URATE OXIDATION BY CUPRIC ION (Cu^{++})

Arnold H. SCHEIN
Department of Biochemistry, University of Vermont College of Medicine, Burlington, Vermont, USA

and

Arthur S. KUNIN
Department of Medicine, University of Vermont College of Medicine, Burlington, Vermont, USA

Received 3 February 1969

Uric acid or urate, a compound of extremely limited solubility produced *in vivo* by degradation of purines from nucleic acids or other endogenous and exogenous sources – has long been considered a metabolic "enfant terrible" of man. Possessing a vestigial uricotelic apparatus but lacking the copper-containing enzyme uricase which oxidizes urate to water-soluble compounds, man teters on the brink of urico-disaster. Although elaborate control mechanisms exist to prevent flooding by urate, when these systems fail for genetic or other reasons body urate pools increase and there is danger of precipitation in soft tissues, cartilage (tophi) and urine (stones). While the major portion (60–70%) of the urate disappearing from the urate pool is excreted as such in urine, the remainder may be converted to CO_2, allantoin, allantoic acid, urea, and NH_3 by bacteria of the gastrointestinal tract [1]. In addition oxygen uptake studies have revealed that urate may be oxidized by Cu^{++} above pH 10 and by the cytochrome oxidase system (Cu^{++}?) at pH 7.9 [2]. Hemoproteins in the presence of peroxide causes breakdown of urate to allantoin, CO_2 and alloxanic acid [3].

We are reporting *in vitro* urate oxidation mediated solely by Cu^{++} at pH 6.0–8.2. In fig. 1 the fall in absorption of a urate solution incubated with Cu^{++} (urate/Cu^{++} ratio 1:4) is shown. Cu^{++} also alters the shape of the urate absorption spectrum drastically, obliterating the second maximum at 237 mμ. H_2S stops the fall in UV absorption caused by Cu^{++}, the centrifuged supernatant fluid has the same absorption spectrum as urate. When uric acid-6-^{14}C was incubated with Cu^{++} in a closed vessel, labeled CO_2 was evolved. This was also the case when Cu^{+} and H_2O_2

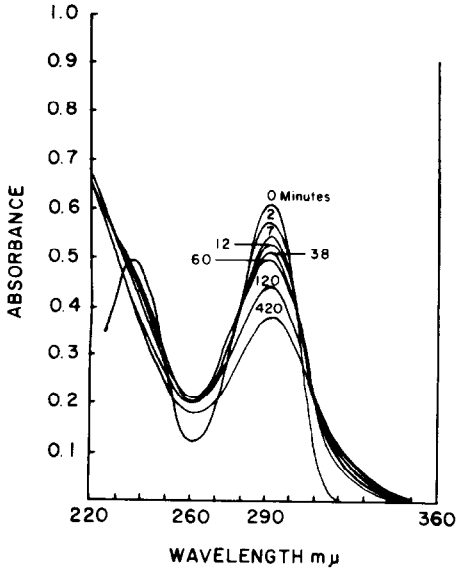

Fig. 1. Time course for the effect of Cu^{++} on urate absorption. Urate = 0.15 μmole, Cu^{++} = 0.60 μmole, vol = 3.0 ml. pH = 6.1, T = 24°. Beckman DB scanning time = 3 min. Cu^{++} omitted = 0 min urate spectrum.

Table 1
Oxiation of urate-6-^{14}C

Flask contents	CO_2 ** (cpm X 10^3)
urate-6-^{14}C (control) *	1.6
urate-6-^{14}C * + EDTA	0.2
urate-6-^{14}C + Cu^{+} (CuCl)	74
urate-6-^{14}C + Cu^{++} ($CuSO_4$)	97
urate-6-^{14}C + Cu^{++} + EDTA	0.2
urate-6-^{14}C + Fe^{++} ($FeSO_4$)	0.7
urate-6-^{14}C + Fe^{+++} ($FeCl_3$)	1
urate-6-^{14}C + H_2O_2	77
urate-6-^{14}C + H_2O_2 + EDTA	1
urate-6-^{14}C + H_2O_2 + Cu^{++}	279
urate-6-^{14}C + H_2O_2 + Cu^{++} + EDTA	1
urate-2-^{14}C (control) *	6.7
urate-2-^{14}C * + Cu^{++}	6.7

Urate and metals = 0.095 μmole, EDTA = 0.95 μmole, total volume = 3.0 ml, pH = 7.5. Solutions were pipetted into rubber-capped flasks equipped with a suspended plastic Hyamine container [4]. Urate-^{14}C was injected through the cap and the flasks were shaken at 100 strokes/min at 37° for 40 min. The reaction was stopped by the addition of 0.5 ml of 0.5 N H_2SO_4 and the shaking was continued for an additional 90 min, which time sufficed to trap 99% of the evolved CO_2 in the Hyamine. The Hyamine container was removed, immersed in a toluene phosphor solution and counted in a Packard Tri Carb liquid scintillation spectrometer.

* 0.25 μc = 5.55 X 10^5 cpm. Efficiency of counting was 87%. Thus 100 X 10^3 cpm represented approximately 30% of the urate-^{14}C initially placed into the flask.

** Radioactive CO_2 was slowly evolved from the substrate controls (urate-6-^{14}C or 2-^{14}C) yielding from several hundred up to several thousand cpm. Such control counts could be lowered when desired by preliminary aeration (or bubbling with N_2) or by addition of EDTA or H^+ to pH 1–3 and significantly lowered by both treatments. Usually these procedures were not necessary; significant differences between control and experimental flasks were clearly evident.

were substituted for Cu^{++}. However, EDTA stopped the reaction with these oxidizing agents. Fe^{+++} and Fe^{++} were not active in the oxidation of urate (table 1). When uric acid-2-^{14}C was substituted for uric acid-6-^{14}C, no $^{14}CO_2$ above background was released.

Cu^{++} has great affinity for anions and amino groups. At pH 7 the evolution of radioactive CO_2

Table 2
Effect of buffers on urate oxidation by Cu^{++}

Flask contents	CO_2 (cpm X 10^3)
urate-6-^{14}C (control)	2
urate-6-^{14}C + Cu^{++}	118
urate-6-^{14}C + NaH_2PO_4	0.3
urate-6-^{14}C + Cu^{++} + NaH_2PO_4	11
urate-6-^{14}C + tris	0.3
urate-6-^{14}C + tris + Cu^{++}	7.5
urate-6-^{14}C + glycylglycine	0.2
urate-6-^{14}C + glycylglycine + Cu^{++}	0.2
urate-6-^{14}C + histidine	1.5
urate-6-^{14}C + histidine + Cu^{++}	1.8

Buffers = 0.05 M, urate-6-^{14}C = 0.095 μmole, 0.25 μc, volume = 3.0 ml, pH 7.5. Procedure as described in legend of table 1.

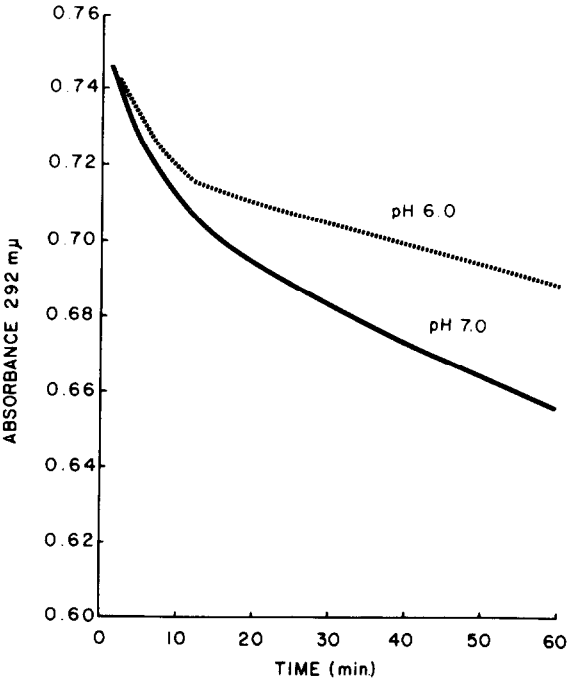

Fig. 2. Time course for effect of Cu^{++} on urate absorption at 292 mμ, pH 6.0 and 7.0. Urate = 0.185 μmoles, Cu^{++} = 0.185 μmoles, vol = 3.0 ml, T = 24°.

Table 3
Effect of pH on Cu^{++} oxidation of urate-6-^{14}C

pH	Flask contents	CO_2 (cpm X 10^3)
3.5	urate-6-^{14}C (control)	0.2
	urate-6-^{14}C + Cu^{++}	0.8
7.5	urate-6-^{14}C (control)	1
	urate-6-^{14}C + Cu^{++}	106
11.5	urate-6-^{14}C (control)	18
	urate-6-^{14}C + Cu^{++}	360

Urate-6-^{14}C = 0.095 μmole, 0.25 μc; Cu^{++} = 0.095 μmoles; volume = 3.0 ml; pH adjusted with dilute HCl or NaOH. Procedure as described in legend of table 1.

from urate-6-^{14}C by Cu^{++} was inhibited by phosphate and tris and completely inhibited by glycylglycine and histidine (table 2). CN^- also effectively blocked the reaction. Fig. 2 shows the rate of urate oxidation by Cu^{++} measured spectrophotometrically at pH 6.0 and at 7.0. At pH 5.5 the reaction was very slow, at pH 5 no reaction could be detected. Above pH 7 a slight haze developed in the Cu^{++} solution rendering it unsuitable for spectrophotometry. Results obtained with urate-6-^{14}C confirmed the diminished Cu^{++} oxidation rate at low pH and the accelerated rate at high pH (table 3). Fig. 3 shows the extent of recovery of urate after zero, three and nine hours reaction with Cu^{++}. Chromatograms containing the products of urate with Cu^{++} were sprayed separately with dimethylaminobenzaldehyde (Erlich's reagent) and diphenylcarbazone. A reactive spot with the same mobility and color as allantoin with both reagents was observed on the chromatograms.

Cu^{++} tightly bound in uricase does not act as a reversible redox system. Mahler et al. have suggested that urate oxidation occurs within a ternary complex of substrate, enzyme and acceptor [5]. The role of Cu^{++} is to chelate and bring into proximity urate and O_2, to allow interpenetration of metal and ligand orbitals and to polarize the electrons to be transferred. After electron transfer the reaction to allantoin involving decarboxylation and ring rearrangement proceeds non-enzymatically. In contrast to its role in uricase, Cu^{++} alone may non-enzymatically oxidize

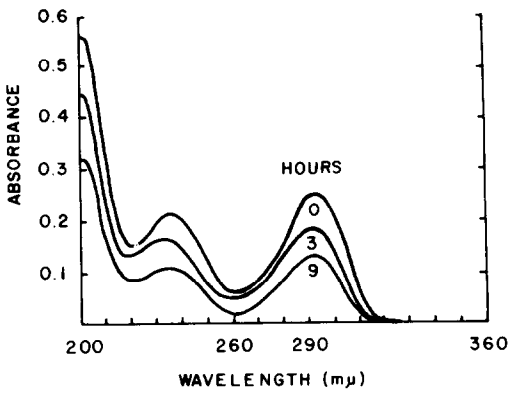

Fig. 3. Ultraviolet absorption spectrum of H_2S treated urate + Cu^{++} solutions at 0, 3 and 9 hours. 1.2 μmoles urate was incubated with 1.2 μmoles Cu^{++}, pH = 7.0, 37°, vol = 5 ml. After gassing with H_2S and centrifuging, 1.0 ml aliquots of the supernatant fluid were chromatographed (descending) on Whatman No. 1 paper with H_2O as solvent. The single UV-absorbing spot was eluted with 0.001 M $NaHCO_3$ for 16 hours at 4° and scanned from 200 to 360 mμ with a Beckman model DB recording spectrophotometer.

urate directly to CO_2 and allantoin at body pH. This may be a mechanism for degradation of urate in organisms lacking uricase.

We thank Mrs. K.Shiverick, Mr. Michael Giancola and Mr. Don Majercik for technical assistance. The research was supported by grants from the National Institutes of Health, the Easter Seal Research Foundation (A.S.K.) and the Atomic Energy Commission (A.H.S.).

References

[1] L.B.Sorensen, (a) Scand. J. Clin. Lab. Invest. 12, Suppl. 54 (1960); (b) Metabolism 8 (1959) 687.
[2] M.J.Griffiths, J. Biol. Chem. 197 (1952) 399.
[3] R.Howell and J.B.Wyngaarden, J. Biol. Chem. 235 (1960) 3544.
[4] A.S.Kunin and S.M.Krane, Biochim; Biophys. Acta 107 (1965) 203;
A.S.Kunin and W.L.Meyer, Arch. Biochem. Biophys., in press.
[5] H.R.Mahler, H.M.Baum and G.Hubscher, Science 124 (1956) 705.