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Urate Oxidase in Primate Phylogenesis

Philipp CHRISTEN, Wendell C. PEACOCK, Anita E. CHRISTEN, and Warren E. C. WACKER

Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, and Division of Medical Biology, Peter Bent Brigham Hospital, Boston, Massachusetts, and New England Regional Primate Research Center, Southborough, Massachusetts

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1. Several genera of New World monkeys have high uric acid concentrations in serum and urine comparable to man and the higher apes. Like man these animals lack urate oxidase activity in liver tissue. The purine metabolism of these non-human primates appears to be similar to that of man as exemplified by fasting-induced hyperuricemia. These South American monkeys should provide an easily accessible experimental system for the study of purine metabolism in a species closely related to man.

2. Old World monkeys and prosimians have low uric acid concentrations in serum and urine. The urate oxidase activity of prosimian liver is remarkably stable whereas that of Old World monkeys was found to be quite unstable, a probable reflection of the gradual degeneration of this enzyme in primate phylogenesis.

It has been generally accepted that man and the anthropoid apes are the only mammals devoid of uricase. This conclusion is based on a remarkably small number of data, the last of them obtained 55 years ago (see *e. g.* the excellent review of Keilin [1]). The studies on non-human primate species have been limited in fact to the chimpanzee, orangutan and three genera of Old World monkeys, *i. e.* Macaca, Papio, and Cercopithecus [2–6].

The uric acid concentrations in serum and urine of several South American monkey species have now been found to be comparable to those of man. Concomitantly, the absence of significant amounts of allantoin in urine and the failure to detect urate oxidase activity in liver tissue of these species indicate the absence of urate oxidase not only in man and the anthropoid apes, but also in some genera of New World monkeys.

In the present studies low concentrations of uric acid were found in serum and urine of Old World monkeys and prosimians in accord with previous reports [1]. However, in contrast to the enzyme from prosimian and rabbit liver, the urate oxidase activity in liver tissue of these species has been found to be highly unstable, perhaps reflecting a partial degeneration of the enzyme in these species.

METHODS

Animals were housed in the New England Regional Primate Research Center (Harvard Medical School, Southborough, Massachusetts). They were fed a diet of Purina monkey chow or Purina "High Protein" chow (for marmosets), obtained from the Purina Company (St. Louis) supplemented with fresh fruits and vitamins. *Cebuella* and *Leontocebus* were housed at the Anthropological Institute of the University of Zurich. Care and diet of these animals have been reported [7].

Blood samples of 1–3 ml were drawn from the superficial femoral vein after sedation with Sernylan (Parke-Davis and Company). Urine specimens were collected on a tray at the bottom of the cage. Serum and urine samples were stored at −20° and analyzed within one week. Uric acid was measured by specific enzymatic conversion to allantoin [8] (Determatube U, Worthington Biochemical Corp.). Allantoin was determined by the method of Larson [9]; creatinine by the method of Folin and Wu [10].

Liver specimens were obtained by open biopsy or from final experiments and stored at 0°. Within 3 hours a portion of liver tissue was homogenized in two parts of 0.1 M sodium borate buffer, pH 9.0, with a Potter-Elvehjem homogenizer. Urate oxidase activity was determined by measuring oxygen consumption with a Warburg manometer at 37°. The manometer flasks were filled with 0.5 ml homogenate and 2.1 ml of 0.1 M borate buffer, pH 9.0. Other conditions were as described by Leone [11]. The manometer readings were corrected for oxygen uptake of controls containing no uric acid. All determinations were made in duplicate and agreed within 20% of the total activity. Nitrogen was determined by a modified Kjeldahl procedure [12].

RESULTS AND DISCUSSION

The concentration of serum uric acid of *Cebus*, *Lagothrix* and *Saguinus* (Table 1) are all comparable to those of man [13]. Two previous reports cited the

Enzyme. Uricase or urate oxidase (EC 1.7.3.3).

Table 1. *Concentration of urate in serum of New and Old World monkeys*

Species	Urate	No. of determinations (individual animals)
	mg/100 ml (mean)	
New World monkeys:		
Cebus albifrons	3.3	3
Cebus apella	2.8	1
Lagothrix lagotricha	3.1	5
Saguinus oedipus	2.1	3
Saimiri sciureus	0.5	3
Old World monkeys:		
Macaca mulatta	0.3	5
Macaca irus	0.5	2
Macaca arctoides	0.3	2
Papio cynocephalus	0.4	2
Prosimian		
Tupaia glis	0.5	3

Table 2. *Concentration of urate in urine of New and Old World monkeys and of prosimia*

Species	Urate	Urate / Creatinine
	mg/100 ml	
New World monkeys		
Lagothrix lagotricha	22.5	1.2
Saguinus oedipus	12.5	1.8
Saimiri sciureus	10.4	0.5
Old World monkeys		
Macaca mulatta	6.2	0.2
Macaca arctoides	10.8	0.3
Prosimians		
Galago senegalensis	25.0	0.4
Tupaia glis	25.8	0.1
Nycticebus coucang	13.3	0.8

Table 3. *Urate oxidase activity of liver*

Species	Urate oxidase activity
	$\mu l\ O_2 \times mg\ N^{-1} \times h^{-1}$
New World monkeys	
Cebus apella	6
Cebus albifrons	8
Aotes trivirgatus	20
Old World monkeys	
Macaca irus	27
Macaca mulatta	18
Prosimian	
Tupaia glis	80
Rabbit	58

average concentration of uric acid in serum to be 1.8 mg-% for *Saguinus oedipus*, 2.7 mg-% for *Saguinus fuscicollis* and *Saguinus nigricollis* [14] and 2.3 mg-% for *Cebus albifrons* [15].

Saimiri is the only South American species examined where low serum uric acid concentrations were found comparable to those of macaques and baboons (Table 1). The serum uric acid concentrations of 0.3—0.5 mg-% found in the Old World monkey species correspond to the values measured in other mammalian orders [16, 17].

The concentration of uric acid in urine (Table 2) correlates to the blood concentration but probably as a result of evaporation during collection of the specimens the difference between New and Old World monkeys is not as pronounced as found in serum. However, the urate/creatinine ratio, eliminating uncontrollable concentration changes during collection, reveals marked differences. The urate/creatinine ratios of *Lagothrix* and *Saguinus* are high, consistent with their elevated serum urate concentration. Again, *Saimiri* resembles the Old World rather than the New World monkeys. The urate/creatinine ratio of all prosimians examined is low.

The allantoin concentrations in the urine of South American species was also determined. The concentration of allantoin was 0.6 mg-% in *Cebuella pygmea* and 1.7 mg-% in *Leontocebus tamarin*, while the concentration of urate was 24.4 mg-% and 23.3 mg-%, respectively. The coincidence of a high concentration of urate in blood and urine and the absence of significant amounts of allantoin in urine suggests that with the probable exception of *Saimiri* and related genera the South American species lack urate oxidase, the enzyme that oxidizes urate to allantoin.

The urate oxidase activity of the liver tissue of two *Cebus* species was very low, whereas that of *Aotes trivirgatus*, of two macaques, and of *Tupaia glis* exhibited considerable activity, about equivalent to that of rabbit liver (Table 3). However, the urate oxidase activity of macaque and *Aotes* livers appears to be highly labile. After storage at $-20°$ for only one day it had fallen to the level found in the South American species. This residual activity as well as the initially low activity of the South American species is stable over many months at $-20°$, suggesting that it is due to non-specific oxidation of urate by other enzymes, *e. g.* peroxidases [18] or cytochrome, cytochromoxidase [19]. The lability of the urate oxidase activity of macaque liver is in marked contrast to that of *Tupaia glis* and of rabbit liver which is remarkably stable.

These data provide new insight into the phylogenetic aspects of purine metabolism. Heretofore, only man and higher primates have been thought to lack urate oxidase. The finding that New World primates also lack this enzyme should provide a useful biochemical parameter for further study of the phylogenetic development of primates, especially the New World monkeys. Within this superfamily the present study has revealed significant differences among various species, thus, the low concentrations of uric acid in serum and urine of *Saimiri* (Tables 1 and 2) and the high urate oxidase activity in *Aotes* liver (Table 3) are in marked contrast with the values found in all other South American species examined.

Investigations of urate oxidase in primates might also serve to visualize the consecutive steps occurring in the degeneration of an enzyme system during evolution. The lability of urate oxidase activity of macaque liver in contrast to that of *Tupaia glis* and of lower species [20] appears to be significant and suggests that the mutations leading to the absence of the enzyme in certain primates are already partially operative at this level of evolution. Urate oxidase has been identified as a component of peroxisomes and

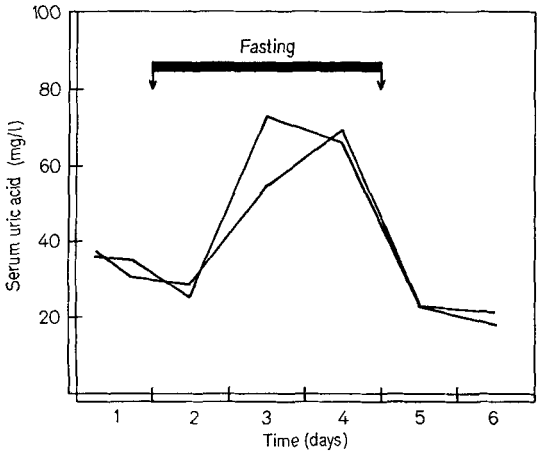

Fig. 1. *Effect of fasting on the concentration of urate in the serum of two wooly monkeys* (Lagothrix lagotricha)

the degeneration of this enzyme in the phylogenesis of primates would seem to represent a particular segment of the process of gradual involution of these subcellular particles, demonstrated to extend over a much longer period of evolution [21].

Except for the great apes, an experimental animal has not been available whose purine metabolism is similar to that of man. The effect of fasting on the uric acid concentration in serum of wooly monkeys *(Lagothrix lagotricha)* (Fig. 1) was investigated in view of the well-known hyperuricemia observed in man consequent to fasting [13]. The response of the serum uric acid on a starvation regimen suggests that the factors affecting uric acid metabolism in man may also be expected to affect that of these New World primates. Studies of renal urate clearance have also demonstrated a close similarity between cebus monkeys and man in regard to the renal transport mechanism of urate and its response to drugs [15].

These non-human primates should serve as invaluable experimental subjects for studies of the regulation of purine synthesis, and the renal excretion of urate, processes which are impaired in human disease such as gout [13] of the X-linked hyperuricemia syndrome [22, 23]. Moreover, access to these readily available experimental animals should provide the opportunity to study the effects of nutrition, electrolyte alterations and drugs on purine metabolism in a species closely related to man. Finally, it is not unreasonable to expect that diseases corresponding either to gout or to the X-linked hyperuricemia syndrome will be found to exist in these species providing an ideal experimental system for the study of abnormal purine metabolism.

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P. Christen's present address:
Biochemisches Institut der Universität
Zürichbergstrasse 4, CH-8032 Zürich, Switzerland

W. C. Peacock
New England Regional Primate Research Center
Southborough, Massachusetts, U.S.A.

A. E. Christen's present address:
Anthropologisches Institut der Universität
Künstlergasse, CH-8001 Zürich, Switzerland

W. E. C. Wacker
Biophysics Research Laboratory
Peter Bent Brigham Hospital
Boston, Massachusetts 02115, U.S.A.