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Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis

(lipid peroxidation/ascorbic acid/primate evolution/erythrocyte aging)

BRUCE N. AMES*, RICHARD CATHCART*, ELIZABETH SCHWIERS*, AND PAUL HOCHSTEIN†

*Department of Biochemistry, University of California, Berkeley, California 94720; and †Institute for Toxicology and Department of Biochemistry, University of Southern California, Los Angeles, California 90033

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ABSTRACT During primate evolution, a major factor in lengthening life-span and decreasing age-specific cancer rates may have been improved protective mechanisms against oxygen radicals. We propose that one of these protective systems is plasma uric acid, the level of which increased markedly during primate evolution as a consequence of a series of mutations. Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals. We show that, at physiological concentrations, urate reduces the oxo-heme oxidant formed by peroxide reaction with hemoglobin, protects erythrocyte ghosts against lipid peroxidation, and protects erythrocytes from peroxidative damage leading to lysis. Urate is about as effective an antioxidant as ascorbate in these experiments. Urate is much more easily oxidized than deoxynucleosides by singlet oxygen and is destroyed by hydroxyl radicals at a comparable rate. The plasma urate level in humans (about 300 μM) is considerably higher than the ascorbate level, making it one of the major antioxidants in humans. Previous work on urate reported in the literature supports our experiments and interpretations, although the findings were not discussed in a physiological context.

Toxicity by oxygen radicals has been suggested as a major cause of cancer, heart disease, and aging (1–13). Oxygen radicals and other oxidants appear to be toxic in large part because they initiate the chain reaction of lipid peroxidation (rancidity). Lipid peroxidation generates various reactive species—such as radicals, hydroperoxides, aldehydes, and epoxides—with the capability of causing damage to DNA, RNA, proteins, cellular membranes, and cellular organization. Aerobic organisms have an array of protective mechanisms both for preventing the formation of oxidants and lipid peroxidation and for repairing oxidative damage. The protective systems include enzymes, such as superoxide dismutase (12) and the selenium-containing glutathione peroxidase (9, 10), and antioxidants and radical scavengers, such as α-tocopherol (vitamin E) and β-carotene in the lipid portion of the cell and glutathione and ascorbic acid in the aqueous phase (9, 10). These protective mechanisms are now being recognized as anticarcinogenic and, in some cases, even as life-span extending (5–7).

A marked increase in life-span has occurred in human evolution during the descent from prosimians over the past 60 million years (4). At the same time an enormous decrease in the age-specific cancer rate has occurred in humans compared to short-lived mammals (14, 15). It seems likely that a major factor in lengthening life-span and decreasing age-specific cancer rates may have been the evolution of effective protective mechanisms against oxygen radicals (2–7, 10). We propose that one such mechanism is high plasma uric acid.

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MATERIALS AND METHODS

γ-Ray Irradiation. Solutions of substrate (0.3 mM) in potassium phosphate buffer (20 mM, pH 7.4) were purged with O_2, N_2, or N_2O, sealed, and irradiated at room temperature with a ^{60}Co γ-ray source [12.7 krads/min (127 grays/min)].

Singlet Oxygen Oxidation. Sensitox II (Chemical Dynamics, South Plainfield, NJ), a polymer-supported Rose Bengal, was added at 10 mg/ml to solutions of substrate (0.3 mM in 25 mM potassium phosphate buffer, pH 7.6). These were then purged with oxygen and irradiated for various times in an ice bath by a tungsten/halogen lamp (Sylvania) at a distance of 15 cm. Aliquots were removed at various times, and the Sensitox was removed by rapid filtration of the sample through a Millipore filter.

Chromatographic Analysis. After treatment with either singlet oxygen or γ-irradiation, the amount of unreacted substrate was determined by high-performance liquid chromatography on a Supelco (Bellefonte, PA) 5-μm C_{18} column. The chromatographic equipment consisted of Waters 440 detector (254 nm), M-6000A pumps, WISP 710B, and 720 System Controller and a Spectra-Physics SP4020 and SP4000 integrator. Chromatographic conditions were: pump A, 25 mM KPO_4, pH 7.0; pump B, acetonitrile/H_2O, 40:60 (vol/vol); flow rate, 1.5 ml/min; 2 min isocratic, 0–10% B 15 min, and 10–30% B in 7 min. Urate (or ascorbate) chromatography was isocratic: the solutions were an 80:20 mixture (95:5 for ascorbate) of 5 mM tetrabutylammonium phosphate (pH 7.5) and 40:60 acetonitrile/water at 2 ml/min.

Heme-Catalyzed Peroxidation. We followed the procedure of Howell and Wyngaarden (16). Experiments were done with hematin or hemoglobin at pH 7.4 and 3 mM *t*-butylhydroperoxide or hydrogen peroxide. Oxidation of 50 μM urate or ascorbate was followed at 292 nm and at 260 nm, respectively.

Hemolysis of Human Erythrocytes. Venous blood (20–60 ml) was obtained from healthy donors and drawn into heparin (14 USP units/ml). The blood was centrifuged and the plasma and buffy coats were removed. The cells were washed three times, each with an equal volume of isotonic buffer (10 mM phosphate, pH 7.4/152 mM NaCl) and resuspended in this buffer to give a 5% hematocrit. The reaction mixture contained 2.5 ml of the resuspended cells and various additions (in isotonic buffer) in a total volume of 5 ml. The mixture was then incubated at 37°C in a 50-ml Erlenmeyer flask in a shaking water bath. At the appropriate time, a 0.5-ml aliquot of the mixture was removed and added to 4.5 ml of isotonic buffer containing 100 mg of NaCN and 300 mg of $K_3Fe(CN)_6$ per liter. After centrifugation (1500 × g, 10 min) to remove unlysed cells, the absorbance of the supernatant at 540 nm was determined. A 100% hemolysis value was determined from an identical reaction aliquot placed in the cyanide solution without the saline. In initial

experiments, the blood was drawn into 0.04 M citric acid/0.07 M sodium citrate/0.12 M glucose.

Lipid Peroxidation in Membrane Ghosts. Washed erythrocytes, prepared as above, were lysed in 10 mM phosphate buffer, pH 7.4. The membranes were pelleted by centrifugation (20,000 × g, 40 min) and then washed twice in 10 mM phosphate buffer: the membranes still contained considerable hemoglobin and were red. Peroxidation of the membrane ghosts was carried out in 1 ml total volume, containing 3 mg protein. Additions were made in phosphate buffer and reactions were for 20 min at 37°C in a shaking water bath and were stopped by addition of 0.5 ml of 20% trichloroacetic acid. After centrifugation, the supernatant was mixed with 2/3 vol of 0.67% thiobarbituric acid and heated for 15 min at 100°C (17). The absorbance was measured at 535 nm.

RESULTS

Urate as a Singlet Oxygen Scavenger. Urate has been found to be an excellent scavenger of singlet oxygen (18) (Fig. 1.). We chose a concentration of urate (300 μM) and a pH (7.6) that are approximately those of plasma. Fig. 1 shows that, after 5 min of exposure to singlet oxygen, 55% of the urate and 20% of the ascorbate were destroyed, compared to only about 10% of the deoxynuanosine and negligible amounts of the other deoxynucleosides.

Sensitivity of Urate to Hydroxyl Radicals Generated During γ-Irradiation. We found uric acid and the four deoxynucleosides to be destroyed by hydroxyl radicals at about the same rate. Samples were irradiated for various times in the presence of nitrous oxide. [Nitrous oxide enhances the yield of hydroxyl radicals in irradiated water (9).] The dose of radiation required to destroy 50% of deoxycytidine or uric acid was 40 krads or 60 krads, respectively. Similar results were obtained when samples were irradiated in either oxygen or nitrogen.

Oxidation of Urate by Hemoglobin and Peroxide. Howell and Wyngaarden (16) showed that urate was oxidized by hydrogen peroxide in the presence of hematin or methemoglobin. We confirmed this result (see *Materials and Methods*) and, in addition, found that urate (50% oxidation in 4 min) is about as effective as ascorbate (50% oxidation in 1.5 min) in this system (pH 7.4, hematin, hydrogen peroxide). *t*-Butylhydroperoxide and hydrogen peroxide were found to give comparable results (50% oxidation of urate in 4 min). The four deoxynucleosides showed no destruction (<1%) under conditions such that >80% of the urate was destroyed.

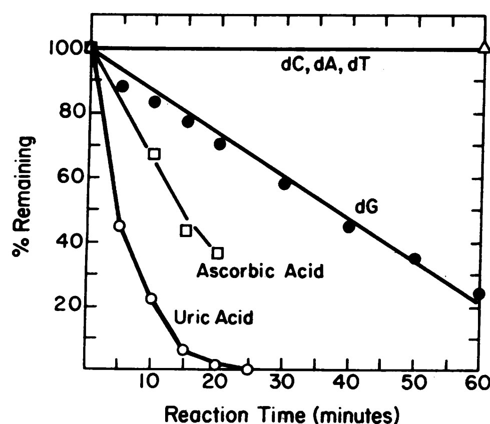

FIG. 1. Destruction of substrates (all at 300 μM) by singlet oxygen. The oxidation was dependent on oxygen, light, and Sensitox, with no measurable destruction observed in the absence of any of these, except with ascorbate which, even in the absence of Sensitox, was destroyed at about 30% of the rate shown (this blank has been subtracted).

Table 1. Lipid peroxidation of erythrocyte membrane ghosts

Additions	Exp. 1 *A*	Exp. 1 %	Exp. 2 *A*	Exp. 2 %
t-Butylhydroperoxide at 1 mM	0.262	100	0.484	100
+ Urate at 30 μM	0.115	44		
50 μM			0.354	73
60 μM	0.060	23		
100 μM			0.119	25
120 μM	0.024	9		
+ Ascorbate at 30 μM	0.092	35		
50 μM			0.144	30
60 μM	0.044	17		
100 μM			0.064	13
120 μM	0.018	7		
+ Glutathione at 30 μM	0.182	69		
120 μM	0.122	47		

Data shown are absorbance at 535 nm in the thiobarbiturate assay for lipid peroxidation products (malondialdehyde and its precursors). The blank (no *t*-butylhydroperoxide) had negligible absorbance. The three reducing agents were shown not to interfere with color formation of a malondialdehyde standard. In 10 experiments, using blood from different individuals, some variation was seen in the amount of lipid peroxidation: with only *t*-butylhydroperoxide, the mean (±SEM) average *A* was 0.47 ± 0.09. There was also variation in the decrease caused by urate (e.g., 25–75% at 100 μM urate). All experiments utilized blood collected in citrate/glucose but similar results were obtained later with blood collected in heparin.

Urate Protects Erythrocyte Membrane Ghosts from Peroxidation. Table 1 shows the protection by urate against peroxidation of erythrocyte membrane ghosts induced by *t*-butylhydroperoxide. Urate and ascorbate were effective in preventing lipid peroxidation at concentrations considerably below those normally found in plasma. Glutathione was also partially effective, although glutathione is not normally free in plasma at a significant concentration (19). The four deoxynucleosides and glucose (see below) were ineffective in inhibiting lipid peroxidation (>95%) at the same concentration as urate (100 μM). Experiments with well-washed erythrocyte membranes, containing no visible hemoglobin, showed little lipid peroxidation and indicate that the hemoglobin is essential.

Similar experiments were carried out using 100 μM cumene hydroperoxide (added as a sonicated suspension in buffer). In these experiments, urate also protected the membranes (50% inhibition at 20 μM) whereas uracil, guanine, and hypoxanthine at 100 μM did not protect appreciably. We preferred to use *t*-butylhydroperoxide as a model initiator because it is soluble in water at the concentrations used.

Urate Protects Intact Erythrocytes from Lysis by Peroxides. Table 2 shows that even small amounts (10 μM) of urate and ascorbate protect intact erythrocytes from lysis by *t*-butylhydroperoxide. The hydroperoxide initiates lipid peroxidation in the membrane which results in cell damage (20). Experiments with cumene hydroperoxide showed similar protection by urate. We also initiated erythrocyte lysis by xanthine oxidase and acetaldehyde and found that urate protects, as reported by Kellogg and Fridovich (21).

In experiments using blood that was collected in citrate/glucose (see *Materials and Methods*), we observed relatively little lysis of the erythrocytes induced by the hydroperoxide. This was found to be due to the presence of the glucose, and we then standardized our blood collection by using only heparin. It seems likely that erythrocyte glucose metabolism results in glutathione regeneration (22) (see *Discussion*) which in turn prevents peroxidation. This interpretation is supported by the following experiment with *N*-ethylmaleimide, which inactivates

Table 2. Effect of urate and ascorbate on t-butylhydroperoxide-induced hemolysis of human erythrocytes

Additions	% hemolysis		
	4 hr	7 hr	20 hr
None	0	0	3 ± 1
t-Butylhydroperoxide at 200 μM	5 ± 1	14 ± 1	100 ± 2
+ Urate at 10 μM	0	4 ± 1	17 ± 2
50 μM	0	0	5 ± 1
100 μM	0	0	6 ± 1
+ Ascorbate at 10 μM	0	2 ± 1	9 ± 2
50 μM	0	0	5 ± 1
100 μM	0	0	4 ± 1

Values are the mean (±SEM) of triplicate determinations from two experiments: 100% = 0.4 A at 540 nm. In a separate experiment, protection was provided by glucose (3% hemolysis at 100 μM) and blood plasma (4% hemolysis at 50% blood plasma). Citrate, allantoin, adenine, xanthine, hypoxanthine, ergothioneine, and dimethylfuran did not protect at 100 μM (>90% hemolysis).

glutathione. Blood was collected in media with glucose (citrate/glucose) and without glucose (heparin). The erythrocytes were then washed in the usual manner except that a portion of each sample was washed and resuspended in isotonic buffer containing N-ethylmaleimide (0.25 mM). Erythrocyte lysis was carried out with 300 μM t-butylhydroperoxide. The preparations from blood collected in heparin (with or without N-ethylmaleimide) and those from blood collected in citrate/glucose and washed in isotonic buffer plus N-ethylmaleimide showed 75% lysis. The blood collected in citrate/glucose and washed with isotonic buffer alone showed only 10% lysis. Erythrocytes were also protected against lysis when glucose was added to the incubation mixtures (legend to Table 2).

DISCUSSION

Protective Role of Urate in Blood. Various heme-containing proteins have been shown to catalyze oxidation of uric acid by hydrogen peroxide or an organic peroxide; these include hemoglobin, myeloperoxidase, catalase, cytochrome c (16, 23), and prostaglandin hydroperoxidase (24).

One of the most important of these reactions in humans is likely to be that involving hemoglobin. A human contains about 750 g of hemoglobin, and it has been estimated that 3% of this (22 g) undergoes autoxidation each day to yield methemoglobin with the concomitant production of superoxide (25). The large quantity of superoxide produced is dismutated by superoxide dismutase to hydrogen peroxide and O_2. The hydrogen peroxide, or possibly lipid hydroperoxides, may react with the iron in the hemoglobin to form oxo-heme oxidants (16), which may be similar to compound I or compound II of catalase and peroxidases (26). These oxo-heme oxidants may be important initiators of lipid peroxidation in erythrocyte membranes. Lipid peroxidation is likely to be a major factor in erythrocyte aging (20). We postulate that one role of urate is to help suppress lipid peroxidation in erythrocytes. Other factors may influence the hemoglobin reaction with peroxides—e.g., methemoglobin reductase (NADH) keeps the steady-state level of methemoglobin in humans below 1%, and glutathione peroxidase destroys peroxides (22). Our experiments with N-ethylmaleimide and glucose [which provides NADPH for glutathione regeneration (20)] emphasize the importance of glutathione peroxidase. Oxygen radicals are also generated in tissues by normal metabolism (e.g., by macrophages) (10).

Urate is presumably protecting not only erythrocytes but also the DNA-containing and longer-lived T and B lymphocytes and macrophages. In addition, urate may also protect other tissues because they presumably are in equilibrium with the blood urate. A transport system has been described that transports urate or hypoxanthine into human erythrocytes (27, 28), and this may ensure the availability of urate at intracellular sites.

Urate as a Scavenger of Singlet Oxygen. Singlet oxygen is an effective initiator of lipid peroxidation (29). It is formed *in vitro* or physiologically by the energy of light being absorbed by dyes and then transmitted to dioxygen (29)—e.g., when light reaches blood pigments (30). It can also be formed by the decomposition of endoperoxides (29). Urate and, to a lesser extent, ascorbate scavenge singlet oxygen very effectively compared to the deoxynucleosides (Fig. 1). Ascorbate, however, is much more easily autoxidized than urate (legend to Fig. 1). Simon and Van Vunakis (18) reported that uric acid was more easily oxidized than guanine in the presence of dye plus light. Although urate is an excellent singlet oxygen scavenger, the toxicological importance of singlet oxygen remains to be determined.

Urate as a Scavenger of Hydroxyl Radicals. Radiation is known to generate the extremely reactive hydroxyl radical from water, and this is thought to be the main species causing DNA damage, membrane peroxidation, and other cell damage (9). Although we show that the sensitivities of the deoxynucleosides and urate to oxidation by hydroxyl radicals are comparable, urate, because of its high concentration, might be important as a hydroxyl radical scavenger *in vivo*. Howell and Wyngaarden (16) showed that hydroxyl radicals generated by hydrogen peroxide plus either $FeSO_4$ or UV light are scavenged by urate.

Urate as a Scavenger of Oxo-Heme Oxidants. Heme is the active site catalyst for many oxidative enzymes, such as cytochrome P-450 and catalase. The extremely reactive oxo-heme intermediates, such as compound I and II of catalase or peroxidase, are thought to be $Fe^{IV}{=}O$ and $Fe^{V}{=}O$ species. Although the role of hemoglobin is that of an oxygen carrier, it can be oxidized to a nonspecific oxidase which may be deleterious for the organism because it is a catalyst for initiating lipid peroxidation.

Howell and Wyngaarden (16) showed that methemoglobin or hematin in the presence of hydrogen peroxide oxidized uric acid to allantoin. They postulated that this was initiated by a one-electron oxidation of urate to a urate radical by a "heme-peroxide" compound. We repeated their experiments and found, in addition, that ascorbate is about as effective as urate and that t-butylhydroperoxide gave results comparable to those with hydrogen peroxide. Howell and Wyngaarden interpreted their results to mean that hydroxyl radicals were not involved in the initial oxidation of urate. Our experiments support this conclusion. Thus, we show that oxo-heme is different in its properties from free hydroxyl radicals in that the former oxidizes urate extremely well and shows no detectable oxidation of pyrimidine deoxynucleosides whereas hydroxyl radicals (from radiation) oxidize both equally well. Our finding that hydrogen peroxide and t-butylhydroperoxide are equally effective in the Howell–Wyngaarden system is also consistent with this interpretation. The lack of oxidation of deoxyguanosine, which is oxidized by singlet oxygen (see Fig. 1), is also evidence against singlet oxygen being involved in the heme system. A hematin/cumene hydroperoxide system can oxidize N-hydroxy-2-acetylaminofluorene, thus mimicking cytochrome P-450 (31). Hawco *et al.* (32) also investigated a lipid peroxide/hematin system and concluded that singlet oxygen was a product, although they could find no evidence for this with a cumene hydroperoxide/hematin system. Rosen and Rauckman (33) investigated the cumene hydroperoxide/hematin system with spin trapping reagents and postulated that the active oxidant could be a cumene hydroperoxy radical.

We have used erythrocyte membrane ghosts as a model sys-

tem for the deleterious effects of oxo-heme in hemoglobin, and we have shown that urate protects from this oxidant. This model combines the methemoglobin/peroxide activity with the erythrocyte membrane and suggests that the oxo-heme oxidant can cause membrane peroxidation with its consequent cytotoxic activity. Table 1 shows that hemoglobin and peroxide both are necessary for lipid peroxidation and that urate and ascorbate protect.

We show in Table 2 that lysis in intact erythrocytes is caused by peroxide and that low levels (10 μM) of urate and ascorbate protect. In intact erythrocytes, lipid peroxidation leads to hemolysis (20). In experiments similar to those shown in Table 2, we have observed a marked and possibly interestng variability in hemolysis with blood from different individuals. A similar observation has been reported by Kellogg and Fridovich (21) who also showed that 0.5 mM urate, among a number of other substances, inhibits hemolysis of erythrocytes caused by the xanthine oxidase/acetaldehyde system. They postulated that this was due to the scavenging of singlet oxygen or hydroxyl radicals.

Allantoin is a major end product of urate oxidation in the peroxide/heme system (16, 23). Because humans lack uricase, which converts urate to allantoin, the allantoin levels in urine (about 25 mg/day) (34) and in blood (about 25 mg per person) (35) may be of considerable interest as an indicator of urate oxidation *in vivo* (34). The source of this allantoin has been attributed mostly to fecal breakdown of urate (34), but we believe this should be reexamined.

Urate and Ascorbate Blood Levels During Primate Evolution. Compared to the blood level of about 5 mg of urate per 100 ml in man, close to the maximum solubility, most mammals, such as the rat or prosimians, have levels <0.5 mg/100 ml (36). The increase in urate level occurred during the course of about 60 million years of evolution and coincided with a large increase in both life-span and brain size. A number of mutations appear to have been necessary for the increased blood urate. Monkeys have a labile uricase and many, but not all, have higher urate levels than prosimians but less than the longer-lived apes (36). Humans (with an even longer life-span and higher urate levels) and apes have completely lost uricase. In addition, humans actively reabsorb urate from their kidneys. In man, >90% of the urate is reabsorbed from the kidney (37). The development of an efficient reabsorption mechanism appears also to have been critical for high plasma urate levels, because human mutants who do not actively reabsorb urate from the kidney have abnormally low urate levels (0.2–1.8 mg/100 ml) (36). It also may be relevant that, in the evolution of prosimians to monkeys, ascorbate synthesis was lost (38). It may be that ascorbate loss and urate gain were related to each other and that urate replaced some (but not all; see following section on brain) and supplemented other of the antioxidant functions of ascorbate during primate evolution. It is possible that the usefulness of ascorbate as an antioxidant may be limited by its propensity, under certain conditions, to autoxidize and generate oxygen radicals (e.g., see Fig. 1 and ref. 39) and mutagens (40).

For comparative purposes the antioxidant levels of various substances in human plasma are shown in Table 3; the level of urate is severalfold higher than that of ascorbate.

Urate Concentration in Blood in Humans Is Determined by Several Factors. Urate is formed from purine degradation; part of the purines come from dietary sources and part from biosynthesis *de novo* (37). Plasma urate values decline about 35% when healthy males are placed on a purine-free diet. Plasma urate can be increased by ingestion of yeast RNA, but not by ingestion of urate, and levels in man increase linearly at about 1 mg/100 ml per g of RNA ingested up to a level of 8 g RNA per day (37).

Table 3. Plasma concentrations of natural antioxidants

Antioxidant	mg/ 100 ml	μM
Urate:		
Males (37)	2.6–7.5	160–450
Premenopausal females (37)	2.0–5.7	120–340
Ascorbate:		
Normal adult (41)	0.9 ± 0.4	50 ± 20
Normal adult (42)	0.7–2.5	40–140
+ 2-g supplement (42)	1.7–2.8	100–160
Glutathione (19)	(65)*	(2100)*
Vitamin E (43)	0.5–1.6	10–40
Carotenoids (43)	0.09–0.12†	2†

* Whole blood; no appreciable amount in plasma.
† Children (molarity calculated as β-carotene).

Urate is present as the sodium salt in plasma at pH 7.4 (uric acid has a pK of 5.8). Its concentration (Table 3) in males approaches the maximum solubility (saturation occurs at about 7 mg/100 ml) and it is well known that people with higher levels than this are susceptible to gout (37, 44). The level in men is higher than that in women (Table 3). The level of urate in women decreases during the first part of pregnancy (45) and increases after menopause. The renal reabsorption and excretion rates are of considerable importance, and extreme examples of this are humans with an inherited defective reabsorption who have low plasma urate levels and who are discussed above. About 500 mg of urate is excreted per day per normal adult male (3 mmol), and the pool of urate is about 1200 mg (37).

Increase in Urate Levels in Response to Oxidative Stress. Some humans (two of six studied) show a marked increase in lipid peroxidation during heavy exercise (46). It is known that urate levels in blood increase with exercise, and we interpret this as a possible physiological mechanism to help cope with the increased oxidative stress. The mechanism of this increase is thought to be the marked inhibition of renal clearance of urate by the lactate and β-hydroxybutyrate that accumulate during exercise (47). Lead exposure, high alcohol ingestion, and obesity all are associated with increased urate levels in man (47). Because each of these has been associated with lipid peroxidation (48–50), there may be a causal connection.

Urate in Alimentary Tract. Saliva contains almost as much urate as does blood, about 3 mg/100 ml in men and 2 mg/100 ml in women (34). It has been estimated that several hundred milligrams, almost one-third of the urate turned over, enters the alimentary tract. The saliva accounts for about 40 mg/day and the rest is from gastric juice (5–10 mg of urate per liter), bile, and pancreatic and intestinal juices (34). Ascorbate is known to be effective as a trap for nitrosating radicals derived from nitrite (51), which can form carcinogenic nitrosamines from secondary amines at the acidic pH of the stomach, and urate might be equally effective. Urate is an effective antioxidant at alkaline and neutral pH (52) and in acid; a standard method for its analysis involves its use as a reductant of the acid phosphotungstate complex to form a blue color (53).

Urate and the Brain. The human brain utilizes about one-fifth of the oxygen taken in each day (54) and contains lipidic material with a high content of unsaturated fatty acids. Thus, antioxidant defense mechanisms against lipid peroxidation in the brain should be of critical importance. The concentration of urate in the human cerebrospinal fluid is about 0.3 mg/100 ml, only 7% of that in plasma (28, 55). The ascorbate concentration in human cerebrospinal fluid, however, is 1.8 mg/100 ml (56), even higher than it is in plasma (Table 3). Thus, ascorbate may be a more important antioxidant in cerebrospinal

fluid than urate, and the role of urate in the brain remains to be evaluated. A connection between plasma urate level and intelligence (57) has been postulated, as well as a connection between RNA ingestion (which increases urate levels) and memory (58); these are difficult areas to evaluate.

Hypouricemia. Evaluation of the medical literature on hypouricemia and xanthinuria and new studies on urate levels as related to longevity, cancer, and other diseases may test some of the ideas presented here.

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