A Study On The Loss Of Uricolytic Activity During Primate Evolution I. Silencing Of Urate Oxidase In A Hominoid Ancestor.

by

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To my parents,

Jorge E. Polanco

Maria S. Polanco

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Abstract

A study on the loss of uricolytic activity during primate evolution. I. Silencing of urate oxidase in a hominoid ancestor.

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This study extends previous findings on the presence, absence and stability of primate hepatic urate oxidase activity and clarifies conflicting published data. Urate oxidase [E.C.1.7.3.3.], a liver specific enzyme, catalyzes the oxidation of uric acid to allantoin, carbon dioxide and hydrogen peroxide. Urate oxidase activity assays used in earlier studies measured the rate of disappearance of uric acid or monitored the consumption of oxygen during the oxidation of uric acid and therefore did not exclude non-specific oxidation of uric acid by other enzymes or non-enzymatic degradation of uric acid. In this study a definitive and ultrasensative microradiochemical assay for urate oxidase activity which measures the production of [^{14}C]allantoin from [^{14}C]uric acid was used (Friedman and Merril, 1973; Friedman and Johnson, 1977).

The results of this study indicate that: 1. All the New World and Old World monkeys examined exhibit stable and easily detectable hepatic urate oxidase activity 2. Primate hepatic urate oxidase has a lower specific activity than mouse and rabbit hepatic urate oxidase 3. Representative species of the five genera of hominoids all lack hepatic urate oxidase activity 4. No detectable activator or inhibitor in whole liver homogenates of urate oxidase activity is present in hominoids, New World

monkeys and Old World monkeys 5. There exist no evidence for intermediate steps in the loss of urate oxidase activity in the hominoids 6. Urate oxidase activity was silenced in an ancestor of the five present day genera of hominoids after the ancestor diverged from the Old World monkeys.

Christen and coworkers (1970a, 1970b) and Wacker (1970) claimed without published data that urate oxidase activity is highly labile in some New World and Old World monkeys. This assertion is the main premise of a model proposed to explain the loss of urate oxidase activity during primate evolution. No evidence of highly labile urate oxidase activity was observed in this study.

Introduction

For many mammalian species, the terminal step in the metabolic degradation of purines is the oxidation of uric acid by urate oxidase [E.C.1.7.3.3] to the excretory product allantoin . The principle subcellular location of urate oxidase is the peroxisome of hepatic tissue(Essner, 1969; Masters and Holmes, 1977; Smith, 1979). Human disorders associated with elevated uric acid levels have prompted examination of serum uric acid levels and urate oxidase activity in a variety of vertebrates including nonhuman primates.

A survey of the literature reveals that hepatic urate oxidase activity levels have been examined in a few New World monkeys, Old World monkeys and hominoids (Wells, 1909; Wells and Caldwell, 1914; Sorensen,1959; de la Iglesia et al., 1966; Christen et al., 1970a and 1970b; Nakajima and Bourne, 1970). Past studies have employed urate oxidase activity assays which measured the rate of disappearance of uric acid or which monitored the consumption of oxygen during the enzymatic oxidation of uric acid. These assays are not particularly sensitive to low levels of urate oxidase. Often proper controls were not included and therefore these studies could not distinguish between urate oxidase activity and non-specific oxidation of uric acid. In this study an ultrasensitive microradiochemical assay measured the rate of synthesis of [^{14}C]allantoin from [^{14}C]uric acid and consequently non-specific oxidation of uric acid can be ruled out (Friedman and Merril, 1973; Friedman and Johnson, 1977). Furthermore, appropriate controls were used to assure that tissue samples were stored properly for maintenance of enzymatic

activity.

At present, the limited data available on humans, orangutans, and chimpanzees indicates that no detectable hepatic urate oxidase activity is found in these hominoids (Wells and Cadwell, 1914; Sorensen, 1959). Urate oxidase activity levels were measured in five different primate species by Christen and coworkers (1970a and 1970b). They reported that two species of New World monkeys completely lacked urate oxidase activity and that one New World and one Old World monkey, Aotes trivirgatus and Macaca mulatta respectively, had highly labile urate oxidase activity although the data was not provided. The unpublished observations on the lability of urate oxidase in Aotes and Macaca formed the basis of postulated multiple steps in the loss of urate oxidase activty during primate evolution.

In the step-wise mutational model, a genetic change in a primate ancestor resulted in reduced and/or labile urate oxidase activity. An additional mutation or series of mutations which followed in time, presumably silenced urate oxidase activity entirely in a hominoid ancestor while the extant genotype that gives rise to low and/or labile urate oxidase activity can be observed in some New and Old World monkeys. Furthermore, additional mutations in some New and Old World monkeys supposedly resulted in : 1. further reduction in urate oxidase activity, 2. a more highly labile variant of urate oxidase activity, or 3. the complete loss of urate oxidase activity. Despite the pausity of supporting data, this postulate of a step-wise series of mutations resulting in the loss of hepatic urate oxidase activity

during primate evolution is widely accepted. (Orowan, 1955; Christen et al.,1970a, 1970b; Wacker, 1970; Kuster et al., 1972; Logan et al.,1976; Roch-Ramel and Peters, 1978; Ames et al., 1981).

The genetic events that occured during the evolution of primates resulting in the loss of urate oxidase activity in hominoids are unknown. However, there exists considerable speculation about the adaptive significance of elevated uric acid levels in primates. Of special interest are the affects on human development which may have resulted from the increase in serum uric acid level which followed the loss of urate oxidase activity. Since uric acid is structurally similar to caffeine and theobromine, Orowan (1955) proposed that it may mimic their effect and act as a cerebral stimulant. According to Orowan, the loss of urate oxidase activity in primates and the concurrent rise of serum uric acid levels produced an abrupt increase in intellectual potential and thus may have been a significant event in man's evolution. Ames and coworkers (1981) suggested that since uric acid is a powerful antioxidant it may act as a sink for free radicals and singlet oxygens present in the serum. Smith (1983) has demonstrated that uric acid is an effective antioxidant for unsaturated fatty acids and that it protects lipids of cell membranes. High serum uric acid levels may have contributed to a lengthened hominoid life span and a decreased cancer rate (Proctor, 1970; Ames et al. 1981; Smith, 1983).

This study re-examines and extends previous findings on primate urate oxidase activity and the stability of primate urate oxidase activity. Seven species of hominoids, which included gorilla and

gibbon, which had not previously been examined for uricolytic activity, were assayed for hepatic urate oxidase activity. The evolutionary relationship among primates and the results, on the presence and absence of urate oxidase activity, from this study were compared in order to estimate at which phase during primate evolution urate oxidase activity was lost.

Materials and Methods

Chemicals

Xanthine-2 [^{14}C] (48mCi/mmole) and uric acid-2[^{14}C] (53mCi/mmole) were acquired from Research Products International, Elk Grove, Illinois and Amersham, Arlington Heights Illinois, respectively. Xanthine, uric acid, allantoin and EDTA were purchased from Sigma and borate from MCB. Polygram cel 300PEI thin layer sheets were supplied by Brinkman Instuments, Westbury, New York.

Liver Samples

The human liver samples used in this study were obtained from patients of Drs. J. Mayle and D. Greenbaum (College of Human Medicine, Michigan State University). Human liver samples were biopsied by percutaneous needle biopsy from patients requiring histological examination to diagnose a suspected liver dysfunction. Only when the amount tissue was in excess of that required for medical examination was the tissue used for assaying urate oxidase and xanthine oxidase activity. Permission from Michigan State University and local hospital research committees was obtained and the guidelines of these institutions regulating informed consent were followed.

Human liver samples were immediately frozen on dry ice and stored at -70 ^{o}C before being assayed for urate oxidase and xanthine oxidase activity. Forty to eighty microliters of deionized water was added to the human liver sample and the tissue was then disrupted in a Kontes Duall glass/glass homogenizer. The mean protein concentration was 7.6mg/ml with a range of 1.1mg/ml to 25mg/ml.

Nonhuman primate liver samples were obtained from six different primate centers in the United States. Liver samples were obtained at autopsy or were biopsied from live animals. For nonhuman primates, depending on the size of the liver sample, deionized water was added to yield a mean protein concentration of approximately 168mg/ml with a range of 113mg/ml to 282mg/ml. Protein concentrations were determined by the Bradford method (Bradford, 1976) using bovine serum albumin as the standard. The sources and conditions of the primates livers are decribed in Table 1.

Enzyme Assays

Urate Oxidase Assay

Urate oxidase activity was assayed by modifications of an ultrasensitive microradiochemical assay procedure which measures the production of [^{14}C]allantoin from [^{14}C]uric acid (Friedman and Merril, 1973; Friedman and Johnson, 1977). The commercially prepared [^{14}C]uric acid was further purified on a cellulose-coated thin layer backed sheet using water as a solvent. This ultrapure [^{14}C]uric acid was used in cases where tissue was suspected of having low or no urate oxidase activity. Optimal assay conditions for urate oxidase activity with respect to EDTA and borate concentration and optimal buffer pH was determined for several primate species (Appendix A).

To assay urate oxidase activity 13.0 microliters of 0.01M borate/ 15.7mM EDTA pH 9.0 buffer, 2.0 microliters of primate liver homogenate and 10.0 microliters of 0.79mM [^{14}C]uric acid were

mixed and incubated in a 37°C water bath. At designated times, 5.0 microliter aliquots were removed from the reaction mixture and spotted on a PEI cellulose thin layer plate. Prior to spotting the reaction mixture, the PEI plates were washed and allowed to air dry and lanes were lightly penciled on the surface. Each sample site was spotted with 5.0 microliters of 16.0 mM allantoin which was visualized with dimethylamionbenzaldehyde. The allantoin served as a marker for identifing the location of [^{14}C]allantoin. One-dimensional chromatography was carried out in an ascending fashion to a height of 10 centimeters using 0.15M NaCl:100% EtOH (4:1) as a solvent. [^{14}C]allantoin spots were cut out and placed in Nalgene filmware scintillation bags with 3.0 milliliters of scintillation cocktail. The scintillation bags were sealed, wiped with 70% ethanol to eliminate static charges and counted in a Beckman LS 7500 liquid scintillation spectrometer. The average counting efficiency for all experimental runs was 97%.

Xanthine Oxidase Assay

An ultrasensitive microradiochemical assay which measures the production of [^{14}C]uric acid from [^{14}C]allantoin was used in this study. The xanthine oxidase assay is similar to the urate oxidase assay with the following exceptions: Ten microliters of primate liver homogenate was combined with 5.0 microliters of 0.1 M cyclohexylaminopropane sulfonic acid buffer (CAPS), pH 11.0 and 10.0 microliters of [^{14}C]xanthine. Five microliters of 2.9mM uric acid served as a marker and was visualized under short wave ultraviolet light. The solvent used consisted of

glycerol:isopropanol:0.15M LiCl in water (1:9:10).

Concurrent Controls

Primate liver homogenates were simultaneously assayed for urate oxidase and xanthine oxidase. At the same time mouse liver homogenates were also assayed for urate oxidase and xanthine oxidase. These two concurrent positive control assays assured that an absence of urate oxidase activity in a particular primate liver homogenate could not be attributed to a technical error in the assay procedure. Primate liver homogenates were also boiled and assayed for urate oxidase and xanthine oxidase activity. Assays using boiled homogenates and assays in which primate liver was omitted from the reaction mixture served as negative controls.

Stability of Urate Oxidase And Xanthine Oxidase Activity

The stability of primate liver urate oxidase activity was examined under the following conditions: 1. After maintaining liver homogenate at 0-4 ^{o}C for 7.5 and 12 hours, 2. After maintaining liver homogenate at room temperature (23 ^{o}C) for 1 and 2.5 hours, 3. After subjecting the liver homogenate to five and ten rapid freeze-thaw cycles (-70 to 23 ^{o}C). Primate liver xanthine oxidase activity was measured after five freeze-thaw cycles. Rabbit and mouse liver homogenates were also subjected to some of the same treatments before being assayed for urate oxidase and xanthine oxidase activity .

Results

All hominoids, New World and Old World monkeys reported in table 2 had xanthine oxidase activity. Xanthine oxidase activity served as a control, since the existence of xanthine oxidase in a primate liver homogenate suggests that the tissue sample was properly preserved and could be assayed for urate oxidase activity with confidence. Xanthine oxidase activity is believed to be present in all primates except humans with xanthinuria, a rare inherited deficiency (Holmes and Wyngaarden, 1983). When a primate liver sample exhibited no urate oxidase and no xanthine oxidase activity, as was the case with one Erythrocebus patas sample, it suggested that the liver sample was not properly stored. Another Erythrocebus patas liver sample could not be obtained to verify the presence of xanthine oxidase and urate oxidase in this primate species. However, a liver sample from a closely related genus, Miopithecus talapoin, was assayed and exhibited easily detectable levels of these two enzyme.

Livers from six humans, three chimpanzees, two orangutans, two gibbons and one gorilla were assayed for urate oxidase and xanthine oxidase activity (Table 2). These represent species from the five living genera of hominoids. No urate oxidase activity was detected in any hominoid liver sample (Table 2). Seven species of Old World monkeys and three species of New World monkeys all had easily detectable levels of urate oxidase activity.

When the specific activity of hepatic urate oxidase in New World and Old World monkeys and that found in rabbit and mouse livers are compared, a significant quantitative difference is observed

(Table 3). The combined average specific activity of urate oxidase from New World and Old World monkey liver homogenates is 16.5% and 28.9% of the urate oxidase activity observed in rabbit and in mouse liver, respectively. The observed specific activity differences could reflect an increased *in vitro* lability of New World and Old World monkey hepatic urate oxidase activity.

The stability of primate urate oxidase activity was investigated under three different sets of conditions (Table 4 & 5). Urate oxidase activity from nine species of Old World and three species of New World monkeys appear to be as stable as urate oxidase activity from rabbit and mouse liver. For example, after ten freeze-thaw cycles or after maintaining liver homogenates at 23 °C for 2.5 hours, the percent of initial *Macaca mulatta* urate oxidase activity remaining was nearly identical to the percent of rabbit or mouse liver urate oxidase activity enduring the same treatment (Table 4 & 5). Primate xanthine oxidase activity was as stable as rabbit or mouse liver xanthine oxidase activity (Table 4 & 5). The most distinctive aspect of these results is that primate liver urate oxidase activity and xanthine oxidase activity, when present, are stable.

Discussion

There are inconsistencies among published reports on absence or presence of hepatic urate oxidase activity in different species of hominoids, New World and Old World monkeys. The following characteristics of the experimental design used in this study make the results reported here definitive and unequivocal:

1. A direct ultrasensative microradiochemical urate oxidase activity assay which measures the rate of synthesis of [^{14}C] allantoin from [^{14}C]uric acid excludes the possibility of non-specific oxidation of uric acid by other enzymes or non-enzymatic degradation of uric acid.

2. To be certain that the primate liver was removed and stored properly soon after death of the animal, a direct ultrasensitive microradiochemical xanthine oxidase activity assay which measures the rate of synthesis of [^{14}C]uric acid from [^{14}C]xanthine was simultaniously performed on all primate liver homogenates that were assayed for urate oxidase activity.

3. At the same time that primate liver homogenates were assayed for urate oxidase and xanthine oxidase activity positive control assays for these two enzymes were carried out on mouse liver homogenates; this monitored any variation of urate oxidase activity during different experimental runs and revealed any technical error.

4. A small sample of each liver homogenate was boiled and subsequently assayed for both urate oxidase and xanthine oxidase activity; these boiled homogenates were used as a negative control and to establish a background level of [^{14}C]allantoin since all enzymatic activity is extinguished by the boiling process.

5. In primate liver homogenates where little or no urate activity was expected the commercially prepared [^{14}C]uric acid was further purified to remove trace contaminants which comigrate with [^{14}C] allantoin; this procedure reduced the background counts of [^{14}C]allantoin and increased the sensitivity of the assay.

The results indicate that representatives of the five living genera of hominoids, adult human, gorilla, chimpanzee, orangutan and gibbon, all lack urate oxidase activity. Seven species of Old World monkeys and three species of New World monkeys have easily detectable urate oxidase activity which are as stable as the urate oxidase activity found in rabbits and mice (table 4 & 5). These results are not in agreement with the unpublished observations made by Christen and coworkers (1970a and 1970b) and Wacker (1970). Christen and cowokers claimed that Macaca mulatta and Aotes trivirgates liver homogenates stored at -20°C for one day lost a substantial amount of the total urate oxidase activity which was previously observed. Wacker (1970) reported, presumably based on the same unpublished observation, that no urate oxidase activity could be detected in liver homogenates from Macaca and Aotes after storage at -20°C for 24 hours. Based on their unpublished observation, these investigators erroneously concluded that urate oxidase activity from Macaca and Aotes was highly labile. Despite the lack of any supporting evidence, the notion that urate oxidase activity is highly labile in primates is often sited. As the underlying premise to the popular multiple step mutational model for the gradual evolutionary silencing of urate oxidase activity in primates, the stability of urate oxidase must be carefully examined.

Christen and coworkers' (1970a and 1970b) postulate on the loss of urate oxidase activity, attributes the increased lability of primate urate oxidase to a mutational event. This first mutation was believed to have occured preceeding the divergence of

hominoids form the Old and New World monkeys. A testable prediction from this hypothesis is that this first mutational event, and therefore its' effect, can be found in present day Old and New World monkeys. The hypothesis further states that the manner by which urate oxidase activity was lost in the different primate species may have been completely independent. Also, Old and New World monkeys which now have different genetic backgrounds, might have different degrees of urate oxidase lability.

This study found no hint, by three criteria, of highly labile urate oxidase activity or xanthine oxidase activity in nine species of Old World monkeys and in three species of New World monkeys (Table 4 & 5). Old World and New World monkey livers stored at -70^{o} C for greater than 52 months and 46 months respectivly, have easily detectable urate oxidase and xanthine oxidase activity (Table 1). The rate of loss, after treatment (Table 4 & 5), of urate oxidase and xanthine oxidase in a whole liver homogenate of Macaca mulatta and Cebus apella was not significantly different from the rate of loss of mouse or rabbit liver urate oxidase and xanthine oxidase activity. The observed variation in the rates of loss of urate oxidase activity, after the various treatments among the different Old and New World monkey liver homogenates, is not significantly different either (Table 4 & 5).

No evidence of highly labile urate oxidase activity was observed in Old and New World monkeys. Furthermore, urate oxidase actvity of all primates appears to be equally stable (Table 1, 4 & 5) and also as stable as urate oxidase from rabbit and mice liver

homogenates. The presence or absence of primate urate oxidase activity was easily demonstrated in liver homogenate preparation and the results were unambiguous and definative. On the basis of the results from this study and with particular consideration of the evolutinary relationship among primates, as judged by morphological, chromosomal, biochemical and molecular evidence (Templeton, 1983), it appears as if a single mutational event silenced urate oxidase activity in a homonoid ancestor after divergence from the Old World monkeys (Fig. 1).

Macaca mulatta hepatic urate oxidase is sufficiently stable to be purified to apparent homogeneity as evidenced by a single major silver stained peptide band in an SDS polyacrylamide gel of a high urate oxidase activity fraction obtained by conventional affinity chromatography procedures (Fig. 2). The evidence presented in this study suggests that primate urate oxidase activity, when present, is stable. The observed variation in specific activity of urate oxidase in the different primate liver homogenates (Table 3) may be due to the differences between and among species, time and temperature of storage of liver tissues prior to assaying, age and sex of the animal (Townsend and Lata, 1969) and medical history and diet of the animal.

Endogenous levels of unknown competitive or non-competitive inhibitors of urate oxidase activity may also modify the in vitro urate oxidase activity levels. When liver homogenates from primates with and without urate oxidase activity were mixed there was no detectable inhibition or activation of urate oxidase activity in either homogenate in the mixture. The predicted

values of urate oxidase activity in the mixtures closely resembled those observed (Table 6).

The specific activity of urate oxidase of primates is lower than that of other mammals, such as the mouse or rabbit (Table 3). Rabbit liver urate oxidase specific activity levels are 4.5 and 10.1 times greater than the specific activity observed in liver homogenates from Cebus apella and Cebus albifrons, respectively. Using Christen and coworkers' (1970a and 1970b) data, similar differences in specific activities can be calculated. The ratio of the urate oxidase activity of rabbits to Cebus apella and Cebus albifrons calculated from Christen and coworkers' data is 9.67 and 7.25, respectively. Christen and coworkers (1970a and 1970b) misinterpreted their own findings when they concluded that urate oxidase activity was absent in Cebus. Based on the results from this study and Christen and coworkers' (1970a and 1970b) findings, Cebus apella and Cebus albifrons have easily detectable levels of stable liver urate oxidase activities and that the specific activities are approximately 21% and 10%, respectively, of that observed in rabbits.

Christen and coworkers (1970a and 1970b) multiple step mutational model for the gradual evolutionary elimination of urate oxidase activity in a hominoid ancestor is not refuted by the results of this study herein reported. However, since no evidence for a highly labile urate oxidase was found in any primates tested (Table 1, 4 & 5 ; Fig. 2) such a complex explanation without any data for intermidiate steps in the loss of detectable urate must be questioned. The simplest explanation, which is consistent with the data , on the presence, absence and stability of urate

oxidase activity and which is in accordance with the genealogical relationships among primates, is that a single mutational event in a common hominoid ancestor resulted in the loss of urate oxidase activity. This single mutational event occurred in a hominoid ancestor after the divergence of hominoids from the Old World monkeys (Fig 1).

To successfully understand the genetic mechanism by which urate oxidase activity was lost in a hominoid ancestor, several plausible explanations must be considered. A mutation in the primate urate oxidase structural gene as well as a mutation in any cis-acting regulatory regions may result in absence of urate oxidase activity. Furthermore, in Drosophila, the presence of urate oxidase activity has been shown to be repressed by the steroid hormone ecdysone (Kral et al. 1982). This finding suggest that the absence of urate oxidase in primates could result from a mutation in any one of several different genes whose product acts upon the primate urate oxidase structural gene or directly on the primate enzyme. Other events which may account for the loss of urate oxidase activity are 1. A chromosomal break involving the urate oxidase structural gene giving rise to an inversion, a deletion or a translocation, 2. An insertion element integrating into a region necessary for the expression of urate oxidase activity.

A polyclonal antibody to Macaca mulatta urate oxidase and a cloned gene for Macaca urate oxidase could be used in experiments designed to distinguish between the various mutational events which may account for the loss of urate oxidase activity in

hominoids.

Recently, Martin and coworkers (1983), examined the molecular basis for the inactivation of the delta globin gene in Old World monkeys. The delta globin gene is present in hominoids, Old World and New World monkeys but is no longer expressed in Old World monkeys. A molecular comparison of the delta globin gene of Macaca and Colobus disclosed three 5' point mutations that are shared by these two Old World monkeys and several other mutations found exclusively in one monkey or the other but not in both. Martin and coworkers (1983) reasoned that one of the three point mutations shared by Macaca and Colobus was responsible for the silencing of the delta globin gene while the mutations unique to each species occurred after the gene was silenced.

The results of the study reported herein indicate that all five genera of hominoids are completely devoid of urate oxidase activity. In addition, all New World and Old World monkeys examined had stable and easily detectable urate oxidase activity. The mutational event which silenced urate oxidase activity occurred in a hominoid ancestor after it diverged from the Old World monkeys (Fig 1).

TABLE 1. SOURCE AND CONDITION OF HEPATIC TISSUE FOR URATE OXIDASE AND XANTHINE OXIDASE ASSAYS

SPECIE	ISOLATION AND STORAGE CONDITIONS(2)	SOURCE(1)
HOMINOIDS		
Homo sapiens	LF/0/	Michigan State University Clinical Center, Sparrow Hospital Ingham County Hospital
Pan troglodytes	LF/00/	Primate Research Institute Holloman Air Force Base, a
Pan panicus	DF/ST20/42.3/	Yerkeys Regional Primate Research Center/Emory University, d
Pongo pygmaeus pygmaeus	DF/ST20/36.3/	Yerkeys Regional Primate Research Center/Emory University, d
Gorilla gorilla	DF/ST20/18	Yerkeys Regional Primate Research Center/Emory University, c
Hylobates lar	LF/00/	Yerkeys Regional Primate Research Center/Emory University, c
Hylobates concolor	DF/ST70/20.5/	National Zoological Park, Department of Pathology, f
CERCOPITHECOIDS		
Papio cynocephalus anubis	DF/ST70/12.2/	Regional Primate Research Center/University of Washington, b
Macaca fascicularis	DF/ST70/13.6/	Regional Primate Research Center/University of Washington, b
Macaca mulatta	DF/ST70/0.4/	Regional Primate Research Center/University of Washington, b
Macaca nemestrina	DF/ST70/0.4/	Regional Primate Research Center/University of Washington, b
Miopothecus talapoin	DF/ST70/52.5/	University of California, Berkley Dr. L. Bromley, c
Presbytis entellus	DF/ST70/16.3/	University of California, Berkley Dr. P. Dohlinow, c
Colobus guereza	DF/ST70/46.1/	National Zoological Park, Department of Pathology, f
PLATYRRHINI		
Cebus apella	DF/ST70/35.7/	University of California, Berkley, c
Cebus albifrons	DF/ST70/0.5/	Oregon Regional Primate Research Center, e
Callicebus moloch	DF/ST70/46.3/	National Zoological Park, Department of Pathology, f
Mus musculus (Swiss ICR)	L/0/	Harlan Inc.
Orchytolagus cuniclus (New Zeland white rabbit)	LF/ST70/0.3/	Bailey Inc. Michigan

Table Legend 1.

(1) The following persons provided liver samples.

a. Dr. C.E. Graham and Dr. L.B. Cummins
b. Dr. J.A. Johnson
c. B. Stewart and Dr. A.C. Wilson
d. Dr. H.M. McClure and Dr. E. Lockwood
e. Sharon Maher
f. Dr. J. Lagenberg, Dr. R. Montali and R. Freeman

(2) Isolation and Storage Condition Code

L = Removed from a live animal, homogenized immediately and assayed for urate oxidase and xanthine oxidase activity.
LF = Removed from a living organism, frozen on dry ice, thawed, homogenized and assayed for urate oxidase and xanthine oxidase activity.
ST70 = Stored at -70 C
ST20 = Stored at -20 C

The second set of two digits following the temperature of storage are the number of months of storage at the designated temperature prior to assaying. A 0 indicates tissue homogenized and used within one hour of biopsie. 00 indicates tissue frozen on dry ice immediately after biopsie, thawed, homogenized and assayed within 24 hours of biopsie. D and DF are identical conditions to L and LF except the tissue was removed from a recently deceased or sacrificed animal. The numbers following D and DF are explained above. The sex of animal(s) is indicated.

TABLE 2. PRESENCE OF URATE OXIDASE AND XANTHINE OXIDASE ACTIVITY IN PRIMATES

SPECIE	URATE OXIDASE ACTIVITY Present or absent(d)	XANTHINE OXIDASE ACTIVITY Present or absent(d)
HOMINOIDS(a)		
Homo sapiens(6)	-	+
Pan troglodytes(2)	-	+
Pan panicus(1)	-	+
Pongo pygmaeus pygmaeus(2)	-	+
Gorilla gorilla(1)	-	+
Hylobates lar(1)	-	+
Hylobates concolor(1)	-	+
CERCOPITHECOIDS(b)		
Papio cynocephalus anubis(1)	+	+
Macaca fascicularis(1)	+	+
Macaca mulatta(3)	+	+
Macaca nemestrina(1)	+	+
Miopithecus talapoin(1)	+	+
Presbytis entellus(2)	+	+
Colobus guereza(1)	+	+
PLATYRRHINI(c)		
Cebus apella(1)	+	+
Cebus albifrons(1)	+	+
Callicebus moloch(1)	+	+
OTHER MAMMALS		
Mus musculus(4)	+	+
Orchytolagus cuniclus(1)	+	+

The number in parenthesis following the species name is the number of liver biopsies from different animals which were assayed for urate oxidase and xanthine oxidase activity.

a) great apes
b) Old World monkeys
c) New World monkeys
d) + = easily detectable levels of activity; - = no detectable activity

TABLE 3. URATE OXIDASE AND XANTHINE OXIDASE SPECIFIC ACTIVITY IN PRIMATES

SPECIE	URATE OXIDASE ACTIVITY Specific activity(d)	XANTHINE OXIDASE ACTIVITY Specific activity(e)
HOMINOIDS(a)		
Homo sapiens(6)	0	596
Pan troglodytes(2)	0	137
Pan panicus(1)	0	13,700
Pongo pygmaeus pygmaeus(2)	0	12,100
Gorilla gorilla(1)	0	12,900
Hylobates lar(1)	0	1,850
Hylobates concolor(1)	0	3,400
CERCOPITHECOIDS(b)		
Papio cynocephalus anubis(1)	64,300	3,200
Macaca fascicularis(1)	58,600	2,850
Macaca mulatta(3)	28,900	4,950
Macaca nemestrina(1)	34,900	10,550
Mionothecus talapoin(1)	36,800	4,650
Presbytis entellus(2)	32,850	2,900
Colobus guereza(1)	28,900	8,300
PLATYRRHINI(c)		
Cebus apella(1)	50,525	3,950
Cebus albifrons(1)	23,550	6,150
Callicebus moloch(1)	34,110	11,940
OTHER MAMMALS		
Mus musculus(4)	135,650	10,600
Orchytolagus cuniculus(1)	237,550	8,950

The number in parenthesis following the species name is the number of liver biopsies from different animals which were assayed for urate oxidase and xanthine oxidase activity.

a) great apes
b) Old World monkeys
c) New World monkeys
d) Specific activity is the initial rate of synthesis of 14 C-allantoin from 14 C-uric acid expressed as the change in CPM per minute of reaction time divided by protein concentration in milligrams per milliliter. At 95% counting efficiency, 112 CPM equals 1 x 10(-6) u moles of 14 C-allantoin.
e) Specific activity is the initial rate of synthesis of 14 C-uric acid from 14 C-xanthine expressed as the change in CPM per minute of reaction time divided by protein concentration in milligrams per milliliter. At 95% counting efficiency, 120 CPM equals 1 x 10(-6) u moles of 14 C-uric acid.

TABLE 4. STABILITY OF PRIMATE URATE OXIDAS

Pg 26 is not missing — — — misnumbered

SPECIE	PERCENT OF URATE ACTIVITY REMAINING AFTER TREATMENT: Freeze thaw 5x	1	
CERCOPITHECOIDS			
Papio cynocephalus anubis	31.4		
Macaca fascicularis	68.6	74.7	
Macaca mulatta(a)	82.8	58.9	41.9
Macaca nemestrina	--	77.6	39.8
Miopithecus talapoin	32.1	38.8	41.7
Presbytis entellus	36.4	29.3	61.6
Colobus guereza	67.3	--	--
PLATYRRHINI			
Cebus apella	47.1	32.1	51.3
Cebus albifrons	52.6	--	49.7
Callicebus moloch	84.8	--	77.6
OTHER MAMMALS			
Mus musculus	62.8	--	56.4
Orchytolagus cuniclus	67.5	--	71.3

One freeze thaw cycle = freezing homogenate at -70 C for 10 minutes, completely thawing sample at room temperature, waiting one minute and freezing at -70 C.

a) Data is the mean of two independant determinations from hepatic tissue of two different animals.

TABLE 5. STABILITY OF PRIMATE URATE OXIDASE ACTIVITY AND XANTHINE OXIDASE ACTIVITY

	PERCENT OF URATE OXIDASE ACTIVITY REMAINING AFTER TREATMENT			
	Hours at 4 C		Hours at 23 C	
SPECIE	2.5 hrs.	12 hrs.	1 hrs.	2.5 hrs.
CERCOPITHECOIDS				
Papio cynocephalus anubis	60.5	27.7	67.7	71.9
Macaca fascicularis	64.3	58.3	45.9	39.1
Macaca mulatta(a)	62.8	53.8	94.2	79.6
Macaca nemestrina	75.6	73.2	--	--
Miopithecus talapoin	42.1	19.1	66.8	51.2
Presbytis entellus	45.8	42.9	28.4	27.6
Colobus guereza	--	--	--	--
PLATYRRHINI				
Cebus apella	51.3	58.9	49.1	--
Cebus albifrons	--	47.9	--	--
Callicebus moloch	--	--	--	--
OTHER MAMMALS				
Mus musculus	--	78.6	--	--
Oryctolagus cuniculus	--	69.7	--	--

a) Data is the mean of two independant determinations from hepatic tissue of two different animals.

TABLE 6. MIXING LIVER HOMOGENATES TO TEST FOR DIFFUSIBLE INHIBITORS OR ACTIVATORS OF URATE OXIDASE ACTIVITY IN HOMINOIDS, OLD WORLD AND NEW WORLD MONKEYS

Experiment #1: *Pongo pygmaeus* mixed with *Miopithecus talapoin*

Experiment #2: *Pan paniscus* mixed with *Cebus albifrons*

Species A	Urate oxidase activity(a)	ug protein in assay	Species B	Urate oxidase activity(a)	ug protein in assay	total ug protein
Pongo	0	0	*Miopithecus*	6691 +263	435	435
	0	180			435	615
	0	450			435	885
	0	720			435	1155
Pan	0	0	*Cebus*	4679 +198	385	385
	0	143			385	528
	0	358			385	743
	0	572			385	957

RESULTS:

% Urate oxidase activity expected if no inhibitor or activator in mixture(b)	% Urate oxidase activity observed in mixture(c)	Urate oxidase activity of mixture(a)
100	100	-
71	79	5294
49	53	3553
38	37	2487
100	100	-
73	61	2866
52	48	2258
40	32	1512

(a) Urate oxidase activity is equal to CPM of 14 C-allantoin produced from 14 C-uric acid per minute of reaction time per ug of protein.

(b) % Urate oxidase activity expected is equal to ratio of ug of protein of species B divided by total ug of protein.

(c) % Urate oxidase activity observed is equal to urate oxidase activity of mixture divided by urate oxidase activity of species B.

Fig. 1.

Phylogram of Platyrrhini. Cercopithecoids and Hominoids. The divergence dates are from Sibley and Ahlquist (1984)

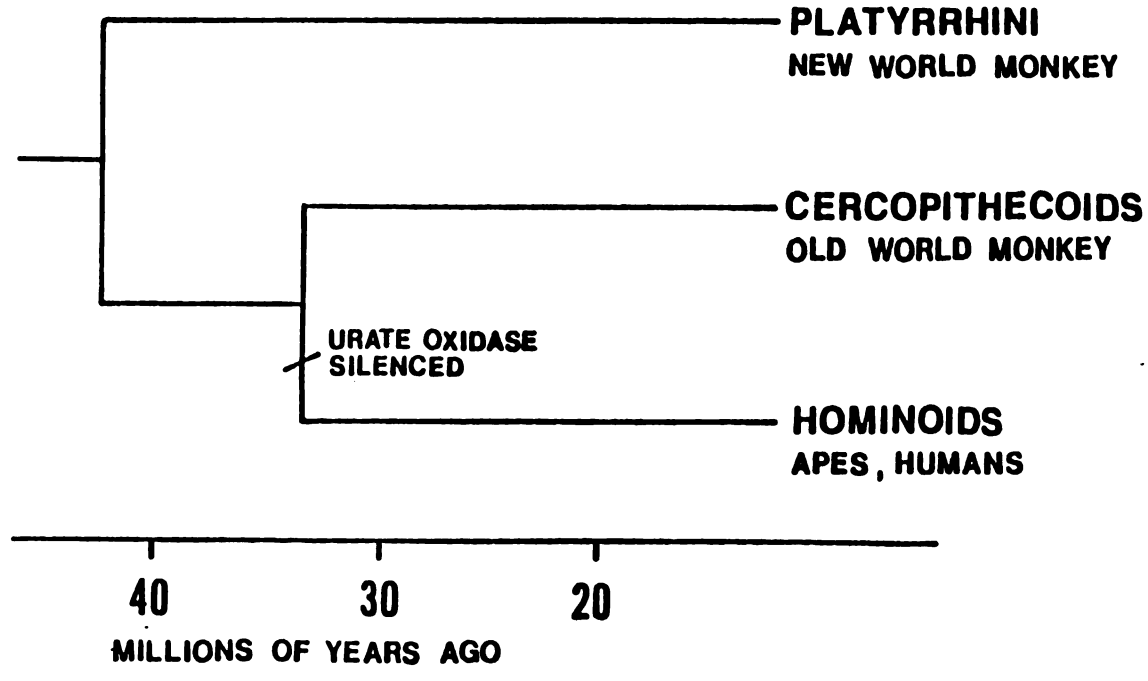
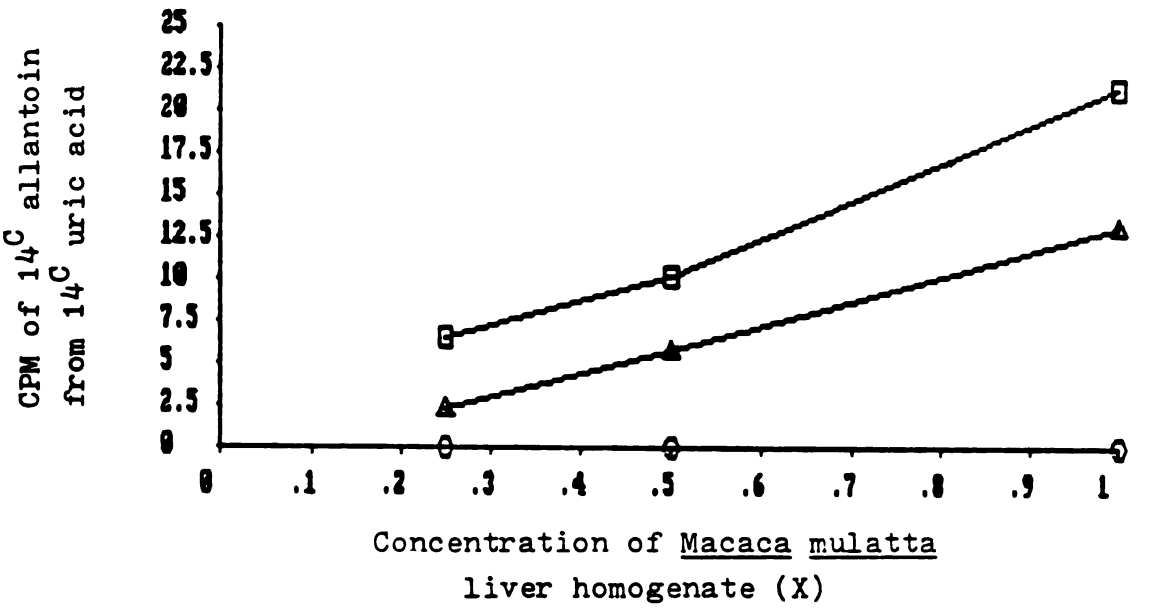


FIG. A URATE OXIDASE ACTIVITY WITH RESPECT TO CONCENTRATION OF MACACA mulatta LIVER HOMOGENATE



○ - CPM after 0 minutes of reaction time
△ - CPM after 5 minutes of reaction time
□ - CPM after 10 minutes of reation time

FIG. B MACACA mulatta URATE OXIDASE ACTIVITY WITH RESPECT TO CONCENTRATION OF EDTA

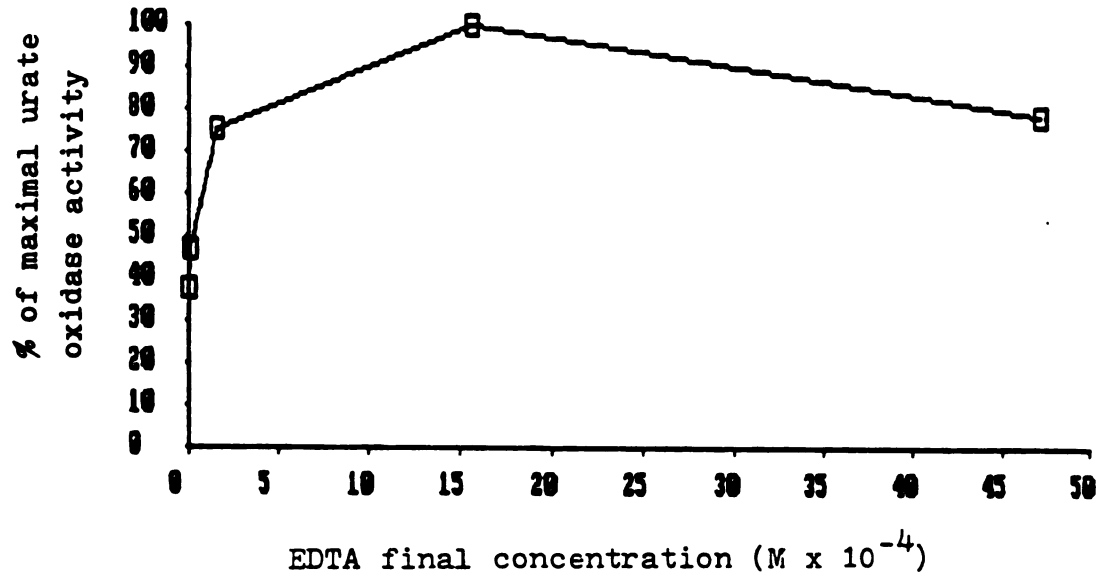
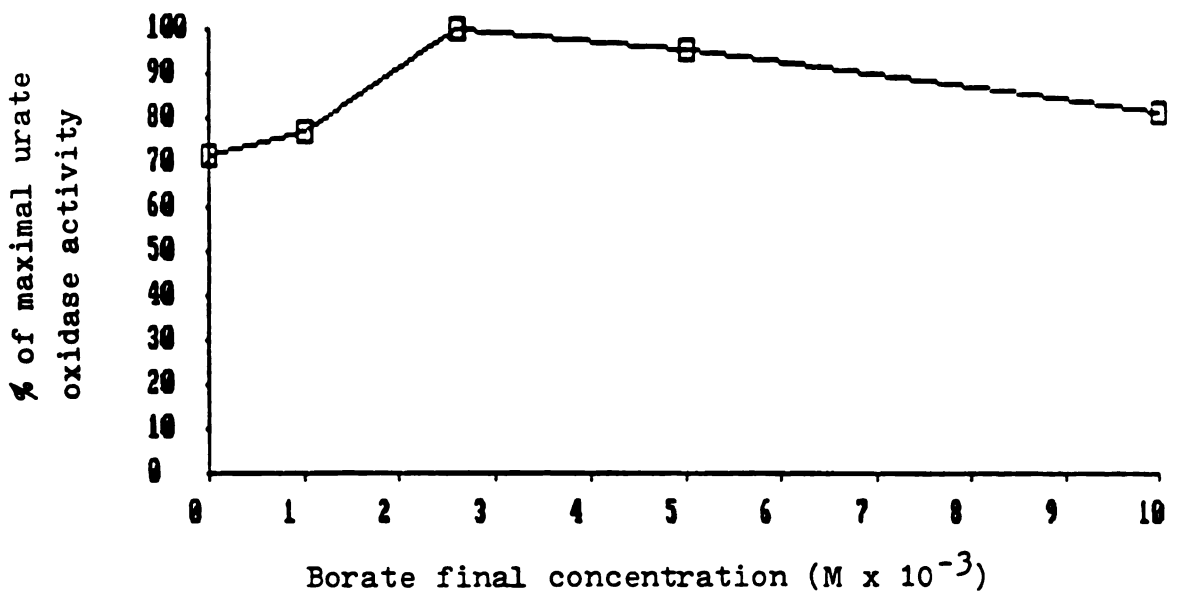


FIG. C MACACA mulatta URATE OXIDASE ACTIVITY WITH RESPECT TO CONCENTRATION OF BORATE



APPENDIX A

PROTOCOL FOR THE PURIFICATION OF URATE OXIDASE FROM MACACA mulatta

1. All operations were carried out at 0-4 °C unless otherwise stated. Two hundred and twenty three grams of Macaca mulatta liver previously frozen and stored at -70 °C was wrapped in several layers of cheeze cloth and aluminum foil and was fragmented, with a hammer, into small pieces.

2. The 223 grams of partially thawed liver were added to a total of 1,858 mls of homogenization buffer (0.25M sucrose, 50 mM tris-HCl,15.7 mM EDTA pH 7.0) and minced in a Virtis triple blade homogenizer (at 10,000 RPM x 2 minutes), liquidized in a Tekmar Polytron Tissuemizer (2 minutes) and reduced into a homogeneous suspension by a drill driven Teflon Potter-Elvejhen homogeninizer (one up and down cycle).

3. The whole liver suspension (W) was seperated into 40 ml aliquots and centrifuged (at 750g x 30 minutes) in a Beckman #J2-21 centrifuge with a JA 20 rotor. The supernatants (S1) were saved and the pellets were washed with a small amount of homogenization buffer, rehomogenized in a Tekman Polytron Tissuemiser as before and centrifuged (at 750g x 30 minutes). The supernatants of this centrifugation were pooled with supernatant S1, collectively called S1b and had a total volume of 630 mls.

4. Forty ml aliquots of S1b were centrifuged (at 48,000g x 30 minutes) and a total of 545 mls of the supernatant, S2, were recovered and saved.

5. To S2, saturated ammonium sulfate was added until it was 40% saturated, centrifuged at 40,000g x 30 minutes, saving the supernatant, to which ammonium sulfate was added to give a final concentration of 55% and centrifuged at 40,000g x 30 minute, saving the pellet, P3.

6. P3 was resuspended in 10 mls homogenization buffer and centrifuged 250,000g x 30 minute. The final supernatant was added to an affinity column to which xanthine was bound.

7. The column fractions were monitored at 280nm by a Pharmacia UV-1 ultraviolet analyzer and recorded on a strip chart. The fractions were assayed for urate oxidase activity by monitoring the decrease in absorbance of a reaction mixture in a Cary 210 spectrophotometer, at 292nm (Friedman and Baker, 1982).

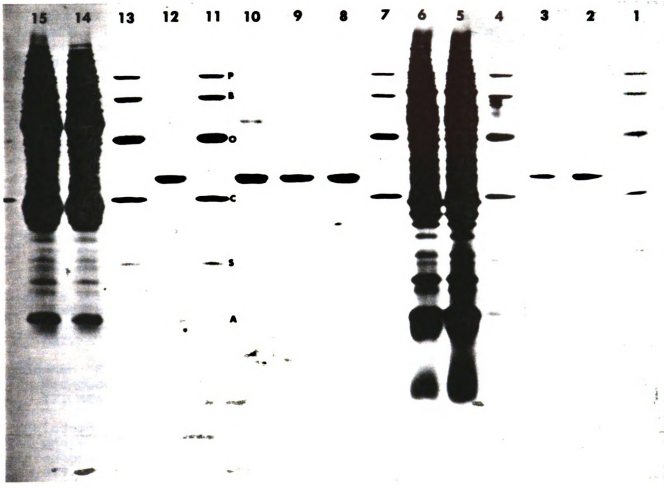
8. High urate oxidase activity fractions were lyophilized, resuspended in Laemmli's sample buffer and electrophoresed in a vertical apparatus (Laemmli, 1970).

APPENDIX B

SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

A 13% separating and a 4% stacking polacylamide slab gel (1.5mm x 100mm x 140xmm) was prepared according to Laemmli (1970). Samples were boiled in 2% SDS and 0.1M mercaptoethanol for 10 minutes and electrophoresis was carried out in the presence of 25mM tris, 19.2mM glycine, 1% SDS buffer, pH 8.3, at a constant current of 10 milliamperes for seven hours at 22 ^{o}C. Proteins were stained with the modified silver stain procedure of Switzer (Friedman and Baker, 1982) which is approximately 100-1000 times more sensative than Coomassie blue R stain. The molecular weight standards were purchased from Pharmacia Fine Chemicals, prepared according to the manufactures directions and further diluted 1/5. The standards and their subunit molecular weights were: (P) Phosphorylase b, 94,000; (B) Bovine Serum Albumin, 67,000; (O) Ovalbumin, 43,000; (C) Carbonic Anhydrase, 30,000; (S) Soybean Trypsin Inhibitor, 20,100; (A) Alpha-Lactalbumin, 14,000.

Fig. 2 SDS Gel Electrophoresis Analysis Of Macaca mulatta Liver Homogenate



Lane 1,4,7,11,13: Molecular weight standards

Lane 2,3: Purified Macaca mulatta urate oxidase (1X)

Lane 5,6: Partially purified Macaca mulatta whole liver homogenate

Lane 8,9,10,12: Purified Macaca mulatta urate oxidase (50X)

Lane 13,14: Macaca mulatta whole liver homogenate

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