

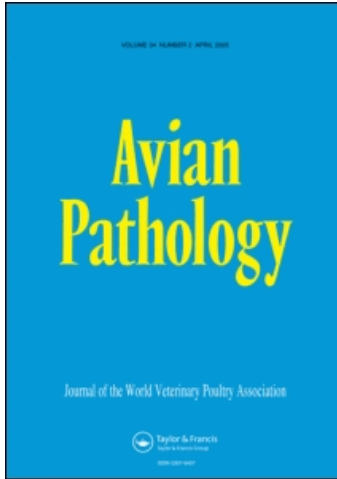
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### Investigations into the uricolytic properties of urate oxidase in a granivorous ( *Columba livia domestica* ) and in a carnivorous ( *Buteo jamaicensis* ) avian species

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# Investigations into the uricolytic properties of urate oxidase in a granivorous (*Columba livia domestica*) and in a carnivorous (*Buteo jamaicensis*) avian species

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**To study the uricolytic properties of urate oxidase in granivorous and carnivorous birds, experiments were conducted in pigeons (*Columba livia domestica*) and Red-tailed Hawks (*Buteo jamaicensis*). Plasma concentrations of allantoin and uric acid were determined at various times in experimental groups before and after receiving 100, 200, and 600 U/kg urate oxidase once daily and were compared with controls. All regimens caused a significant decrease in plasma uric acid concentrations within 2 days after the first administration, when compared with controls. Furthermore, both doses used in Red-tailed Hawks (200 and 100 U/kg) caused a significant postprandial suppression of plasma uric acid concentrations. Plasma allantoin concentrations were significantly higher when compared with controls. The ability of urate oxidase to prevent the physiological postprandial hyperuricaemia in Red-tailed Hawks and to reduce plasma uric acid concentrations to undetectable levels, shows the great potential of this drug for treating avian hyperuricaemia.**

## Introduction

When plasma uric acid concentrations are elevated above the solubility of sodium urate in plasma, urates are deposited within the body, causing gout. Hyperuricaemia can be caused by renal disorders, protein oversupplementation of the diet, and miscellaneous causes. Two forms of gout can be identified: visceral (acute form, uric acid deposits in and on the internal organs and peripheral joints) and articular gout (chronic form, uric acid deposits in peripheral joints). The chronic form of the disease may run a protracted course, extending over months, while the acute form is considered to be fatal for an individual bird within a short period of time, because of the underlying renal failure (Lumeij, 2001). Visceral and articular gout can occur both separately and concurrently. It has been suggested by Siller (1981) and Shivaprasad (1998)

that visceral gout can be chronic, but no substantial proof has been provided. The long-term existence of uric acid deposits on visceral surfaces has not been documented by endoscopic studies.

Articular gout is caused by a chronic condition whereby the concentration of sodium urate in plasma remains just above the solubility in plasma and therefore precipitates at predilection sites (e.g. peripheral joints) (Lumeij, 1994). In an attempt to find a drug to treat avian hyperuricaemia, various studies have been performed investigating the effects of allopurinol in a raptor hyperuricaemic model (Lumeij & Redig, 1992; Lumeij *et al.*, 1998; Poffers *et al.*, 2002). The controlled studies performed thus far have resulted in toxicity (100 mg/kg and 50 mg/kg allopurinol s.i.d.) or no effect (25 mg/kg allopurinol s.i.d.) (Lumeij & Redig, 1992; Lumeij *et al.*, 1998; Poffers *et al.*, 2002). The effect of allopurinol is explained by the (competitive) inhibition of xanthine

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oxidase resulting in accumulation of the uric acid precursors xanthine and hypoxanthine. The poor results obtained with allopurinol in controlled studies in birds prompted the investigation into a drug that causes a further breakdown of uric acid.

While the formation of uric acid is common to all vertebrates, the further degradation varies between vertebrate classes. In principle, the uric acid degradation pathway is composed of five enzymatic reactions, catalysed by urate oxidase (uricase, enzyme classification number (EC) 1.7.3.3) (definition and databanks can be found in Bernstein *et al.*, 1977; *Enzyme Nomenclature*, 1992; Bairoch, 1993), allantoinase (EC 3.5.2.5), allantoinase (EC 3.5.3.4), ureidoglycolate lyase (EC 4.3.2.3), and urease (EC 3.4.1.5), respectively. Degradation products are allantoin, allantoinic acid, ureidoglycolate, urea and ammonia, respectively. The uricolytic pathway seems to have been progressively lost during vertebrate evolution (Wood, 1993). Humans and, most probably, birds do not have a functional uricase gene (Colloch *et al.*, 1997) and excrete uric acid as the end-product of purine metabolism (Christen *et al.*, 1970). Furthermore, in birds, uric acid is not only formed by purine metabolism, but also by protein catabolism via transformation of amino acids into inosine monophosphate (Giminger & Scanes, 1986). From a theoretical point of view, and based on experiences in humans, it is to be expected that urate oxidase (uricase) has uricolytic potential in birds.

When administered to humans, urate oxidase degrades the excess of poorly hydro soluble uric acid to allantoin, which is 10 times more soluble than uric acid and has a higher renal clearance. In humans, the administration of urate oxidase results in a more rapid and larger decrease in plasma uric acid levels when compared with allopurinol (Piu *et al.*, 1997). Uric acid depositions can be resolved initially, causing more severe hyperuricaemia at the start of treatment. Urate oxidase is highly specific for uric acid and urates, and does not interfere with the metabolism of other purines, whereas allopurinol blocks the purine metabolic pathway (Jankovic *et al.*, 1985). Furthermore, there is no substrate accumulation of xanthine. It is reported to be well tolerated, both locally and systemically, while a good renal function is maintained. In humans, urate oxidase is a fast-acting, highly potent drug and a more effective uricolytic agent than allopurinol (Mahmoud *et al.*, 1998). Side effects after iatrogenic urate oxidase administration in humans are few and are mainly of an allergic nature (Piu *et al.*, 1997). Masera *et al.* (1982) suggest combining allopurinol and urate oxidase, which, acting by two different mechanisms, might have a synergistic effect. In contrast, Jankovic *et al.* (1985) do not consider the combination of the two drugs rational, because allopurinol, by blocking the purine metabolic pathway, might possibly reduce the substrate for urate oxidase and, in the meantime, provoke xanthine accumulation.

Based on experience in humans (Masera *et al.*, 1982; Jankovic *et al.*, 1985; Piu *et al.*, 1997; Mahmoud *et al.*, 1998), it was decided to study the effect of urate oxidase in avian hyperuricaemia. To obtain a guideline for an avian dose, initial studies were performed in racing pigeons (*Columba livia domestica*). The physiological postprandial increase of plasma uric acid concentration in raptors (*Buteo jamaicensis*) was then used in a second study to investigate the effects of urate oxidase in a hyperuricaemic avian model (Lumeij & Remple, 1991).

Significant postprandial increases in plasma uric acid and plasma urea concentrations are observed in raptorial birds. Postprandial uric acid concentrations are similar to those in granivorous birds suffering from hyperuricaemia and gout, and are well above the theoretical limit of solubility of sodium urate in plasma (600  $\mu\text{mol/l}$  at 43°C) (Lumeij & Remple, 1991).

## Materials and Methods

### Urate oxidase

Urate oxidase (Uricozyme®; Sanofi Winthrop, Milano, Italy) was extracted, purified and lyophilized from industrial cultures of *Aspergillus flavus*. Urate oxidase can be given intramuscularly or intravenously. One unit corresponds with the amount of the enzyme that breaks down one-half of 100  $\mu\text{g}$  substrate in 10 min at 30°C and pH 8.5 (Sanofi Winthrop, 1995). The dosages used in the racing pigeons were extrapolated using human dosage regimens reported by the manufacturer (Sanofi Winthrop, 1995) as well as the literature (Masera *et al.*, 1982; Jankovic *et al.*, 1985; Piu *et al.*, 1997; Mahmoud *et al.*, 1998), and transformed by metabolic weight ( $\text{BW}^{0.75}$ ). The dosages used in the Red-tailed Hawks were extrapolated from dosages used in the pilot study with racing pigeons and were transformed by metabolic weight.

### Birds and experiments

Eighteen apparently healthy racing pigeons (*C. livia domestica*) were used for the pilot study. Two experimental groups and one control group of six pigeons each were used. One experimental group was given 200 U/kg urate oxidase intramuscularly (i.m.) in the pectoral muscle. The other experimental group was given 600 U/kg urate oxidase i.m. The pigeons came from a group maintained for research and educational purposes at the Division of Avian and Exotic Animal Medicine at the University of Utrecht. Both female and male birds were used; age ranged from 2 to 10 years and weights ranged from 398 to 490 g. Both water and food (Hope Farms, Woerden, Holland, 2400 P40 Pigeon pellets, serial number 00356, mixed in equal parts with Witte Molen, WiMo Duivenvoerders, Meeuwen, Holland, a grain mixture of barley, wheat, millet, red dary, white dary, paddy, kardiseed and white seed) were given *ad libitum* and changed daily. Seventeen days prior to the experiment, the racing pigeons were housed individually so that they could get accustomed to their new environment. They were kept on gratings where they could hear, see and smell each other. The radio was playing constantly and lights were on from 07:00 to 19:00.

Eleven Red-tailed Hawks (*B. jamaicensis*) were used for the experiments in carnivorous birds. Two experiments were conducted 1 month apart using the same 11 birds. The first experiment included an experimental group of six Red-tailed Hawks receiving 200 U/kg urate oxidase i.m. and a control group of five Red-tailed Hawks. The second experiment included an experimental group of six Red-tailed Hawks receiving 100 U/kg urate oxidase i.m. and a control group of five Red-tailed Hawks. In case no significant differences were present (repeated-measures analysis of variance, unpaired Student's *t* test) between both control groups, they were combined as one. Both experimental groups were compared with this combined control group, thus merging the results of two experiments into one group of results. Only five

Red-tailed Hawks were used in the control groups because one of the birds did not get accustomed to living in a cage. He refused to eat during the acclimation period and was therefore excluded from the experiments.

The birds were maintained for research purposes at The Raptor Center at the University of Minnesota. All these birds had been admitted to the Center with wing fractures. None of the birds could fly well enough for release into the wild, but all were judged to be otherwise healthy. Both female and male birds were used, with a great diversity and variation between birds. Weight ranged from 1050 to 1590 g. Water was given *ad libitum* and changed daily. Food was only supplied once after administration of urate oxidase, namely 74 h after the first administration. The birds were fed rats at 10% of their body weight. Four days prior to the experiment, the hawks were housed individually in plastic cages, receiving food daily, so that they would be able to get accustomed to their new environment. The hawks were kept on gratings and rope-perches, and they could hear, see and smell each other. The lights were on from 07:00 to 20:30.

Birds from the experimental groups were given urate oxidase i.m., halfway down the keel, 1 cm lateral to the carina, alternating sides every day, for 4 days at 09:00. Control birds were treated with an i.m. injection of an equal volume of saline. There were seven measuring points in time; blood samples were collected just before any dosing, and 5, 10 and 24 h after the last urate oxidase administration.

Blood was collected in heparinized tubes and kept at 0 to 4°C during transport and preparation to block enzymatic degradation of uric acid by urate oxidase *in vitro*. Immediately after sampling, the tubes were centrifuged at 3500 to 3690 r.p.m. for 5 min at 0 to 4°C.

In the Red-tailed Hawks, catheters were placed in the basilic vein, with the rare exception of two catheters in the medial metatarsal vein. Catheters were placed just after the first administration of urate oxidase, using isoflurane anaesthesia. Catheters used were 25 × 0.095 mm<sup>2</sup>, 22 gauge, 1-inch long (Johnson & Johnson Medical Limited, Gargrave, Skipton, Arlington, Texas) with intermittent infusion plugs (Sherwood Medical, Norfolk, Madison County, NA).

#### Reagents and assays

Phenylhydrazine (hydrazinobenzene C<sub>6</sub>H<sub>5</sub>NH-NH<sub>2</sub>) was used as a solution of 17.5 mg in 5 ml distilled water. Potassium ferricyanide (Potassium hexacyanoferrate III, K<sub>3</sub>Fe(CN)<sub>6</sub>) was used as a solution of 85 mg in 5 ml distilled water. Allantoin (A-7878 25 g, lot 37H2507; Sigma-Aldrich Chemie GmbH, Steinheim, Germany, Sigma) solutions were prepared with 158 mg allantoin in 100 ml distilled water. Diluting this solution 100 times gives a 100 μM allantoin standard. NaOH in an amount of 0.8 g was solved in 100 ml distilled water, giving 0.2 M NaOH. This solution was then diluted one to five (15 ml NaOH + 75 ml distilled water).

Plasma uric acid concentrations were determined by spectrophotometry (Beckman Synchron CX® systems, Brea, CA, USA) with methods described in the Beckman Synchron CX® systems' manual (Beckman Instruments Inc., 1995). Pigeon and hawk blood samples were analysed at different laboratories (University of Utrecht and University of Minnesota, respectively). The minimum detectable concentration was 30 μmol (0.5 mg/dl) allantoin/l for the pigeons and 59 μmol/l (1 mg/dl) for the Red-tailed Hawks. The reference range of uric acid in racing pigeons was 150 to 765 μmol/l or 2.5 to 13 mg/dl (Lumeij & De Bruijne, 1985). The reference range of uric acid in Red-tailed Hawks was 478 to 992 μmol/l or 8.1 to 16.8 mg/dl (Johnson-Delaney, 1994).

Allantoin was determined via spectrophotometry by the Rimini-Schryver reaction (Young & Conway, 1942). The protocol was obtained from the Department of Experimental Surgery of Dijkzigt Hospital (Dr van Schalkwijk), Rotterdam, The Netherlands. A protein-free filtrate of 100 μmol was taken from the blood samples by means of Amicon Microcon filters (Microcon YM-30, clear filter, catalogue number 42410, lot number R9SM51425; Millipore Corporation, Bedford, USA) (Millipore Corporation, 1997), centrifuging for 30 min. The formed complex was not stable so spectrophotometry was performed as quickly as possible. The Rimini-Schryver reaction involved condensation of hydrazine derivatives with keto acids to give a coloured product. Allantoin was converted into allantoic acid in the presence of 600 μmol NaOH at 100°C for 8 min, after which it was

cooled in ice for 5 min. Glyoxyl acid was formed out of the allantoic acid when 100 μmol of 1 N HCl was added. Glyoxyl acid was then converted to phenylhydrason by adding 100 μmol phenylhydrazine at 100°C for 4 min, and was also cooled afterwards in ice for 5 min. Phenylhydrason was converted by 100 μmol potassium ferricyanide to a chromophore complex at 37°C for 20 min, and was measured via spectrophotometry at 520 nm (Chen, 1996). Every sample run had a separate calibration curve. All calibration curves displayed a linear relationship between the extinction and the allantoin concentration. The minimum detectable concentration was 10 μmol allantoin/l.

The formulae used to calculate allantoin concentrations from the measured extinctions were as follows:

Standard – blank = X

Sample – blank = Y

Y/X × constant = plasma allantoin concentration

#### Statistics

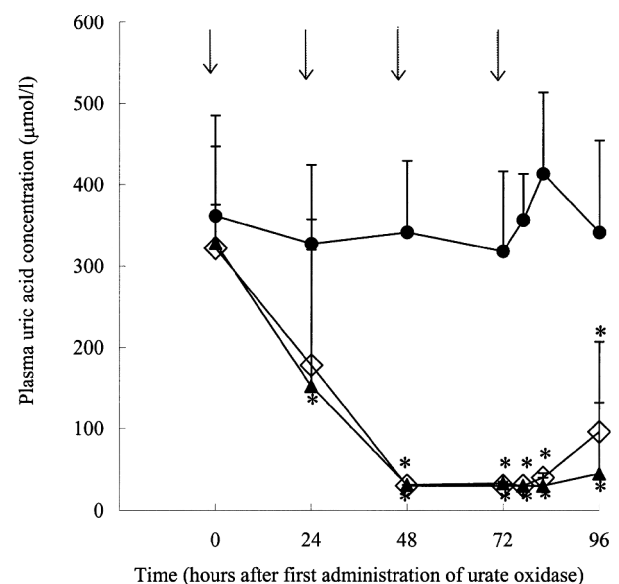
A repeated-measures analysis of variance (ANOVA) was performed on all data. If the ANOVA gave a significant difference between groups, a Student's *t* test for unpaired observations was used to further analyse the data. In all calculations, significance was assumed at *P* < 0.05. Some of the pigeons and hawks had uric acid levels below detectable limits. These data were handled as being equal to the lowest detectable value. The control groups in the two experiments with the Red-tailed Hawks were combined as one, since no significant differences were present (repeated-measures ANOVA, unpaired Student's *t* test).

## Results

### Plasma uric acid concentrations in pigeons

Initial plasma uric acid concentrations were not significantly different between experimental and control groups. The control group showed no significant changes in plasma uric acid concentrations throughout the duration of the experiment (Figure 1).

Both dosages (200 and 600 U/kg) of urate oxidase caused a significant decrease in plasma uric



**Figure 1.** Plasma uric acid concentrations (mean ± standard deviation) in pigeons, fed *ad libitum*, before (T = 0) and after administration (↓) of urate oxidase (◇, 200 U/kg; ▲, 600 U/kg) compared with placebo (●). \* Significantly different compared with controls. n = 6, *P* < 0.05.

acid concentrations when compared with controls. The higher dose of urate oxidase caused a significant decrease in plasma uric acid concentrations by the first day, whereas the lower dose caused a significant decrease in plasma uric acid concentrations by the second day, when compared with controls. After 2 days, no significant differences existed between both dosages. The decline in uric acid was similar in both experimental groups, except there was marked variability in the low dose group on the first day. In addition, no statistically significant difference was present between the means of the two treatment groups on the first day. Both dosages caused plasma uric acid concentrations to drop below measurable levels ( $<30 \mu\text{mol/l}$ ) within 2 days. Although the effect of the higher dose tended to last longer, the difference was not significant (Figure 1).

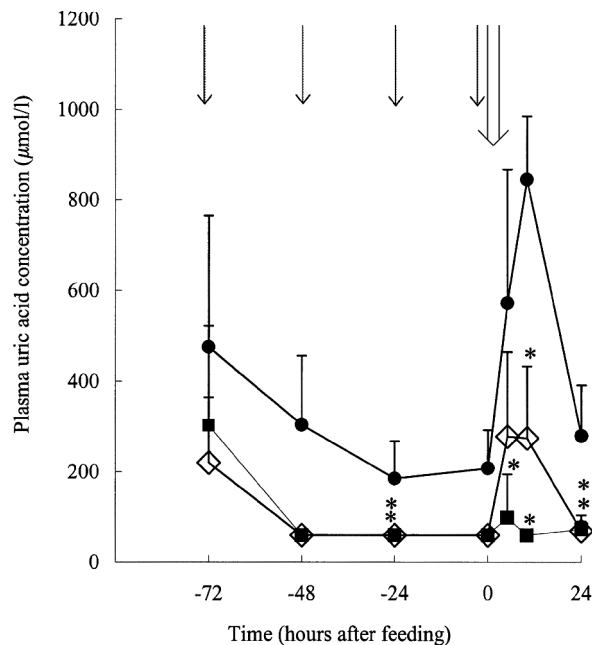
#### Plasma uric acid concentrations in Red-tailed Hawks

No significant differences were present between both control groups; therefore, they were combined as one. Both experimental groups were compared with this combined control group, thus merging the results of two experiments into one group of results.

Initial plasma uric acid concentrations were not significantly different between experimental groups and controls. After administration of urate oxidase (100 and 200 U/kg), plasma uric acid concentrations in both experimental groups were significantly lower than those in the controls (Figure 2) within 2 days, even though the controls also showed a decrease in uric acid concentration caused by fasting. Just before feeding time there was no significant difference between the two experimental groups; plasma uric acid concentrations of both groups were suppressed. After feeding, the controls immediately showed a rapid and significant physiological increase in plasma uric acid concentrations, with a maximum reaching  $1003 \mu\text{mol/l}$  10 h after feeding. After this maximum, uric acid concentrations decreased again, reaching concentrations similar to those during fasting at 24 h after feeding (Figure 2).

The lower dose of urate oxidase (100 U/kg) caused significantly lower uric acid levels throughout the whole experiment. Even 10 h after feeding, plasma uric acid concentrations were still at undetectable levels (e.g.  $< 59 \mu\text{mol/l}$ ) (Figure 2).

The higher dose of urate oxidase (200 U/kg), however, resulted in plasma uric acid concentrations of  $277 \mu\text{mol/l}$  at 5 h after feeding, even resulting in the loss of significant differences when compared with controls. Nevertheless, 10 and 24 h after feeding, uric acid concentrations of the experimental group were again significantly lower than the controls. Because the plasma uric acid concentrations reached by the placebo were maximal 10 h after



**Figure 2.** The uricolytic effect (mean  $\pm$  standard deviation) of urate oxidase ( $\diamond$ , 200 U/kg;  $\blacksquare$  100 U/kg) in Red-tailed Hawks compared with placebo ( $\bullet$ ). The placebo group shows a physiological postprandial increase of uric acid concentration after feeding ( $\downarrow$ ; T = 0). Urate oxidase is administered just before taking blood samples ( $\downarrow$ , T = -72, -48, -24 and 0 h). \* Significantly different compared with controls. Experimental birds, n = 6; controls, n = 10; P < 0.05.

feeding (mean,  $856 \mu\text{mol/l}$ ), this point in time was considered to be the critical point of observation. Twenty-four hours after feeding, plasma uric acid concentrations of both experimental groups were close to undetectable levels again (Figure 2).

#### Plasma allantoin concentrations in pigeons

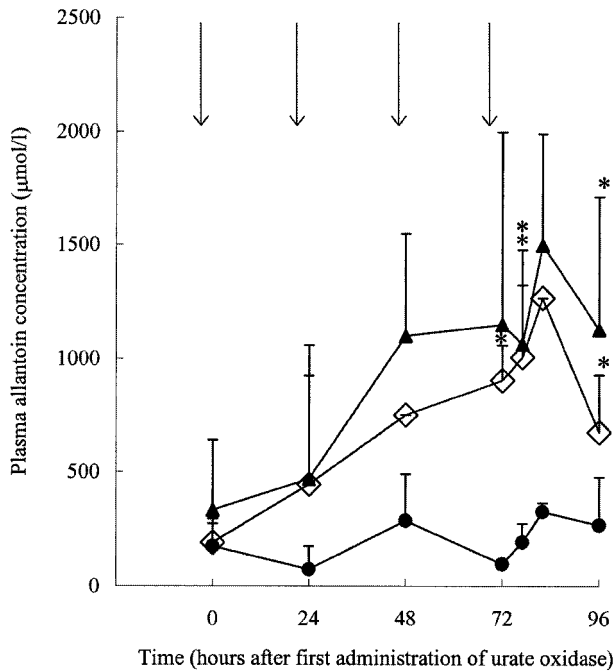
Initial plasma allantoin concentrations were not significantly different between experimental and control groups. The control group showed no significant changes during the entire experiment.

Plasma allantoin concentrations of both experimental groups were significantly higher when compared with the control group after 77 and 96 h, and when compared with initial values. Only the lower dose caused a significantly higher plasma allantoin concentration after 72 h (Figure 3).

#### Plasma allantoin concentrations in Red-tailed Hawks

No significant differences were present between both control groups, so therefore they were combined as one. Both experimental groups were compared with this combined control group, thus merging the results of two experiments into one group of results.

Initially plasma allantoin concentrations were not significantly different between experimental groups and controls. The controls showed no significant changes during the entire experiment, when com-



**Figure 3.** Plasma allantoin concentrations (mean  $\pm$  standard deviation) in pigeons, fed ad libitum, before ( $T = 0$ ) and after administration ( $\downarrow$ ) of urate oxidase ( $\diamond$  200 U/kg;  $\blacktriangle$  600 U/kg) compared with placebo ( $\bullet$ ). \* Significantly different compared with controls.  $n = 6$ ,  $P < 0.05$ .

pared with initial plasma allantoin concentrations (Figure 4).

Plasma allantoin concentrations of both experimental groups were significantly higher within 2 days after the first administration, when compared with the controls. With the exception of the third day of the lower dose, the plasma allantoin concentrations stayed significantly elevated throughout the experiment. With the higher dose even showing twice the effect of the lower dose (Figure 4).

Five hours after feeding as well as 24 h after feeding, the higher dose caused significantly higher plasma allantoin concentrations, when compared with the lower dose.

The drug was well tolerated and no adverse reactions were observed.

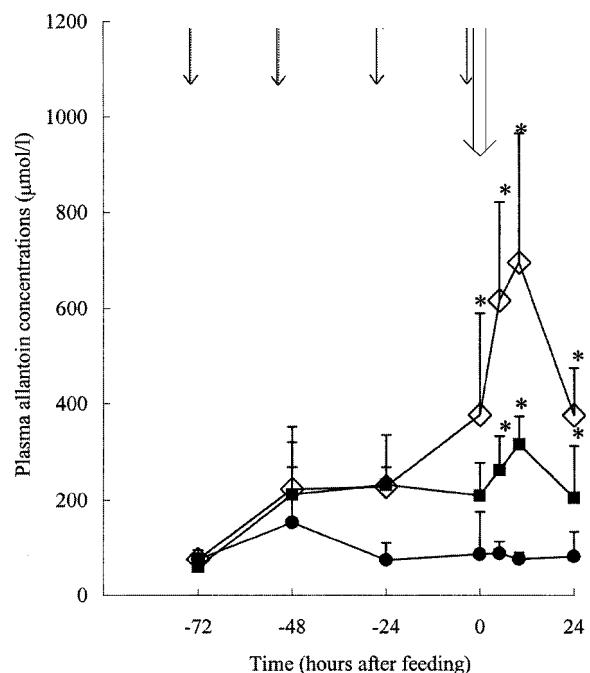
## Discussion

The results of the present experiments show that urate oxidase was highly effective in reducing plasma uric acid levels in granivorous and carnivorous birds. All regimens caused a significant decrease in plasma uric acid concentrations within 2 days after the first administration, when compared with controls. Furthermore, both doses used in Red-tailed Hawks (200 and 100 U/kg) caused a significant postprandial suppression of plasma uric acid concentrations, even though the controls reached physiological plasma uric acid concentrations as high as  $1003 \mu\text{mol/l}$  (17 mg/dl). Postprandial uric acid concentrations in control raptors

were similar to those in granivorous birds suffering from hyperuricaemia and gout, and were well above the theoretical limit of solubility of sodium urate in plasma ( $600 \mu\text{mol/l}$  at  $43^\circ\text{C}$ ) (Lumeij, 1994). It is not clear why, under normal circumstances, urate deposits do not occur in raptors, which show hyperuricaemia for at least 12 h after ingesting a natural meal (Lumeij & Rempel, 1991).

The ability of urate oxidase to keep plasma uric acid concentrations at undetectable levels during expected postprandial increase in Red-tailed Hawks shows the great potential of this drug for treating avian hyperuricaemia. The important clinical implications of this new therapeutic approach can also be seen in the time course and the extent of decreased uric acid concentration, as compared with those achieved with allopurinol therapy. Urate oxidase is much more effective compared with allopurinol. Urate oxidase is a fast-acting, highly potent drug, lowering plasma uric acid concentrations below  $59 \mu\text{mol/l}$  (1 mg/dl) at least 2 days after the first administration. The minimal dose of urate oxidase that gives a maximal effect could be lower or equal to the lowest dose used in the present study.

The lower dose of urate oxidase in Red-tailed Hawks (100 U/kg) caused significantly lower uric acid levels throughout the whole experiment. Even 10 h after feeding, plasma uric acid concentrations were kept at undetectable levels (e.g.  $< 59 \mu\text{mol/l}$ ). In contrast, plasma uric acid concentrations in hawks given the higher dose (200 U/kg) showed an



**Figure 4.** Plasma allantoin concentrations (mean  $\pm$  standard deviation) in Red-tailed Hawks, before and after feeding ( $\downarrow$ ;  $T = 0$ ). Urate oxidase ( $\diamond$  200 U/kg;  $\blacksquare$  100 U/kg) is administered just before taking blood samples ( $\downarrow$ ,  $T = -72, -48, -24$  and  $0$  h) and compared with placebo ( $\bullet$ ). \* Significantly different compared with controls. Experimental birds,  $n = 6$ ; controls,  $n = 10$ ;  $P < 0.05$ .

increase to 277  $\mu\text{mol/l}$  (4.7 mg/dl) 5 h after feeding, even resulting in the loss of significant differences when compared with controls. Because the plasma uric acid concentrations reached by the placebo were maximal 10 h after feeding (1003  $\mu\text{mol/l}$ ), this point in time was considered to be the critical point of observation. The standard deviation increased 5 h after feeding, caused by the variance in ingestion and digestion between birds, leading to erratic results. The higher uric acid concentrations reached in the hawks on the higher dose might be due to increased variability due to dietary influences and to increased variability between birds in the high-dose experimental group. One of the explanations could also be a presence of a threshold or an optimum level of urate oxidase beyond which it may not be effective.

Effects of isoflurane anaesthesia on plasma uric acid concentrations have been reported in American kestrels (Dressen *et al.*, 1999). Hepatic uric acid synthesis or renal tubular urate excretion, or both, decrease during isoflurane anaesthesia. Isoflurane anaesthesia resulted in a decrease in plasma uric acid values when compared with controls (Dressen *et al.*, 1999). Isoflurane anaesthesia was only used once in the present study to put in catheters. Moreover, since all the birds went through the same procedure, they were all exposed to the same effect. Therefore, the effect is not considered of influence on the final conclusion.

The human kidney easily eliminates allantoin. The hypothesis was that the avian kidney would also eliminate allantoin at a fast rate. By contrast, plasma allantoin concentrations of both experimental groups of pigeons were significantly higher than the control group after the last administration of urate oxidase, and already significantly higher within 2 days in Red-tailed Hawks. This indicates that the avian kidney is not able to eliminate the urate oxidase metabolite allantoin within 24 h after the last administration, at least not with the dosages given in these studies. Nevertheless, it took 4 days to reach significantly elevated plasma allantoin concentrations in pigeons. Hence, the elimination rate in the initial phase was fairly adequate, although not efficient enough to prevent the realization of a plasma allantoin level. Based on the fact that in Red-tailed Hawks the higher dose caused twice as high plasma allantoin levels than the lower, a lower dose might result in plasma allantoin levels that the avian kidney could eliminate more easily.

The assay used for the determination of allantoin showed a basal level of absorption, which is probably innate to plasma of Red-tailed Hawks, as the control group could not have allantoin in their blood.

Although no side effects were observed, the lower dosage seems preferable for use in clinical cases of hyperuricaemia. Because of its documented potential for allergic reactions in humans, urate oxidase should be used repeatedly only after

careful consideration. Side effects after iatrogenic urate oxidase administration in humans are few, and are mainly of an allergic nature (urticaria, bronchospasm, and hypoxemia) (Piu *et al.*, 1997). Despite its therapeutic potential, urate oxidase needs further investigation into long-term effects with regard to allergic reactions after repeated use, as well as lower dosages and tolerance in hyperuricaemic cases. The long-term fate of uric acid metabolites such as allantoin, allantoic acid, urea, and ammonia, should also be studied. Another experiment with more measuring points in time after the last administration of urate oxidase might shed some light on the elimination rate of allantoin in birds.

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## RÉSUMÉ

**Investigations des propriétés uricolitiques de l'oxydase d'urate chez des espèces aviaires: un granivore (*Columba livia domestica*) et un carnivore (*Buteo jamaicensis*)**

Pour étudier les propriétés uricolitiques de l'oxydase d'urate chez des oiseaux granivores et carnivores, des expérimentations ont été entreprises chez les pigeons (*Columba livia domestica*) et les buses à queue rouge (*Buteo jamaicensis*). Les concentrations en allantoiné et en acide urique du plasma ont été déterminées à des temps différents dans des groupes expérimentaux avant et après avoir reçu 100, 200, et 600 U/Kg d'oxydase d'urate une fois par jour, comparés à des témoins. Toutes les doses ont entraîné une diminution significative des concentrations d'acide urique dans le plasma, dans les deux jours après la première administration, comparées aux témoins.

De plus, les doses de 200 et 100 unités/kg administrées aux buses à queue rouge ont entraîné une suppression postprandiale importante des concentrations d'acide urique plasmatique. Les concentrations d'allantoiné plasmatique ont été significativement supérieures, comparées aux témoins. Le fait que l'oxydase d'urate prévienne l'hyperuricémie physiologique postprandiale chez les buses à queue rouge et réduise les concentrations d'acide urique plasmatique à des niveaux indécétables, montre la potentialité importante de ce médicament pour le traitement de l'hyperuricémie.

## ZUSAMMENFASSUNG

**Untersuchungen zu den urikolytischen Eigenschaften von Uratoxidase in einer granivoren (*Columba livia domestica*) und einer carnivoren (*Buteo jamaicensis*) Vogelspezies**

Zur Untersuchung der urikolytischen Eigenschaften von Uratoxidase in grani- und carnivoren Vögeln wurden Experimente in Tauben (*Columba livia domestica*) und Rotschwanzbussarden (*Buteo jamaicensis*) durchgeführt. Plasmakonzentrationen von Allantoin und Harnsäure wurden in den experimentellen Gruppen zu verschiedenen Zeiten nach Erhalt von 100, 200 und 600 U/kg Uratoxidase einmal täglich bestimmt und mit den Kontrollen verglichen. Alle Behandlungsregime verursachten im Vergleich zu den Kontrollen einen signifikanten Abfall der Harnsäurekonzentrationen innerhalb von zwei Tagen nach der ersten Verabreichung. Überdies führten beide in den Rotschwanzbussarden angewendeten Dosierungen (200 und 100 U/kg) zu einer signifikanten Unterdrückung des Harnsäureanstiegs im Plasma nach Nahrungsaufnahme. Die Plasma-Allantoinkonzentrationen waren signifikant höher als in den Kontrollen. Die Fähigkeit von Uratoxidase die physiologische Hyperurikämie nach Nahrungsaufnahme in Rotschwanzbussarden zu verhindern sowie Plasma-Harnsäurekonzentrationen unter die Nachweisgrenze zu senken, zeigt das große Potential dieses Arzneimittels für die Behandlung aviärer Hyperurikämie.

## RESUMEN

**Estudios de las propiedades uricolíticas de la urato oxidasa en especies aviares granívoras (*Columba livia domestica*) y carnívoras (*Buteo jamaicensis*)**

Con la finalidad de estudiar las propiedades uricolíticas de la urato oxidasa en aves granívoras y carnívoras, se realizaron experimentos en palomas (*Columba livia domestica*) y Gavilanes colirrojos (*Buteo jamaicensis*). Se determinaron las concentraciones plasmáticas de ácido úrico y allantoina en los grupos experimentales a diferentes tiempos antes y después de ser administrados 100, 200 y 600 U/kg de urato oxidasa una vez al día, y se compararon con los controles. Todas las pautas causaron una reducción importante de las concentraciones plasmáticas de ácido úrico a los dos días después de la primera administración en comparación con los controles. Además, dos de las dosis empleadas en Gavilanes colirrojos (200 y 100 unidades/kg) causaron una importante supresión postprandial de las concentraciones plasmáticas de ácido úrico. Las concentraciones plasmáticas de allantoina fueron significativamente superiores cuando se compararon con las de los controles. La capacidad de la urato oxidasa para evitar la hiperuricemia fisiológica postprandial en Gavilanes colirrojos y para reducir las concentraciones plasmáticas de ácido úrico a niveles no detectables, demuestra el gran potencial de este medicamento para el tratamiento de la hiperuricemia aviar.