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Ascorbic acid in a New World monkey family: species difference and influence of stressors on ascorbic acid metabolism

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Summary: Like other simian primates, the New World monkey *Callithrix jacchus,* marmoset, and *Saguinus fuscicollis,* tamarin, require ascorbic acid as an essential nutrient. For adult marmosets, a daily intake of 15 mg/kg metabolic body weight was found to be necessary to obtain a serum level above the kidney threshold. A survey of the serum ascorbic acid level of marmosets and tamarins in a breeding colony resulted in a vast divergence between the two species, indicating a higher ascorbic acid requirement for tamarins. Unaccustomed trial conditions or additional stressors resulted in a higher catabolism of ascorbic acid to CO_2 in both species, measured with ^{14}C labeled material, compared to a higher rate of renal excretion when the animals were accustomed to the metabolic cage. These isotope excretion studies suggest a different metabolic behavior of ascorbic acid in the two species. This is supposedly caused by a higher sensitivity of the tamarins when subjected to the same conditions as marmosets.

Zusammenfassung: Die Neuweltaffen *Callithrix jacchus,* Marmosets, und *Saguinus fuscicollis,* Tamarine, sind, wie andere Affen auch, auf die externe Zufuhr von Ascorbinsäure angewiesen. Um bei Marmosets einen Serumspiegel oberhalb der Nierenschwelle zu erreichen, müssen ihnen täglich 15 mg/kg metabolischem Körpergewicht zugeführt werden. Die Nierenschwelle liegt bei ihnen im gleichen Bereich wie beim Menschen. Ein Vergleich des Ascorbinsäurespiegels zwischen den beiden Spezies aus einer Zuchtkolonie ergab einen gravierend niedrigeren Wert bei den Tamarinen. Bei beiden Spezies wurde Ascorbinsäure durch ungewohnte Versuchsbedingungen oder zusätzliche Streßfaktoren zu einem höheren Prozentsatz zu CO_2 abgebaut, als wenn die Tiere an den Stoffwechselkäfig gewöhnt waren. Diese Isotopenexkretionsversuche mit ^{14}C-markierter Ascorbinsäure weisen auf ein unterschiedliches Stoffwechselverhalten von Ascorbinsäure zwischen den beiden Spezies hin, was vermutlich durch die höhere Streßanfälligkeit der Tamarine bei vergleichbaren Bedingungen verursacht ist.

Key words: ascorbic acid metabolism, vitamin C serum level, stress, *Callitrichidae*

Schlüsselwörter: Ascorbinsäurestoffwechsel, Vitamin C Serumgehalt, Streß, *Callitrichidae,* Krallenaffen

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Introduction

Some species of New World monkeys are used in biochemical research, such as the *Aotus, Saimiri,* and mainly *Callithrix* and *Saguinus.* The latter two belong to the family *Callitrichidae,* and only those will be dealt with in this paper, specifically *Callithrix jacchus,* the white-eared marmoset, and *Saguinus fuscicollis,* the black-headed tamarin.

Callitrichidae are small monkeys with a body weight of roughly 400 g. Their habitat is the tropical rain forest in South America, where they utilize mostly the lower rain forest layer between 2 and 10 m, but they can be seen as high up as 20 m. They are day-active monkeys and live in family groups of 4–8 members, joining loosely with other related species to build groups of about 20 individuals. The females cycle regularly after 28 days *(Callithrix jacchus)* or 22 days *(Saguinus fuscicollis),* respectively. When housed with other adult females, only the α-individual will be fertile. Gestation period lasts for almost 5 months. Uniquely within the simian primates, *Callitrichidae* often give birth to twins, which are highly chimerical. In case of a heterosexual pair, both siblings remain fertile. At the age of about 24 months they are fully grown. Their main food intake consists of fruits, insects and other anthropods, leaves, buds, and nuts. A great part of the feeding time is spent with the licking of tree sap, which seems to be an important calcium source, or with the chewing of resin.

Like other New World monkeys, marmosets and tamarins cannot utilize vitamin D_2, but need vitamin D_3 (5). Additionally, the marmosets have been found to have an unusually high serum level of 1,25(OH)D_3, the active form of the vitamin, without developing hypercalcemia, due to an abnormal receptor system (11). In our own therapeutical trials clear clinical symptoms of osteomalacia in marmosets disappeared or were reduced after the application of 25(OH)D_3, probably due to the formation of 24,25(OH)D_3. No improvement resulted from the treatment with 1,25$(OH)_2D_3$ (14). In respect to purine metabolism, we found that *Callitrichidae* and other New World monkeys are somewhere between *Hominoidea* and *Cercopithecoidea,* in that the enzyme uricase is certainly existent, but does not work as effectively as in *Hominoidea* (9).

This paper gives the most characteristic features of a series of experiments, which were carried out to find the optimal ascorbic acid (AA) supply for *Callitrichidae* held in captivity.

Material and Methods

For the determination of the AA requirement of *C. jacchus* varying degrees of AA were incorporated in the pelleted diet (4 000, 2 000, 500 and 250 ppm; see (4)). AA was analyzed in serum and urine of six trial and two control animals. Blood samples of colony animals for the analysis of AA in serum were taken over a longer period of time on several occasions, when the animals had been on the staple diet with 2 000 or 4 000 ppm AA, respectively, for at least 12 months. Studies on the AA metabolism with the isotope excretion method were performed with the oral application of single doses (58 kBq/100 g body weight; on an average 400 g) of 1-^{14}C-AA (Amersham, Braunschweig). One animal of each species was given the labeled AA when fed a high (4 000 ppm) or a low (250 ppm) level of AA in diet, and under conditions of stress (eigher unfamiliar circumstances or additional disturbances or accidental body weight loss) or when more or less used to the trial conditions. The exhaled

$^{14}CO_2$ was analyzed continuously for radioactivity by a modified tritium monitor during the first 8 h. Additionally, $^{14}CO_2$ was captured by ethanolamine (1) for altogether 48 h and activity was measured in aliquots taken from there at definite intervals. The first two days after application the trial animals were placed in a special gas-proof metabolic chamber and were moved thereafter to a larger metabolic cage. Urine and feces were collected periodically during 12 or 4 days, respectively, and analyzed for radioactivity by a liquid scintillation counter (Tri-Carb, Model 3385; Packard). Ascorbic acid was analyzed by the dinitrophenylhydrazine method by HPLC (10). Only young adult males were used for the experiments with the exception of the serum testing of adult colony animals of both sexes.

Results

The minimal AA requirement of adult *C. jacchus* in captivity is 15 mg/kg metabolic body weight daily, which was provided by the diet with 500 ppm AA. This intake will sustain the serum level above the kidney threshold, which is between 0.76 and 1.06 mg AA/100 ml serum. These values were obtained on the diets with 250 ppm and 500 ppm, respectively. The excretion of AA in urine relative to intake was roughly the same when the diets with 4 000, 2 000 and 500 ppm were fed (see Fig. 1), but on the diet with 250 ppm the relative and the absolute amount of AA in urine was reduced to a minimum. A single oral dose of AA resulted in rapid urinary excretion, whereby two-thirds were eliminated within the first 6 h after application.

A comparison of the serum AA level between the two species *C. jacchus* and *S. fuscicollis*, housed and fed identically in a breeding colony (details in (3)), resulted in a remarkably large difference (Table 1). The average value of *S. fuscicollis* was below the kidney threshold when fed the 2 000

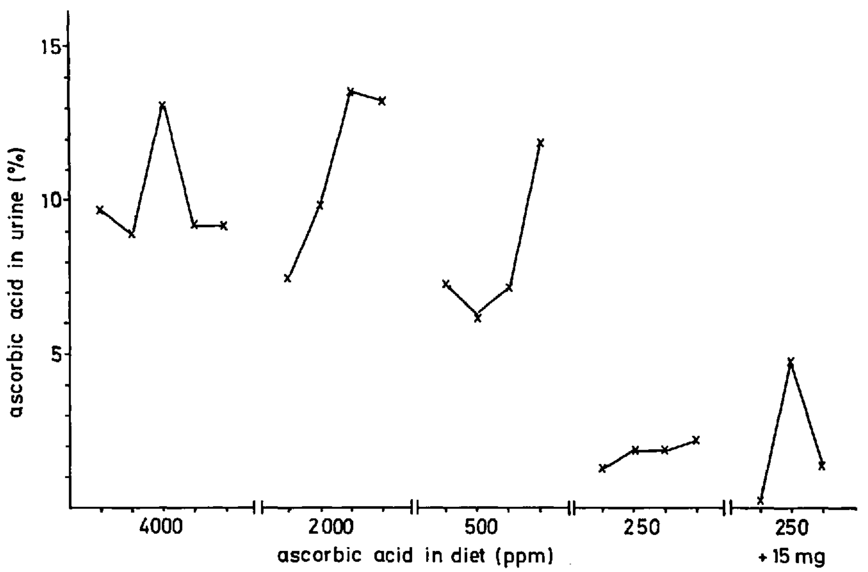

Fig. 1. Ascorbic acid excretion in urine of *Callithrix jacchus*, in percent of intake, when fed diets with varying ascorbic acid content and after a single oral dose.

Table 1. Ascorbic acid content in serum (mg/100 ml) of two species of *Callitrichidae* when the staple feed contained either 2000 or 4000 ppm ascorbic acid.

Ascorbic acid in diet	Callithric jacchus	Sanguinus fuscicollis
2000 ppm healthy	n = 19 2.56 ± 1.07	n = 21 0.54 ± 0.29
sick	n = 5 0.47 ± 0.66	n = 2 0.13 (0.08; 0.18)
4000 ppm healthy	n = 26 2.43 ± 0.54	n = 16 2.06 ± 0.55
sick	n = 18 2.20 ± 0.37	n = 10 1.77 ± 0.45

The total dietary ascorbic acid content was about half the amount indicated when the loss due to pelleting is considered as well as the lower content in the banana and baby pap supplement.

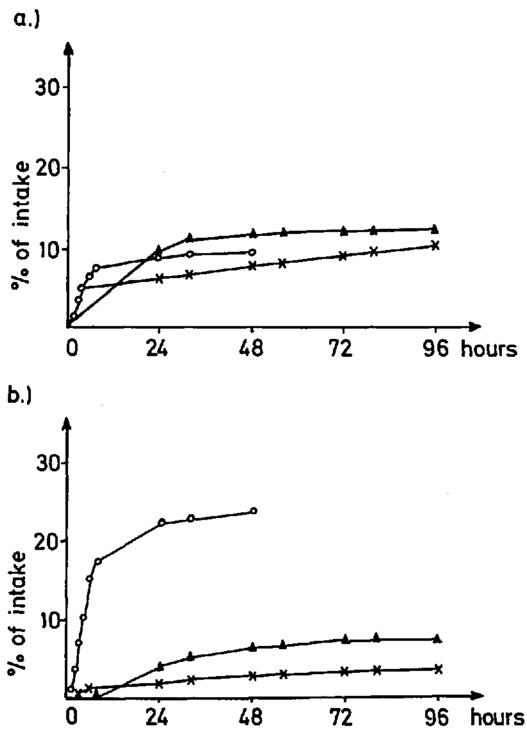

Fig. 2. Cumulative excretion of ^{14}C in percent of a single oral dose of 1-^{14}C-ascorbic acid in a male *Callithrix jacchus* under normal conditions (2a) and when physically stressed by a transitory, unidentified sickness (2b) with a body weight loss of 23 % as the only symptom. The feed contained 250 ppm ascorbic acid. Symbols: ^{14}C excretion in exhaled air (circles), urine (triangles), and feces (asterisk).

ppm-diet. The enormous divergence between the two species diminished after the dietary AA was doubled, but was still evident. Animals sick for various reasons, either short term surgically or long term "metabolically" ill patients (also females with recent abortion) had much less AA in serum than healthy animals, when fed the 2 000 ppm-diet. With a high AA supply, the effect of sickness was obvious only in *S. fuscicollis* this time.

The trials with labeled AA exhibited a difference between the two animals of the two species. In trials under comparable conditions, *S. fuscicollis* exhaled about double the amount of $^{14}CO_2$ than *C. jacchus*, with, however, the urinary portion of ^{14}C being less for *S. fuscicollis*. When the AA supply was high, both animals eliminated most of the recovered ^{14}C in urine (between 24 % and 67 %). About two-thirds of the activity eliminated via urine consisted of unmetabolized AA, for *C. jacchus* in the first 8 h and for *S. fuscicollis* only in the first 3 h, the rest were metabolites. On the diet with a low AA content, $^{14}CO_2$ was the main excretion pathway in most trials (10 %–25 %). This dietary AA content resulted in a lower total ^{14}C recovery (30 %–41 %) within 12 days than the high AA diet (51 %–72 %). Stress, as judged by behavioral criteria, increased the metabolization of AA to CO_2 at the expense of the renal excretion of AA or its metabolites. The speed of total ^{14}C excretion, however, was not correlated to the state of stress. An example for the ^{14}C excretion pattern during physical stress caused by a disease, in comparison to that under normal conditions, is illustrated in Fig. 2.

Discussion

The parameter which actually defines the AA requirement is not yet settled and the extent of the metabolic actions of AA is not fully understood. Therefore, it is difficult to determine the minimal requirement of vitamin C. It seems advisable to take the highest amount of AA which still is used by the body. Provided there is a continuous supply, this value is indicated by the first appearance of unmetabolized AA in urine. AA is filtered into the primary urine and is reabsorbed until it reaches the kidney threshold. In *C. jacchus* this threshold is similar to that in man (0.8–0.9 mg/100 ml serum; (6)). Data from other animals are not known. The required amount must be incorporated in the main feed or given several times a day, since a single oral dose will be eliminated within a few hours in the urine. As a consequence a greater need for a single oral supplement as compared to a constant supply had been found in macaques (12). The requirement of *C. jacchus,* as resulted from our trial (15 mg/kg metabolic body weight) corresponds to the value given for another small New World monkey, the *Saimiri sciureus* (7, 2), but is much higher than the value given for man with 100 mg/day (= 4 mg/kg metabolic body weight; (6)). The unusual high requirement for vitamin C may well be due to the lack of evolutionary pressure towards a low requirement in a biotop with constant yearround high supply of AA. Requirement data for Old World monkeys vary between 3 mg/kg body weight (= 4.5 mg/kg metabolic BW; (12)) and 10 mg/kg (= 15 mg/kg metabolic BW; (8)).

Although not yet proven by experiment, it is obvious from the data of AA in the sera of colony animals that *S. fuscicollis* have a higher AA requirement than *C. jacchus*. When the staple feed contained 2000 ppm, which in fact seems to be an adequate amount, the average serum level in this species was below the kidney threshold and was only one-fourth that of marmosets, although both species were held and fed identically. The 2000 ppm AA were added to the feed mixture prior to pelleting, a loss of one-fourth to one-third can be calculated as due to processing and short time storage. It is generally accepted that stress of all kinds increases the AA requirement. This fact probably is the key in understanding the differing AA metabolism between the two species. This differing excretion pattern is the same as is the case in marmosets, that is regarding the individual states of stress and "normal" behavior. Rhesus monkeys also had an enormously increased rate of CO_2 formation when submitted to confined conditions (13). The example illustrated in Fig. 2 shows an extreme expression of the influence of stress on the AA metabolism. It is obvious that the rate of AA degradation to CO_2 directly reflects the state of stress. Due to the continuous display of the $^{14}CO_2$ rate within the first 8 h, it could be demonstrated in another trial that short term disturbances like annoyance, a series of flashing light or the noise of a nearby pump, increased the $^{14}CO_2$ excretion in one animal by about twice as much within a maximum of 10 min. Within a few minutes after the stressing factors had stopped the $^{14}CO_2$ level had returned to almost the former level. We therefore conclude that stressors increase the AA metabolization to CO_2 drastically, thus indicating a higher AA requirement.

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