Zeitschrift für
Ernährungswissenschaft Z Ernährungswiss 29:192–196 (1990)

Studies on the ascorbic acid metabolism of callitrichid monkeys by ^{14}C isotope excretion technique*)

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Summary: Recently it has been found that the two monkey species Callithrix jacchus and Saguinus fuscicollis, both belonging to the same New World monkey family Callitrichidae and held in the same colony under identical conditions, had extremely different serum ascorbate levels. To examine the ascorbic acid metabolism the ^{14}C-excretion of orally given 1-^{14}C-ascorbic acid was studied under conditions of marginal and abundant vitamin C supply and under intentional stress. There were large differences in the mode of ^{14}C excretion between low and high ascorbate supply. The differences were smaller between stress/no stress conditions intraindividually than between the two species, but they were in the same manner. In comparable trials S. fuscicollis reacted such that a higher status of stress can be supposed in this species.

Zusammenfassung: Bei den beiden Affenarten Callithrix jacchus und Saguinus fuscicollis, die der gleichen Neuweltaffenfamilie der Krallenaffen angehören, wurden extrem unterschiedliche Serumascorbatspiegel gefunden, obwohl die Tiere unter identischen Bedingungen in derselben Kolonie gehalten wurden. Die ^{14}C-Ausscheidung von oral verabreichter 1-^{14}C-AA wurde unter marginaler wie auch reichlicher Vitamin-C-Versorgung sowie unter Streßbedingungen untersucht. Zwischen niedriger und hoher Vitamin-C-Versorgung traten große Unterschiede im ^{14}C-Ausscheidungsmodus auf. Der Speziesunterschied war dabei geringer als der Unterschied zwischen individuellen Streß-/Nichtstreß-Bedingungen, aber in beiden Fällen war er gleichartig. In vergleichbaren Versuchen reagierte S. fuscicollis derart, daß ein höherer Streßzustand bei dieser Spezies angenommen werden kann.

Key words: Ascorbic acid metabolism, Callitrichidae, stress, $^{14}CO_2$-excretion

Schlüsselwörter: Ascorbinsäurestoffwechsel, Krallenaffen, Streß, $^{14}CO_2$-Ausscheidung

Introduction

Ascorbic acid (AA) is synthesized endogenously by most animal species, but all simian primates need the external supply of this substance as a vitamin. The minimal vitamin C requirement of the common marmoset Callithrix jacchus in captivity was found to be around 500 ppm (2). We assumed this requirement to also be valid for tamarins (Saguinus species),

*) In memoriam Prof. Dr. H. Zucker

which belong to the same New World monkey family Callitrichidae. Testing serum ascorbate of Callithrix jacchus and Saguinus fuscicollis held under identical conditions, however, yielded much lower levels for the tamarins (3). The average serum ascorbate of the marmosets was 4–5 times higher than that of the tamarins, the latter being below the kidney threshold. The feed, the same for both species, contained 2000 ppm AA at that time. The blood samples were taken several weeks after the animals had been moved to another building. Particularly, Saguinus had been noted as being excited about the new surroundings. Generally, the daily routine and manipulations or other disturbances also evoke a higher degree of visible nervousness in tamarins, compared to marmosets. The purpose of this work was to study the reflection of this behavioral difference between the two species on the AA metabolism. This preliminary report is part of a more detailed description of studies on the ascorbic acid metabolism of Callitrichidae.

Material and methods

A cylindric plexi metabolic chamber with controlled air flow had been constructed. The space for the animal had a volume of 21 L. Feces and dropped pellets fell through screens and were held back by a closer-meshed wire screen. From there solid constituents (feces, dropped pellets) were removed manually with long tweezers. Water was used evacuated to rinse the urine which the animals chambers had in small portions, while in the drain system 5 % metaphosphoric acid was used to stabilize AA. The lockable openings to allow access to the inside. Air was allowed to come in continuously by a punched annular copper tube lying on the bottom of the cage, and the air was exhausted at the top of the cage behind a home sheet.

The animals were fed a self-made natural ingredient pelleted diet (slightly modified from (4)). The trials were designed to supply the animals either with high (4000 ppm) or low (250 ppm) dietary AA (added to diet prior to pelleting). The animals should either be stressed by the unfamiliar, restricted trial conditions or irritated additionally by flashing lights and teasing, or they should be more or less adapted. The loss of AA during pelleting was determined to be about one-fourth. One male young adult each of *Callithrix jacchus* (marmoset) and *Saguinus fuscicollis* (tamarin) were used in the study.

The labeled 1-^{14}C-AA had a specific activity of 4.4 MBq/mg (Amersham, Braunschweig). 58 kBq/100 g body weight were given orally by syringe, immediately after having been diluted with a 10 % solution of sucrose.

Urine and feces were collected periodically. Urine was analyzed for radioactivity in a scintillation counter for liquids, feces were lyophilized and pulverized before being burned in a sample oxidizer. ^{14}C in breath was analyzed at definite intervals in a scintillation counter, after trapping CO_2 in an absorbing liquid (method of (1)). During the first 8 h the exhaled air was additionally passed continuously through a tritium monitor, which was modified for ^{14}C measurement. $^{14}CO_2$ measurement was discontinued after 48 h.

Results

The main route of ^{14}C excretion of an orally given ^{14}C-AA was the exhaled air, when the animals were in a low AA status (Table 1). Most of total $^{14}CO_2$ was excreted within the first 8 h. When the animals had a high AA supply level, by far most of the excreted radioacitvity was detected in

Table 1. ^{14}C excretion of oral given ^{14}C-ascorbic acid in Callithrix jacchus (C.j.) and Saguinus fuscicollis (S.f.) within 48 h, in % of intake

species	C.j.	S.f.	C.j.	C.j.	C.j.	S.f.	S.f.
AA[1] in diet, ppm	4000	4000	250	250	250	250	250
stress conditions	+	+	+	++	++[2]	+	(+)
$^{14}CO_2$ in breath	2.6	4.1	9.4	11.8	23.8	17.7	15.1
^{14}C in urine	48.8	16.2	8.7	3.9	2.7	6.3	10.4
^{14}C in feces	9.8	21.7	11.7	6.4	6.4	11.5	6.5

[1] ascorbic acid; [2] animal had lost 23 % of body weight before the trial;
+ = not used to the trial conditions
++ = additional stress
(+) = animal was tried, unsuccessfully; did not adapt to the trial conditions

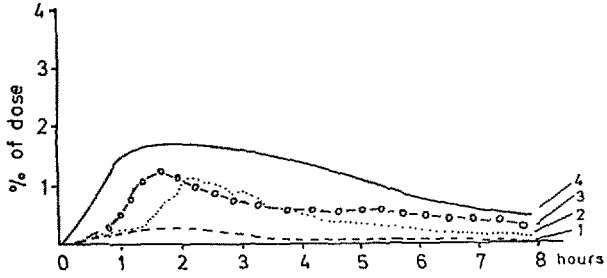

Fig. 1. Exhaled $^{14}CO_2$, in percent of oral ^{14}C-ascorbic acid intake, in Callithrix jacchus.
The amount of percentage has been calculated every 200 s.
1 = 4000 ppm ascorbic acid
2 = 250 ppm ascorbic acid
3 = 250 ppm ascorbic acid; additional stress
4 = 250 ppm ascorbic acid; loss of 23 % of body weight.

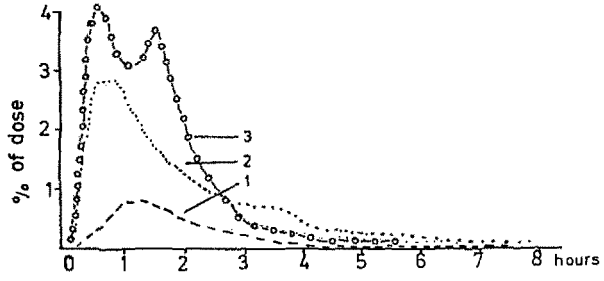

Fig. 2. Exhaled $^{14}CO_2$, in percent of oral ^{14}C-ascorbic acid intake, in Saguinus fuscicollis.
The amount of percentage has been calculated every 200 s.
1 = 4000 ppm ascorbic acid
2 = 250 ppm ascorbic acid, semi accustomed
3 = 250 ppm ascorbic acid.

urine (C. jacchus) or in feces (S. fuscicollis). At this level, up to 71 % of the radioactivity recovered in urine of *C. jacchus* was unmetabolized AA within the first 8 h, while in *S. fuscicollis* specific activity in urinary AA was detected only for 3 h and up to 63 % at the most. After 11 days activity was still detected in urine, feces, and breath, although in negligible amounts, the highest ^{14}C rate then being excreted in urine.

Intentionally administered stress, as well as accidentally occurring stress, caused a shift of ^{14}C excretion from urine to exhaled air. Concomitantly, the peak in $^{14}CO_2$ activity appeared earlier in these cases (Figs. 1 and 2). In comparable trials the $^{14}CO_2$ excretion of the tamarin was almost twice as much as that of the marmoset. Additionally, the CO_2 peaks occurred at 1 h post-tracer in the tamarin as compared to about 2 h in the marmoset.

Discussion

According to published data, there are fundamental differences between species including man in the excretion pattern of AA metabolites after the administration of labeled AA. It is not the uncapability to synthesize AA endogenously, however, which separates the types from each other. For the defect mutant guinea pig, as well as for the rat, which is not dependent on external vitamin C supply, the main route of AA metabolization is the decay to CO_2 (5, 6). In man, who is vitamin C dependent as are all simian primates, most of the radioactivity is excreted in urine and only little $^{14}CO_2$ can be detected in breath (7), while in another primate, the Old World macaque monkey, the degradation of an oral dose of AA to CO_2 varies between 2 % and 90 % (8). The CO_2 excretion in man and in macaque monkeys peaks after 5 h post-tracer, while in the guinea pig the peak appears already within the first hour and in the rat in hour 2–3. The New World monkey species investigated here deviate from the observations made in the other primates mentioned above. *Callitrichidae* began to exhale $^{14}CO_2$ already several minutes after the intake of the labeled AA, the peak appearing only 1 to 2 h later. Although the $^{14}CO_2$ excretion declines rapidly, a small amount can be detected for at least several days, indicating the decay of AA to CO_2 to be a real metabolic pathway.

The rate of $^{14}CO_2$ formation in our animals was highly influenced by the state of stress and by the level of AA supply. The decay of AA to CO_2 was increased and accelerated when the animal was in a low AA status and when it was stressed. This pattern of response to stress partly matches with that in macaques; they exhibited an extreme increase in $^{14}CO_2$ excretion up to 90 % of total ^{14}C output when exposed to an intense restraint. The speed of CO_2 formation, however, was not affected in this species (8).

The animals of the two Callitrichid species differed in the ratio of $^{14}CO_2$ excretion and its peak time, and, correspondingly inverse, in the portion of ^{14}C in urine. When the tamarin was submitted to the same trial conditions as the marmoset, it developed a higher rate of $^{14}CO_2$ formation from AA, with the peak occurring earlier. The height of the CO_2 rate has been reported to be an indication for a condition of stress in macaques (8). There is no comparable report of other AA dependent animals. The results of the

study show clearly that the tamarin was much more excited about the circumstances contingent on the trial than the marmoset. This is underlined by the failure to get the tamarin accustomed to the metabolic chamber within 2 weeks. Macaques could be calmed down within 7 days under even more confined conditions (8).

We do not have a conclusive explanation for the result that the CO_2 rate was higher when the animals were in a marginal AA status. Is this low AA supply already a stress factor?

Saguinus may have a lower capability of AA absorption, as there is a higher ^{14}C-activity in feces. This might in part result from a contamination of the occasionally soft feces with urine. The peak time of radioactivity recovered in these two excretions, however, differed considerably, thus reducing the possibility of an incorrect value in feces.

Acknowledgement

We thank Prof. Dr. F. Deinhardt, head of Max von Pettenkofer Institute, University of Munich, for animals of his colony to work with. Many thanks to Dr. W. E. Kollmer, GSF, Neuherberg, for his valuable support and criticism of this work.

Part of this work was supported by the Deutsche Forschungsgemeinschaft.

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Received July 26, 1990

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