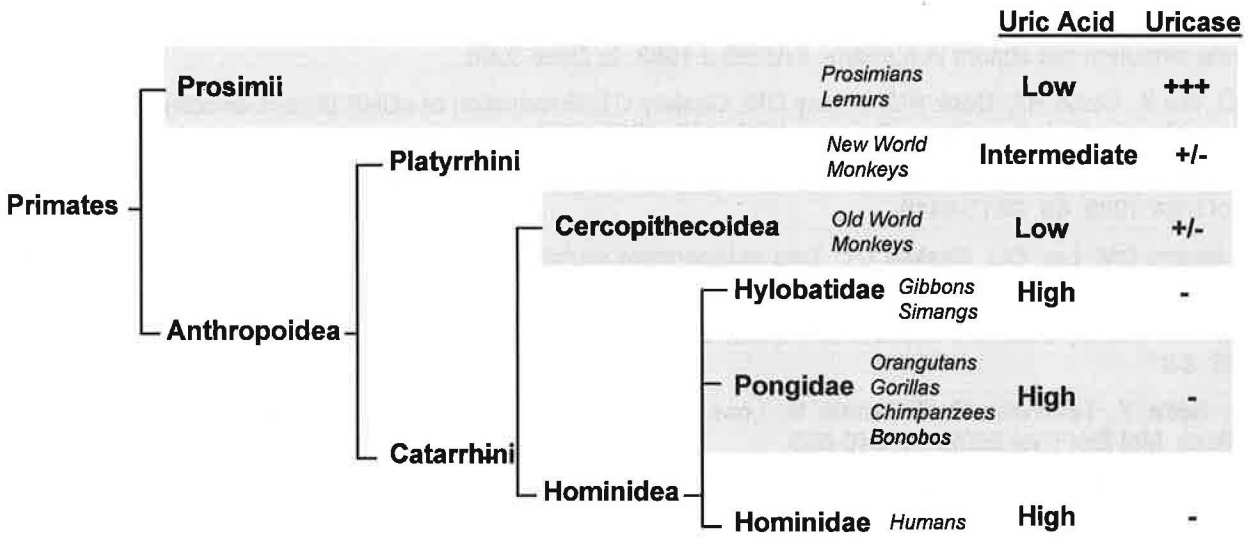
EVOLUTION OF URICOTELISM

The switching on or off of uricotelism is achieved in short term biochemically by altering enzymes that control the traffic of nitrogen between the ammonia, urea, and uric acid pathways. This is particularly true for enzymes of uric acid production and degradation. Over a short period of time such as the migration of a given organism between wet and dry land either as seasonal or developmental (e.g. larvae to adult) transition, changes in metabolism can be achieved by regulating the activity of existing enzymes. On an evolutionary time scale, genes coding for these enzymes are activated or inactivated. The figure summarizes some of these genetic changes in a broad sense. Gene inactivation is a prevalent theme in uricotelism.



Uricotelism in the phyla *Arthropoda, Mollusca, Annelida, Chordata* (Sub-Phylum *vertebrata*) is achieved by inactivation of genes coding for enzymes of uric acid metabolism. Loss of: 1. All enzymes of uric acid catabolism. 2. Urease. 3. Allantoinase and allantoicase. 4. Uricase.

Most species in the class Mammalia are ureotelics. One highly intriguing phenomenon has been what appears to be the "re-emergence" of uricotelism in higher primates. The loss of uricase was achieved by silencing of the uricase gene via accumulation of promoter base changes as well as several splice, missense, and frame-shift mutations. Since other authors have recently expounded on the silencing of uricase, this manuscript will merely provide a précis of the phenomenon and offer an analysis of the potential gain. While uric acid levels are still relatively low in the Old World Monkeys, uricase deficiency is no doubt evident. In higher primates with full loss of uricase expression, part of the compensation to prevent a uric acid flood is reduced expression of xanthine oxidase (uric acid synthase).



Loss of uricase and elevation of plasma uric acid levels in higher primates.

Over some 30 million years, multiple mutations on the uricase gene clearly cumulated in complete lack of its expression. While accumulation of base changes is slow, once polymorphism is established, changes in allelic frequency can proceed at a fast pace in response to environmental selection pressure. The best-known group of genes is the ones evoked by the "malaria hypothesis". 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 the 1940's. 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.

What can be the basis for the positive selection of uricase inactivation in primate evolution? Clearly, primates are thriving well as ureotylics. The uric acid synthetic pathway is probably necessary for purine breakdown but what is the advantage in the truncation at the uricase step and not allowing the reaction to proceed to equally inert but soluble allantoin and allantoic acid? Logic would oblige one to conclude that uric acid serves functions other than merely an insoluble form of nitrogenous waste that can be excreted without an aqueous carrier.

References for Evolution of Uricotelism

Balinsky JB. Phylogenetic aspects of purine metabolism. S Afr Med J 1972; 46: 993-997.

Bodansky M. Introduction to Physiologic Chemistry. 4th. New York, NY: John Wiley and Sons; 1938. pp. 454-

Balinsky JB, Chemaly SM, Currin AE, Lee AR, Thompson RL, Van Der Westhuizen DR. A comparative study of enzymes of urea and uric acid metabolism in different species of amphibia, and the adaptation to the environment of the tree frog chiromantis xerampelina peters. Comp Biochem Physiol B 1976; 54: 549-555.

Shoemaker VH, Baldin D, Ruibal R. Uricotelism and low evaporative water loss in a South American frog. Science 1972; 175: 1018-1020.

Christen P, Peacock WC, Christen AE, Wacker WE. Urate oxidase in primates. Folia Primatol (Basel) 1970; 13: 35-9.

Varela-Echavarria A, Montes de Oca-Luna R, Barrera-Saldana HA. Uricase protein sequences: conserved during vertebrate evolution but absent in humans. FASEB J 1988; 2: 3092-3096.

Lee CC, Wu X, Gibbs RA, Cook RG, Muzny DM, Caskey CT. Generation of cDNA probes directed by amino acid sequence: cloning of urate oxidase. Science 1988; 239: 1288-1291.

Wu X, Lee CC, Muzny DM, Caskey CT. Urate oxidase: primary structure and evolutionary implications. Proc Natl Acad Sci USA 1989; 86: 9412-9416.

Wu X, Muzny DM, Lee CC, Caskey CT. Two independent mutational events in the loss of urate oxidase during hominoid evolution. J Mol Evol 1992; 34: 78-84.

Johnson RJ, Titte S, Cade JR, Rideout BA, Oliver WJ. Uric acid, evolution and primitive cultures. Semin Nephrol 2005; 25: 3-8.

Oda M, Satta Y, Takenaka O, Takahata N. Loss of urate oxidase activity in hominoids and its evolutionary implications. Mol Biol Evol 2002; 19: 640-653.

Christen P, Peacock WC, Christen AE, Wacker WE. Urate oxidase in primate phylogenesis. Eur J Biochem 1970; 12: 3-5.

Muxfeldt M, Schaper W. The activity of xanthine oxidase in heart of pigs, guinea pigs, rabbits, rats, and humans. Basic Res Cardiol 1987; 82: 486-92.

Harrison R. Human xanthine oxidoreductase: in search of a function. Biochem Soc Trans 1997; 25: 786-91.

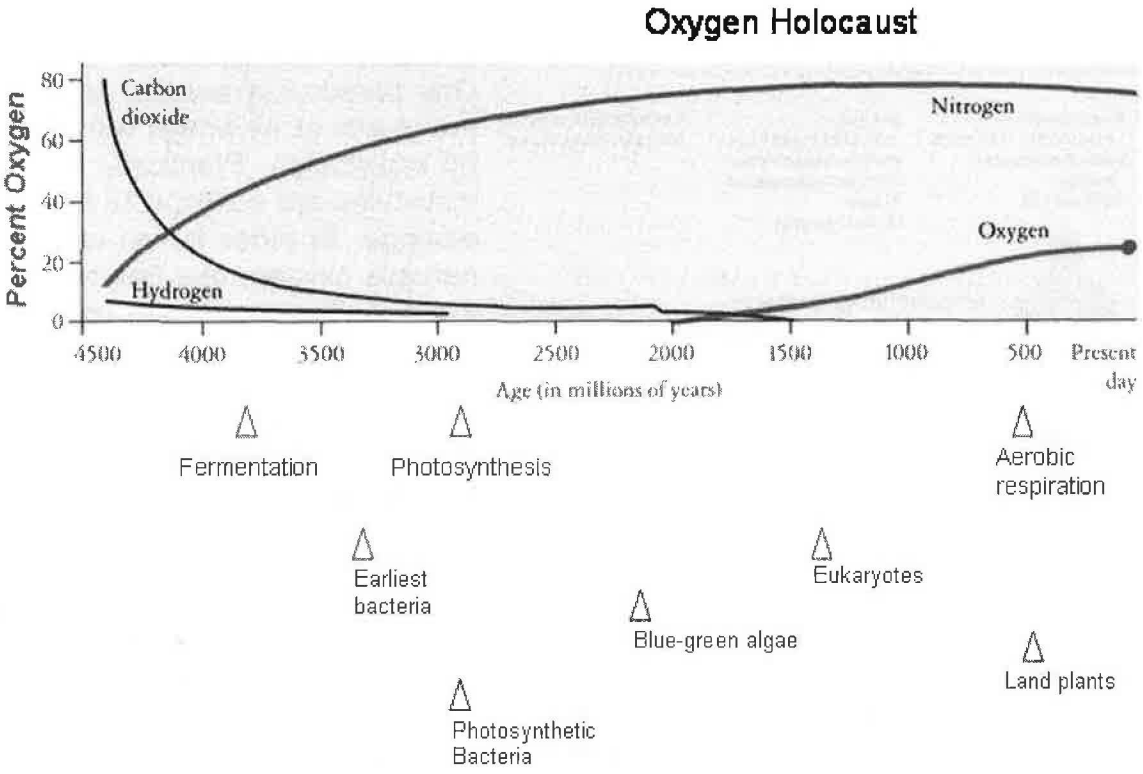
Carter R, Mendis KN. Evolutionary and historical aspects of burden of malaria. Clin Microbio Rev 2002; 15: 564-594.

Haldane JBS. The rate of mutation of human genes. Hereditas 1948; 35: 267-273.

URIC ACID AS AN ANTIOXIDANT

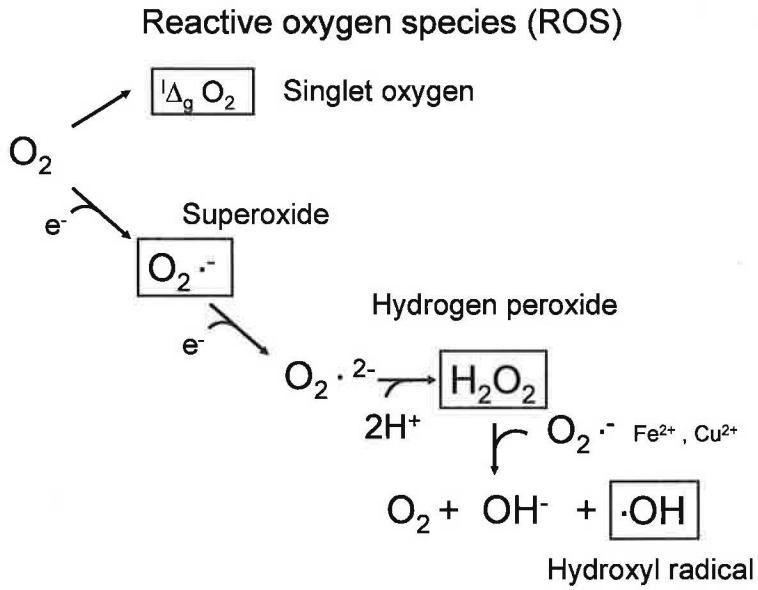
The Earth started 4.6×10^9 years ago as a waterless mass of rock with a primordial primary atmosphere of hydrogen and helium which was stripped by the solar wind. The existing atmosphere is entirely secondary formed by importation, outgassing, and then lastly by biological processes. The early secondary atmosphere consisted of water vapour, carbon dioxide, carbon monoxide, nitrogen, hydrogen chloride and hydrogen. Oxygen freed from water by photodissociation was sequestered surface compounds, and converted to ozone (O_3). Free O_2 did not exist in any substantial quantity.

Life on Earth began around 3.5×10^9 years ago. Procaryotic fermenters fixed carbon in the form of organic compounds and the hydrolytic cyanobacteria (the blue-greens) used water as a source of the electron donor and released O_2 as a waste product. Life started to have a major impact on the environment once photosynthetic organisms evolved. These organisms fed off atmospheric carbon dioxide and converted much of it into marine sediments consisting of the innumerable shells and decomposed remnants of sea creatures. While photosynthetic life reduced the carbon dioxide content of the atmosphere, it also started to produce oxygen. The initial generation of oxygen released was squenched by earth's massive oxygen sinks, forming deposits of banded iron (iron oxides). By 2.0×10^9 years ago, oxygen sinks were gradually approaching saturation but the biologic O_2 production showed no signs of deceleration and atmospheric O_2 levels began to rise. Oxygen levels approached the present atmospheric level around 1.6×10^9 years ago.

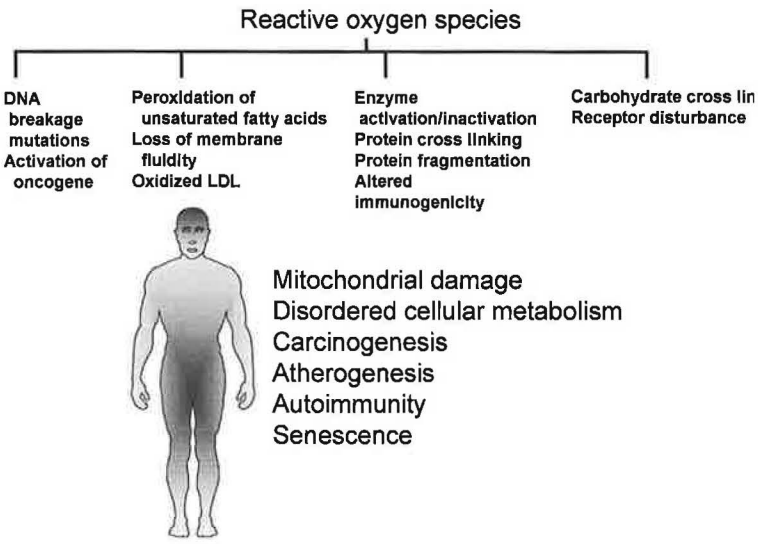


Evolution of the earth's atmosphere and relationship to major biologic events

While biologic life forms drastically changed the earth's atmosphere, evolving atmosphere impacted equally dramatic changes on life. With this new found superior electron acceptor also is extremely toxic. Infusion of toxin of this magnitude is expected to cause drastic changes in life forms. Those that cannot cope will be extinct. Oxygen practically extinguished the majority of life forms. This was the first environmental disaster on the planet and has been termed "Oxygen holocaust". Those that can tolerate will survive and those that can take advantage of the new atmosphere will flourish. The ability to utilize oxygen is not enough to survive. Contemporaneously, the organisms must evolve a way to deal with toxicity.



Harmful by products of oxygen: ROS are boxed



Damage by reactive oxygen species

The notion of uric acid being an antioxidant is well known for decades. One paradox of aerobic being is that the vitality of air brings with it lethality by intoxication. Practically all biologic molecules are susceptible to oxidative damage. In order for an organism to harness oxygen, one has to deal with the oxidative damage to protein, lipid, carbohydrate and nucleic acids. The success of aerobic life was never possible without antioxidant defense in terms of containment of generation of reactive oxygen species as well as countermeasures against the damaging effects of these highly toxic compounds. An excellent account on this general topic has been presented by Benzie. Of the different modes of antioxidation, uric acid along with ascorbic acid, α-tocopherol, and glutathione, function as expendable scavengers of the highly toxic reactive oxygen species. The *in vitro* scavenger action by uric acid of singlet oxygen, hydroxyl free radical, and to some degree peroxide is corroborated by protection from peroxidation of cell membranes and DNA damage by uric acid.

Table 1 Types of antioxidant action

	Action	Example
Prevention	Protein binding/ inactivation of metal ions	transferrin, ferritin, caeruloplasmin, albumin
Enzymatic diversion/ neutralisation	Specific channelling of ROS[1] into harmless products	superoxidase dismutase, catalase glutathione peroxidase
Scavenging	Sacrificial interaction with ROS by expendable (replaceable or recyclable) substrates	ascorbic acid, alpha tocopherol, uric acid, bilirubin, glutathione
Quenching	Absorption of electrons and/or energy	alpha tocopherol, beta carotene

Taken from Benzie

	Exp. 1	
Additions	*A*	%
t-Butylhydroperoxide at 1 mM	0.262	100
+ Urate at 30 μM	0.115	44
50 μM		
60 μM	0.060	23
100 μM		
120 μM	0.024	9
+ Ascorbate at 30 μM	0.092	35
50 μM		
60 μM	0.044	17
100 μM		
120 μM	0.018	7
+ Glutathione at 30 μM	0.182	69
120 μM	0.122	47

Taken from Ames et al. Uric acid and ascorbate both protect RBC ghost from lipid peroxidation

Of interest is the fact that L-gulono-lactone oxidase (GLO) which catalyzes biosynthesis of ascorbate is present in prosimians but is inactivated by mutations in higher primates. The lack of endogenous production of this most critical antioxidant was compensated by adequate dietary intake of ascorbate in the early hominid diet, a reproducibly observed inverse relationship between GLO and superoxide dismutase, and finally the loss of uricase giving rise to high plasma uric acid levels. Cutler studied 22 species of primates and made the empirical

observation that serum and brain urate levels correlates positively with maximum lifespan with H. sapiens basking in the pinnacle with the longest maximal lifespan and highest serum levels of 200-450 μM. Ironically, no intra-species correlations have yet been described between uric acid levels and longevity. While the antioxidant function of uric acid seems solid, there is no proof that this confers sufficient survival and/or reproductive advantage to extinguish the expression of uricase.

References for Antioxidation

Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis. Proc Natl Acad Sci USA 1981; 78: 6858-6862.

Benzie IF. Evolution of antioxidant defense mechanisms. Eur J Nutr 2000; 39: 53-61.

Cutler RG. Urate and ascorbate: their possible roles as antioxidants in determining longevity of mammalian species. Arch Gerontol Geriatr 1984; 3: 321-348.

Cutler RG. Antioxidants and aging. Am J Clin Nutr. 1991; 53(1 Suppl):373S-379S.

Cutler RG. Antioxidants and longevity of mammalian species. Basic Life Sci. 1985;35: 15-73..

Lopez-Torres M, Perez-Campo R, Rojas C, Cadenas S, Barja G. Maximum life span in vertebrates: relationship with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true alondialdehyde, in vivo H202, and basal and maximum aerobic capacity. Mech Ageing Dev 1993; 70: 177-199.

Nandi A, Mukhopadhyay CK, Ghosh MK, Chattopadhyay DJ, Chatterjee IB. Evolutionary significance of vitamin C biosythesis in terrestrial vertebrates. Free Radical Biology & Medicine 1997; 22: 1047-1054.

Pauling L. Evolution and the need for ascorbic acid. Proc Natl Acad Sci USA 1970; 67: 1643-1648.

CENTRAL NERVOUS SYSTEM EFFECTS

Orowan, who was an engineer from MIT first came up with the provocative hypothesis in an article in *Nature* in 1955 that uric acid enhances intelligence. This was based on the pattern of evolution in higher primates and the fact that uric acid shares some biochemical properties with caffeine and theobromine as cerebral stimulants. The associative and conjectural nature of this argument was countered almost immediately by Haldane. Asaka and coworkers used modeling to analyze selected families with twin children and found a significant correlation between plasma uric acid level and intelligence quotient suggests a contribution of partly common gene loci to the two quantitative traits. This remains an associative finding that was visible only after extensive mathematical manipulation. The "intelligence theory" remains unproven. However, the neuroprotective effect is likely related to the antioxidant properties of uric acid.

From Church and Ward.

Dopamine oxidation rate constant in substantia nigra in human brain homogenate

Subjects	Added	k (x 10^2 min^{-1})
Normal	None	2.82 ± 0.87
	Uricase	8.82 ± 7.10
Parkinson	None	4.57 ± 0.78
	Uric acid	0.323 ± 0.123

Church and coworkers examined postmortem tissue samples from human Parkinsonian patients and showed lower uric acid and dopamine levels compared to age-matched controls in the substantia nigra while ascorbic acid levels were not significantly different from the controls in either brain region. Tissue homogenate from Parkinsonian patients oxidized dopamine faster and addition of uric acid decreased the rate of dopamine oxidation, while addition of uricase increased the rate of dopamine oxidation. The authors hypothesized that uric acid may be a critical chemical to preserve dopamine concentrations. This neuroprotective effect could well

be related to the antioxidant action of uric acid as described above.

Nitric oxide can play a dual role in physiology. By interacting with the iron-containing prosthetic group of guanylate cyclase, it has a regulatory function as endothelial-derived relaxing factor. It can also be converted to other nitrogen oxides and thus become a toxic or inflammatory agent. Superoxide converts nitric oxide to the powerful oxidant peroxynitrite (or peroxynitrous acid):

$$NO\bullet + O_2\bullet^- \rightarrow ONOO^-$$

Interesting is the ability of uric acid to scavenge not just reactive oxygen species but peroxynitrite (generated by the reaction of nitric oxide and superoxide), an action that is not accomplished by ascorbate. This biochemical property of uric acid was demonstrated by its superior ability to protect animals against experimental allergic encephalitis compared to ascorbate. This notion is compatible in the epidemiologic finding in humans where there is an inverse correlation between the occurrence of multiple sclerosis and serum uric acid levels.

References for Central Nervous System Effects

Orowan E. The origin of man. Science 1955; 175: 683-684.

Haldane JBS. Origin of man. Nature 1955; 176: 169-170.

Inouye E, Park KS, Asaka A. Blood uric acid level and IQ: a study of twin families. Acta Genet Med Gemellol 1984; 33: 237-242.

Park KS, Inouye E, Asaka A. Plasma and urine uric acid levels: heritability estimates and correlation with IQ. Jpn J Human Genet 1980; 25: 193-202.

Church WH, Ward VL. Uric acid is reduced in the substantia nigra in Parkinson's disease: effect on dopamine oxidation. Brain Res Bull 1994; 33: 419-125.

Church WH, Fong YT. Changes in uric acid during acute infusion of MPP+, 6-OHDA, and $FeCl_3$. Molec Chem Neuropath 1996; 27: 131-144.

Church WH, Rappolt G. Nigrostriatal catecholamine metabolism in guinea pigs is altered by purine enzyme inhibition. Exp Brain Res 1999; 127: 147-150.

Hooper DC, Spitsin S, Kean RB, Champion JM, Dickson GM, Chaudhry I. Uric acid, a natural scavenger of peroxynitrite, in experimental allergic encephalomyelitis and multiple sclerosis. Proc Natl Acad Sci USA 1998; 95: 675-680.

Kean RB, Spitsin SV, Mikheeva T, Scott GS, Hooper DC. The peroxynitrite scavenger uric acid prevents inflammatory cell invasion into the central nervous system in experimental allergic encephalomyelitis through maintenance of blood-central nervous system barrier integrity. J Immunol 2000; 165: 6511-6518.

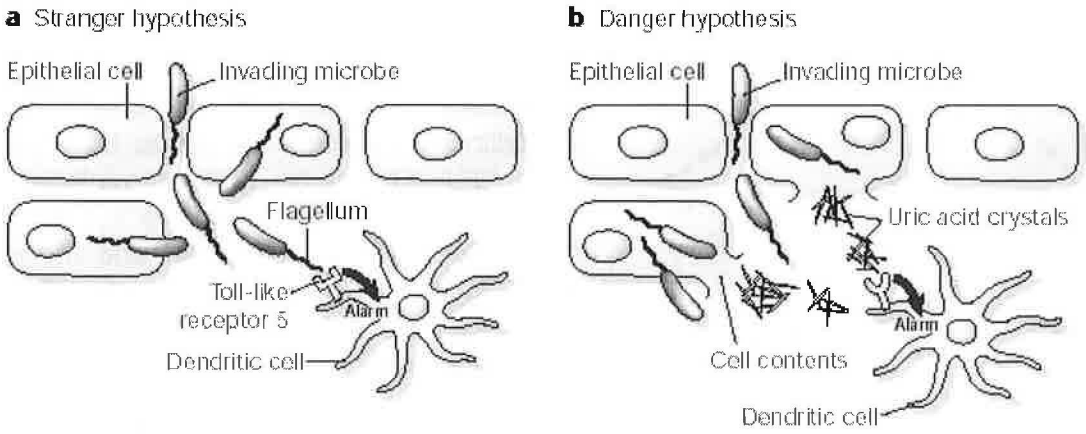
Spitsin S, Scott GS, Mikheeva T, Zborek A, Kean RB, Brimer CM, Koprowski H, Hooper DC. Comparison of uric acid and ascorbic acid in protection against EAE. Free Radical Biology & Medicine 2002; 33: 1363-1371.

Spitsin S, Hooper DC, Mikheeva T, Koprowski H. Uric acid levels in patients with multiple sclerosis: analysis in mono- and dizygotic twins. Multiple Sclerosis 2001; 7: 165-166.

Drulovic J, Dujmovic I, Stojavljevic N, Mesaros S, Andjelkovic S, Miljkovic D, Peric V, Dragutinovic G, Marinkovic J, Levic Z, Stojkovic MM. Uric acid levels in sera from patients with multiple sclerosis. J Neurol 2001; 248: 121-126.

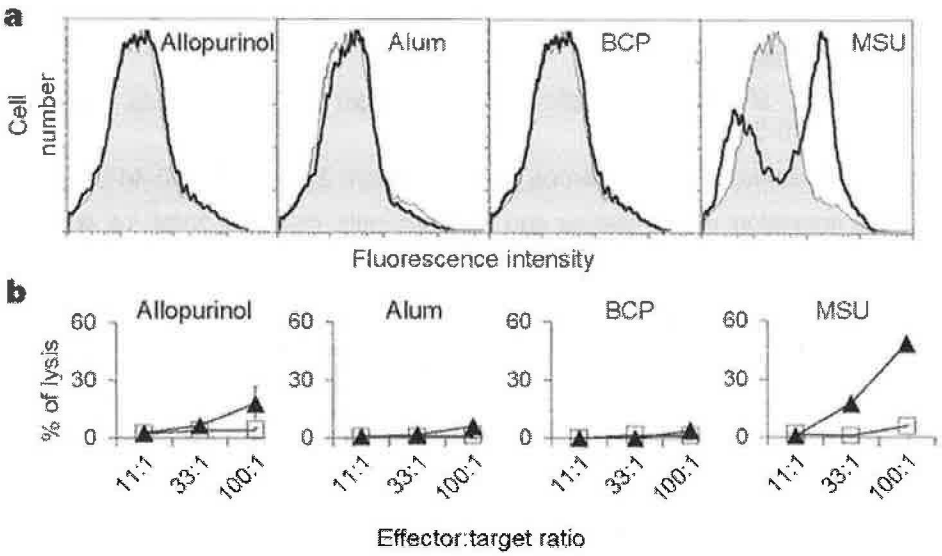
IMMUNE SIGNALING MOLECULE

Rather than a simplistic self vs. non-self model of immune recognition, Matzinger has suggested that the immune system is capable of sensing any form of danger regardless of whether it is a result of a foreign microbe invasion; the so called "danger hypothesis".



Model of uric acid as "Danger signal" taken from Heath and Carbone

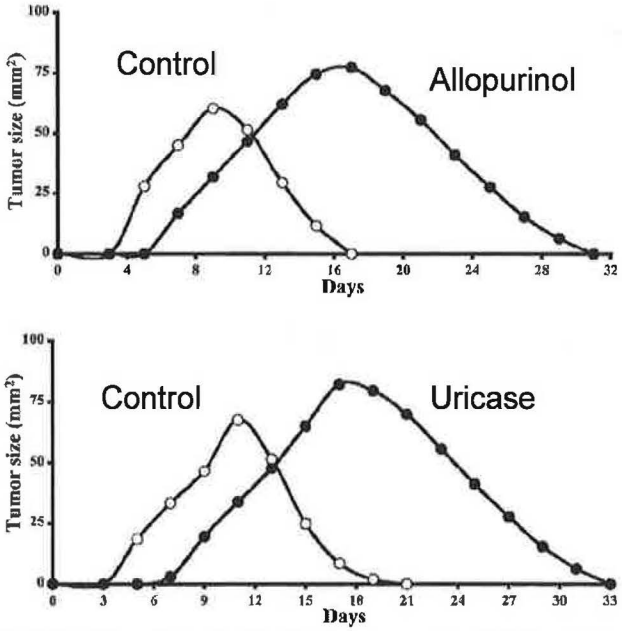
Hence antigen presenting cells can and should sense any form of cellular stress or tissue damage rather than the microbe *per se*. This will then result in the alarm of the troops for battle stations.



Taken from Shi et al. (A) BALB/c bone-marrow-derived dendritic cells were incubated for 6 h with of crystals of allopurinol, alum, BCP, or monosodium urate (MSU), or, CD86 expression is shown in FACS. (B) BALB/c mice were immunized with the indicated crystals and assayed for lysis of targets by CTLs.

The novel and provocative nature of the seminal paper by Shi, Evans and Rock triggered numerous editorial commentaries about this finding. Candidates such as heat shock proteins and nucleotides were proposed but the definitive evidence came from Shi and coworkers. They identified uric acid in a cytoplasmic fraction from dead cells that greatly enhances

cytotoxic T cell responses to be the long sort "danger signal". Identical results can be reproduced by highly purified uric acid. Addition of allopurinol and uricase reduced the active cytoplasmic fraction.



Taken from Hu et al. *In vivo* tumor rejection was delayed but not abrogated by allopurinol or uricase

Further support of uric acid serving as the internal danger signal can be seen experimentally where immune related tumor rejection is blunted by allopurinol and accelerated by uric acid administration. Only crystalline uric acid was active but not soluble urate [51]. Therefore, quite reminiscent of the excretory system, the immune system exploits the low solubility of uric acid and monosodium urate. This has to the case or else the danger signal will be constantly alarming due to the high serum uric acid.

References for Immune Signaling

Shi Y, Evans JE, Rock KL. Molecular indentification of a danger signal that alerts the immune system to dying cells. Nature 2003; 425: 516-521.

Heath WR, Carbone FR. Immunology: dangerous liaisons. Nature 2003; 425: 460-461.

Pulendran B. Immune activation: death, danger and dendritic cells. Curr Biol 2004; 14: R30-R32.

Jerome KR, Corey L. The danger within. N Eng J Med 2004; 350: 411-412.

Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994; 12: 991-1045.

Hu DE, Moore AM, Thomsen LL, Brindle KM. Uric acid promotes tumor immune rejection. Cancer Res 2004; 64: 5059-5062.

Hu DE, Dyke SO, Moore AM, Thomsen LL, Brindle KM. Tumor cell-derived nitric oxide is involved in the immune-rejection of an immunogenic murine lymphoma. Cancer Res 2004; 64: 152-161

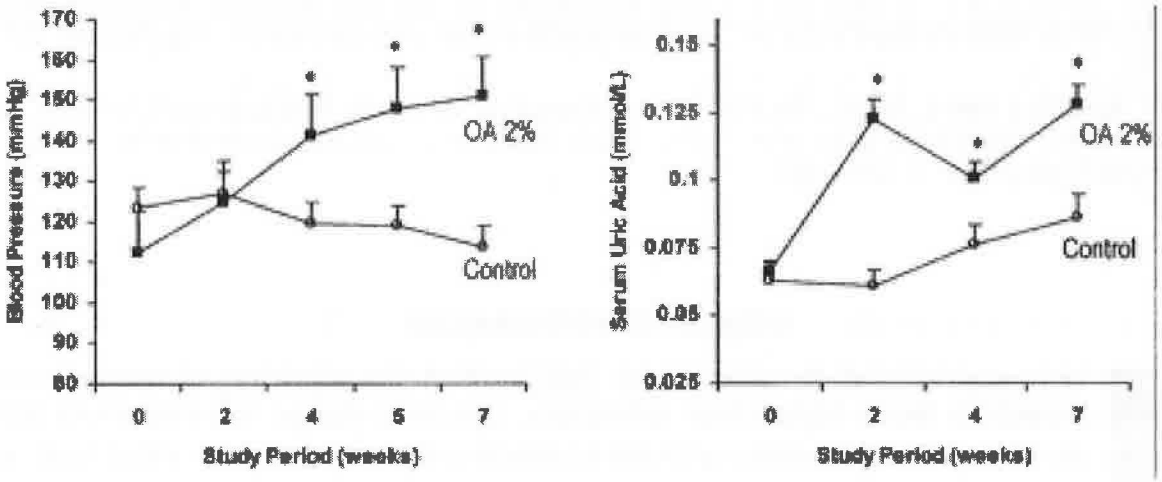
CARDIOVASCULAR EFFECTS

A most interesting and provocative theory has been submitted about uric acid and cardiovascular physiology. Based on a combination of epidemiologic associations, animal studies whether uric acid levels were manipulated and surrogate cell culture models of vascular biology, Johnson and colleagues proposed that high serum uric acid protected hominoids by maintaining a normal blood pressure while subsisting on very low salt diet through renal and vascular mechanisms. This is akin to the classical theory that organisms

with highly developed antinatriuretic mechanisms though millions of years of evolution are assaulted with high dietary salt for less than 10 thousand years resulting in a mismatch between the evolved physiology and the new lifestyle so "overcorrection" of the parameter manifests as hypertension.

The provocative nature of uric acid as a "cardiovascular defender" as a "left-over" from the Miocene epoch is paralleled by the proposal that uric acid in modern humans is an independent causative agent for cardiovascular and renal disease.

Epidemiologic data have shown a correlation between serum uric acid levels and blood pressure.



Taken from Mazzali. Experimentally-induced hyperuricemia elevated blood pressure in rats

Rodents are true ureotylics with very little uric acid their system. When rats were placed on low salt diet, blood remained constant initially but gradually decreased with time. In rats with experimentally induced hyperuricemia, blood pressure and uric acid levels began to rise after about 4 weeks of induced hyperuricemia. This elevation of blood pressure is associated with renal urate deposition and a renal arteriolopathy. Although the findings are clear, it is quite uncertain at the present moment whether the hypertensive response is adaptive for survival on a low salt diet, or maladaptive as predicted when hyperuricemia is induced in a ureotelic organism. The studies of Johnson and colleagues suggest a renal mechanism by which uric acid elevated blood pressure. Cell culture experiments of vascular smooth muscle have also provided plausible models to provide a vascular mechanism for uric acid to increase blood pressure.

The current model assumes that the underlying trait, in our case, high serum uric acid, is still adaptive and favorable, but the superimposition of a high salt diet is rendering the system maladaptive. However, it is important to note conceptually that the uric acid hypothesis extends beyond this paradigm. In addition to salt intake that is considerably higher than 20-30 million years ago, the panel of enzymes of purine metabolism that we possess was never subjected to the same purine load as in the modern human.

At the present time, we have a lot of clinical data showing associations and some rodent data showing that elevation of uric acid in a species that is not accustomed to having uric acid around causes significant morbidity. However, the current body of data does not permit one to

drawn the conclusion of whether: 1. Uric acid is a physiologic defender of blood pressure. 2. Uric acid is causative for cardiovascular or renal disease.

References for Cardiovascular Effects

Cannon PJ, Stason WB, Demartini FE, Sommers SC, Laragh JH. Hyperuricemia in primary and renal hypertension. N Engl J Med. 1966; 275: 457–464.

Jossa F, Farinaro E, Panico S, Krogh V, Celentano E, Galasso R, Mancini M, Trevisan M. Serum uric acid and hypertension: the Olivetti heart study. J Hum Hypertens. 1994; 8: 677–681

Selby JV, Friedman GD, Quesenberry CP. Precursors of essential hypertension: pulmonary function, heart rate, uric acid, serum cholesterol, and other serum chemistries. Am J Epidemiol. 1990; 131: 1017–1027

Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, Lan HY, Kivlighn S, Johnson RJ. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension. 2001; 38: 1101–1106.

Mazzali M, Kanellis J, Han L, Feng L, Xia Y-Y, Chen Q, Kang D-H, Watanabe S, Nakagawa T, Lan HY, Johnson RJ. Hyperuricemia induces a primary arteriolpathy in rats by a blood pressure-independent mechanism. Am J Physiol Renal Physiol. 2002; 6: 991–F997.

CONCLUDING REMARKS

Uric acid with all its advantages that justified the silencing of uricase, may be present in levels higher than necessary. This then places uric acid along with salt as accomplices in elevating blood pressure in modern humans. While solid phase monosodium urate maybe critical for danger signals in immune actions, these same crystal in the synovial fluid will beget gouty arthritis. Uricosuria will compromise ureotelic if the system is not meant to handle "physiologic uric acid crystalluria". There is no doubt that uric acid serves more function than just an insoluble end product of nitrogen and purine metabolism that permits solid phase nitrogen excretion. The high levels of uric acid in body fluids and tissue were selected by many years of evolution and this small purine serves numerous functions in the body. As with most biologic substances, more of a good thing may not always be better. Too much of a good thing in the wrong place and wrong time may be quite deleterious. Even Iris Benzie, the great expert and protagonist of antioxidants (including uric acid) warned against the possible ill effects of having too much uric acid in our system [59]. Gouty arthritis, renal stones, and the more controversial cardiovascular ill effects maybe the price the organism has to pay as a trade-off to possess this simple purine.