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Nitrogen requirements of the didelphid marsupial *Caluromys philander*

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Abstract The use of fruit-based diets by a small arboreal marsupial, the woolly opossum (*Caluromys philander*) was studied to elucidate the mechanisms used to compensate for a low dietary protein concentration. The passage of a liquid phase digesta marker (Cr-EDTA) through the gut was significantly faster when animals were fed a diet containing 0.45% N compared to that measured on a diet containing 0.90% N. The size of the gut of the two groups was similar except that the caecum of animals fed 0.45% N was significantly larger than in those animals fed 0.90% N. Animals fed a diet of 0.45% N ate significantly more food than those fed higher levels of nitrogen but there was no significant difference in the dry matter digestibility of the diet. The maintenance nitrogen requirement of the animals was 176 mg dietary N or 146 mg truly digestible nitrogen per kg metabolic body mass, with low losses of non-dietary faecal nitrogen being notable. There was no significant difference between diets in any measured parameter of urea metabolism and all animals recycled between 60% and 80% of the endogenously synthesised urea.

Abbreviations *NDFN* non-dietary faecal nitrogen · *SCFA* short chain fatty acids

Introduction

Most wet tropical forests contain a seasonal abundance of fleshy fruits that provide a potentially rich source of energy for small terrestrial or arboreal mammals. Some small mammals consume these fruits when they are unripe but many consume ripe pulp or pulp and seeds (Charles-Dominique et al. 1981). From a nutritional point of view, fruit pulp provides large quantities of potentially digestible soluble carbohydrates and variable quantities of lipids. Although there are some notable exceptions (e.g. Herbst 1986; Atramentowicz 1988), most fruits contain relatively low concentrations of protein and even when fruit is abundant, most small mammals spend time searching for insects and other protein-rich foods. Exclusive frugivory is a relatively rare phenomenon amongst non-volant mammals and is largely restricted to members of the Carnivora (e.g *Potos flavus*; Julien-Laferriere 1993, *Nandinia binotata*; Charles-Dominique 1978).

In both regrowth and primary forests in French Guiana, didelphid marsupials including the woolly opossum (*Caluromys philander*) are the major consumers of ripe fruits (Charles-Dominique et al. 1981; Julien-Laferriere and Atramentowicz 1990). The woolly opossum weighs between 300 g and 450 g, is strictly nocturnal and arboreal and has a simple stomach but a modestly developed caecum (Charles-Dominique et al. 1981). We were interested in how small non-volant mammals like the woolly opossum compensate for a low protein concentration in fruit pulp. In the absence of deterrent plant secondary metabolites, animals may simply eat more when protein concentrations are low but this may result in washing out symbiotic microbes in the hindgut (Björnhag 1989). Therefore we studied the volume of the digestive tract and the passage of food through the gut when the protein content of a diet of mango pulp varied. In order to understand the importance of different excretory routes on nitrogen retention, we also measured the amount of nitrogen required for maintenance of woolly opossums and compared this with other species. Recycling of endogenously produced urea back to the gut is one way that urinary nitrogen losses may be reduced and so we measured the importance of this process using labelled urea.

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Materials and methods

Animals and their husbandry

Adult male woolly opossums (0.35–0.45 kg) were caught in cage traps set at 20 m in the canopy of secondary forest near Cayenne, French Guiana. Detailed descriptions of the vegetation, the trapping procedures and the life history of the animals can be found in Charles-Dominique et al. (1981) and Julien-Laferriere and Atramentowicz (1990). The opossums were housed indoors at Institut Pasteur de la Guyane Français at an ambient temperature of 24–27 °C and under a 12L:12D light regime.

Each opossum was held in an individual metabolism cage equipped with a nest box as described by Foley and Hume (1987). Urine and faeces were collected on a mesh screen and plastic tray twice daily (0800 hours and 1800 hours). The efficiency of urine collection from these trays was between 96% and 98% and so measured urine volumes were multiplied by a factor of 1.03 to correct for these losses.

Diet and feeding

We could not collect sufficient wild indigenous fruits at the time of our experiments for the whole study. Instead, we designed three diets based on the pulp of mango (*Mango indica*) fruit which is often eaten by wild woolly opossums. The diets were designed to be of three nitrogen contents: 0.45% N, 0.90% N and 2.5% N which bracket the nitrogen concentration of fruits consumed by wild woolly opossums. Mango fruit was collected and the pulp extracted and mixed with sufficient casein to give the desired nitrogen level. We used sucrose to directly replace different amounts of casein so that the diets had similar energy content. Sucrose is the dominant sugar in mango pulp (> 70% of total water soluble sugars; Chan and Kwok 1975; Freeman and Worthington 1989) and so this procedure would not have altered the types of carbohydrates that the animals received. A mineral and vitamin supplement was added as 1.5% of dry matter and the entire diet was mixed, divided into daily portions and stored at −20 °C. All diets contained on average 20% dry matter, 92% organic matter, 17% neutral detergent fibre, 12% acid detergent fibre and 4% acid lignin.

Animals were fed ad libitum at 1800 hours each evening. The animals were strictly nocturnal so to avoid possible end-point errors in excreta collections, all food refusals, faeces and urine were collected at 0800 hours each morning. Urine and faeces were also collected at 1800 hours if present but this was rarely necessary.

Experiment 1: dietary nitrogen and digesta passage

Eight woolly opossums (mean mass 0.35 kg) were divided among two groups and fed diets of either 0.90% N or 0.45% N for 10 days. At 1500 hours on the 11th day, they were given an oral dose of a liquid phase marker, Cr-EDTA, mixed with a small amount of mango pulp (Foley et al. 1995). The dose was consumed within 3 min by all animals and so can be regarded as a pulse dose. The animals were then fed their usual diet and faeces, if present, were collected twice every hour for the next 48 h and then at 12 h intervals for the next 120 h.

The faeces were dried to constant mass at 55 °C and the concentration of Cr was measured by atomic absorption spectrometry at Monash University, Australia. Standards were prepared in faeces collected before dosing. Mean retention time was calculated by the PCD procedure of Warner (1981).

At the end of this experiment, the animals were killed with an intraperitoneal injection of sodium pentabarbitone. The gut was removed quickly and clamps were used to divide it into four sections: stomach, small intestine, caecum and colon. The mass of each section was measured to the nearest 0.001 g and the contents were removed by rinsing with saline (0.145 $mol \cdot l^{-1}$). Each section was lightly blotted to remove excess saline, re-weighed and then dried to constant mass at 55 °C.

Experiment 2: maintenance nitrogen requirements

Six woolly opossums (mean mass 0.33 kg) were fed diets of mango fruit pulp, casein and sucrose that varied in nitrogen concentration (0.45%, 0.90% and 2.5% of dry matter) so that their maintenance nitrogen requirements could be determined. The experiment was designed as a combination of two 3×3 Latin squares (Cochran and Cox 1957). Each period lasted 15 days of which the first 8 days served as an adaptation period. Quantitative collections of faeces and urine were made on days 8–14. On day 15 we measured the production of urea in the body as the rate of dilution of a single injection of [^{14}C] labelled urea by following the excretion of the label in samples of urine voided naturally. We checked for excreted samples every 30 min and the natural frequency varied between 13 samples and 28 samples per day. We later calculated what proportion of endogenous urea production was degraded in the gut using the methods of Cocimano and Leng (1967). The diets were then changed so that one animal of each pair received one of the other two diets. Therefore, all opossums received each diet once and the possibility of carry-over effects could be assessed statistically.

The nitrogen content of food, food refusals, faeces and urine was determined by a semi-micro Kjeldahl procedure (Ivan et al. 1974) and total sugars were measured on hot water extracts by the dinitrosalycylic acid method (Englyst and Cummings 1988). Urea in urine was assayed using the diacetyl monoxime method of Crocker (1967). The concentration of non-dietary faecal nitrogen (NDFN) was measured by the method of Mason (1969) following extraction of whole faeces with neutral detergent. Mason's (1969) method assumes that the only undigested dietary nitrogen is that associated with plant cell walls and so NDFN is that fraction of the faecal nitrogen that is of endogenous origin (e.g. sloughed and microbial cells). Higher fibre diets and rapid passage of food through the gut usually result in elevated losses of NDFN (Mason 1969). Truly digestible N intake was calculated as (dietary N intake minus NDFN).

Sampling and collection procedures

The dry matter content of the food offered was determined each day by taking duplicate samples of each diet and drying it at 80 °C to constant mass. Higher temperatures led to charring of the samples. A separate sample of the diet offered each day was stored at −20 °C and later freeze-dried for analysis. Food refusals were combined for each animal over the collection period and stored at −20 °C. This material was thoroughly mixed and a subsample taken for determination of dry matter content at the end of each collection period. The remainder was freeze-dried. Faeces were combined over each 7-day collection period in Experiment 2 and stored at −20 °C and the whole sample was later dried to constant mass at 55 °C. Urine was collected into plastic bottles containing 2 ml glacial acetic acid to prevent the possible loss of nitrogen as ammonia. Urine was collected daily, bulked for each animal over each 7-day collection and stored at −20 °C.

Statistical analysis

Comparisons of treatment means for parameters of digesta passage and gut mass in Experiment 1 were made using Students *t*-test. Repeated measures ANOVA was used to test whether treatment means of parameters of food intake and nitrogen metabolism in Experiment 2 were affected by the nitrogen concentration of the diet. All tests were made using SYSTAT software (Wilkinson et al. 1996). The relationship between body mass and food intake included the exponent 0.75 and so all parameters have been expressed in terms of metabolic body mass ($kg^{-0.75}$) to facilitate comparisons with other studies. Details of body mass are provided to allow alternative treatments.

Results

Experiment 1: dietary nitrogen and digesta passage

Opossums fed a diet of 0.45% N had a significantly shorter mean retention time ($P < 0.01$) of Cr-EDTA than those fed a diet of 0.90% N. Despite this difference in retention time, dry matter digestibility was not significantly different on the two diets. There was little difference in the mass of the gut of animals fed diets differing in nitrogen concentration with the exception of the caecum; the dry mass ($P < 0.05$) and the wet mass ($P = 0.05$) of the caecum of animals fed the low-nitrogen diet were greater than in opossums fed a high-nitrogen diet (Table 1).

Experiment 2: maintenance nitrogen requirements

The most striking observation in Experiment 2 was the significantly greater food intake ($P < 0.05$) of opossums fed the low-nitrogen diet (0.45% N) compared with those fed higher levels of nitrogen (Table 2). This meant that although animals fed the high-nitrogen diet had a higher nitrogen intake, the difference was not significant. In spite of the difference in dry matter intake, there was no significant difference in the apparent dry matter digestibility of the three diets. Since water-soluble sugars made up the majority of dietary dry-matter, the digestibility of water-soluble sugars reflected that of the overall dry matter and was not significantly different among treatments.

The only effect of diet on nitrogen excretion was the significantly greater ($P < 0.05$) excretion of non-dietary faecal nitrogen in animals fed the low-nitrogen diet. This was true whether NDFN excretion was expressed in units of g $Nkg^{-0.75} \cdot day^{-1}$ or as a function of dry matter intake (data not shown).

There was a linear relationship between dietary nitrogen intake and nitrogen balance ($P < 0.001$) and truly digestible nitrogen intake and nitrogen balance ($P < 0.001$; Fig. 1). This allowed the estimation of maintenance nitrogen requirements as 176 mg of dietary N and 146 mg of truly digestible N per day per unit metabolic body mass. There were no significant differences between diets in the rate of synthesis of endogenous urea or in the rate of degradation of this urea and all animals recycled between 60% and 64% of the endogenously produced urea to the gut.

Discussion

Mechanisms of increased food intake

The woolly opossums modified their food intake sufficiently to maintain nitrogen balance on all three diets. Animals fed the low-nitrogen diet ate more food than those fed the medium- and high-nitrogen diets and this compensated for the differences in nitrogen content of the diets, such that there was no significant difference in the nitrogen intake of the animals. Their ability to increase their food intake would be important when only low-nitrogen diets are available in the wild. The ability to increase food intake may also be important in lactating animals. Atramentowicz (1992) showed that female woolly opossums with more than one young ate significantly more in late lactation than in early lactation.

Some small mammals fed fibrous diets are able to increase their food intake to meet increased energy needs

Table 1 Mean retention time of Cr-EDTA and the mass of the gut of six woolly opossums (*Caluromys philander*) fed two diets of different nitrogen content (Experiment 1). Mean ± SE ($n = 4$ for each diet)

	Nitrogen content of diet		Significance of difference between treatments
	0.45 (%)	0.90 (%)	
Body mass (kg)	0.34 ± 0.01	0.36 ± 0.01	ns
Body mass change ($g \cdot day^{-1}$)	−1 ± 1	+2 ± 2	ns
Dry matter intake ($g \cdot kg^{-0.75} \cdot day^{-1}$)	44.1 ± 2.8	35.5 ± 1.9	**
Mean retention time (Cr-EDTA) (h)	13.6 ± 2.0	20.7 ± 1.9	**
Wet mass (g)			
Stomach	3.86 ± 0.15	3.56 ± 0.17	ns
Small intestine	13.18 ± 0.41	12.10 ± 0.34	ns
Caecum	3.38 + 0.26	2.38 ± 0.19	*
Colon	3.07 ± 0.31	2.79 ± 0.34	ns
Dry mass (g)			
Stomach	0.85 ± 0.03	0.80 ± 0.02	ns
Small intestine	2.29 ± 0.23	2.35 ± 0.25	ns
Caecum	0.62 ± 0.03	0.46 ± 0.03	*
Colon	0.59 ± 0.06	0.58 ± 0.06	ns

* $P < 0.05$
** $P < 0.01$

Table 2 The intake, excretion and digestibility of dry matter, nitrogen and water-soluble sugars in six woolly opossums (*C. philander*) fed three diets of different nitrogen content (Experiment 2). All values (except where indicated are in $g \cdot kg^{-0.75} \cdot day^{-1}$ and are the mean ± SE ($n = 6$ each diet)

	Nitrogen content of diet (%)		
	0.45	0.90	2.50
Body mass (kg)	0.33 ± 0.03[a]	0.33 ± 0.02[a]	0.33 ± 0.02[a]
Body mass change ($g \cdot day^{-1}$)	−2 ± 1[a]	+4 ± 5[a]	+5 ± 2[a]
Dry matter intake	46.0 ± 2.4[a]	37.8 ± 1.8[b]	33.5 ± 3.1[b]
Apparent digestibility of dry matter (%)	84.1 ± 1.8[a]	84.0 ± 0.8[a]	87.1 ± 1.0[a]
Nitrogen intake	0.19 ± 0.01[a]	0.18 ± 0.03[a]	0.23 ± 0.05[a]
Faecal nitrogen	0.13 ± 0.00[a]	0.11 ± 0.01[a]	0.13 ± 0.01[a]
Urinary nitrogen	0.05 ± 0.00[a]	0.07 ± 0.02[a]	0.06 ± 0.00[a]
Nitrogen balance	0.01 ± 0.01[a]	0.00 ± 0.02[a]	0.04 ± 0.04[a]
Non dietary faecal N	0.11 ± 0.00[a]	0.08 ± 0.00[b]	0.08 ± 0.00[b]
True digestibility of N	88.9 ± 2.0[a]	84.2 ± 3.8[a]	75.5 ± 5.5[b]
Truly digestible N intake	0.17 ± 0.01[a]	0.15 ± 0.03[a]	0.18 ± 0.05[a]
Urea N synthesis	0.08 ± 0.01[a]	0.10 ± 0.01[a]	0.14 ± 0.02[a]
Urea N degradation	0.05 ± 0.01[a]	0.06 ± 0.01[a]	0.09 ± 0.01[a]
Total sugar intake	33.6 ± 2.7[a]	25.7 ± 1.8[b]	21.8 ± 1.5[b]
Apparent digestibility of sugar (%)	92.3 ± 3.4[a]	94.9 ± 2.1[a]	95.8 ± 2.9[a]

[a,b] Means on the same line bearing different subscripts differ significantly ($P < 0.05$)

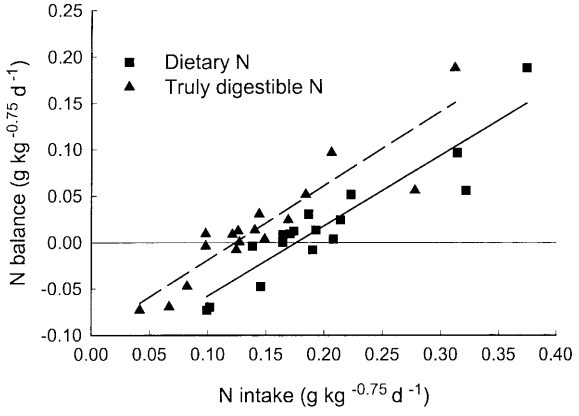

Fig. 1 The relationship between dietary nitrogen intake (NI), truly digestible nitrogen intake (TDNI) and nitrogen balance (NB) in six woolly opossums (*Caluromys philander*) fed diets of fruit pulp varying in nitrogen concentration. Regression eqs.: (i) NB = −0.133 + 0.758 NI; [$r^2 = 0.86$; Residual standard deviation (RSD) = 0.024; $P < 0.001$; *solid line*] (ii) NB = −0.114 + 0.780 TDNI; ($r^2 = 0.82$; RSD = 0.027; $P < 0.001$; *broken line*)

in cold conditions or during lactation (Gross et al. 1985; Hammond and Wunder 1991). The most important factors mediating this response are an increase in the size of the gut (Gross et al. 1985; Hammond and Wunder 1991; Foley and Cork 1992) and in the number of nutrient transporters in the gut (Toloza et al. 1991). Passage rate remains constant and the larger gut is able to accommodate a greater mass of digesta without a corresponding decline in digestibility. This response has been documented in a wide range of small herbivorous mammals and birds feeding on fibrous diets (Foley and Cork 1992).

However, our data suggest that on low fibre diets such as the pulp of mango fruits, increased food intake is achieved to a small extent through changes in gut size but principally via changes in the rate of passage of food through the gut. The digestibility of dry matter and nitrogen did not differ between low- and medium-nitrogen diets in spite of the faster passage of food through the gut on the low-nitrogen diet. However, the fruit pulp diet was very digestible and so on low-fibre diets such as these we would not anticipate that passage time, gut size and digestibility would be so closely linked as they are when small mammals are fed fibrous diets (Gross et al. 1985; Hammond and Wunder 1991).

One feature that may be important in enabling animals to increase the size of their hindgut is the production of fatty acids through the fermentation of dietary fibre or other substrates (Sakata and Tamate 1994). Substantial concentrations of short chain fatty acids (SCFA) were present in the caecal contents (~110 $mmol \cdot l^{-1}$: W.J. Foley, unpublished observations) and although no measurements were made of their production rates, this suggests an active fermentation in the caecum of the woolly opossums studied here; butyric acid in particular stimulates the division of cells within the gut epithelium (Sakata and Tamate 1994).

A more rapid digesta passage is not without its costs. The majority of nitrogen lost in the faeces is not undigested dietary nitrogen but nitrogen resulting from sloughed gut cells, enzyme secretions and microbes that have been washed out of the caecum (Mason 1969). Animals fed the low-nitrogen diet lost greater amounts of this non-dietary nitrogen in the faeces. Although the levels of NDFN on the mango pulp diets were low compared with those of marsupials fed fibrous browse (Foley and Hume 1987), they were related to the intake of dry matter and therefore greater on the low-nitrogen diet than on the medium- or high-nitrogen diets.

Nitrogen requirements

The maintenance nitrogen requirement of the woolly opossums in these experiments was 176 mg dietary N $kg^{-0.75} \cdot day^{-1}$ and 146 mg truly digestible N $kg^{-0.75} \cdot day^{-1}$. These values are substantially below

those measured in a range of herbivorous marsupials fed forage or browse diets (240–500 mg dietary N $kg^{-0.75} \cdot day^{-1}$: summarised in Hume 1999) but greater than those measured in the marsupial sugar glider (*Petaurus breviceps*) fed pollen and nectar (87 mg dietary N $kg^{-0.75} \cdot day^{-1}$: Smith and Green 1987). Marsupials have basal rates of metabolism that are below those of eutherian mammals and even though the relationship between basal metabolism and body protein turnover is perhaps not as close as in eutherian mammals (White et al. 1988), this should still result in low losses of endogenous urinary nitrogen.

Urinary nitrogen losses are even further reduced by a high level of urea recycling but the value of this to the animal is not clear. In herbivorous mammals, urea recycling is responsive to the degree of fermentation occurring in either the foregut or the hindgut. In mammals such as the woolly opossums studied here, the caecum is only moderately developed but it is possible that some soluble material escaped digestion in the small intestine and was fermented in the caecum. Milton and McBee (1983) measured very rapid fermentation rates in the caecum of howler monkeys fed fruit pulp. If a rapid fermentation did occur in the caecum then a high rate of recycling of urea to the gut could be anticipated. This urea would then be hydrolysed in the caecum by bacterial urease and the resulting bicarbonate could buffer some of the SCFA being produced while the ammonium would be used for microbial growth. Our data suggest that urea recycling is not necessarily an adaptive response to low nitrogen diets but is likely to be extensive in all mammalian species that have even a limited microbial fermentation.

The second factor that reduced the N requirement of the opossums studied here is its low losses of NDFN. In comparison with animals fed browse, the losses of NDFN were low (Foley and Hume 1987; Wallis and Hume 1992). This is a function of the absence of soluble phenolics and the low degree of lignification of the fibre of fruit pulps (Milton et al. 1980). In all experiments, NDFN formed the majority of faecal nitrogen and the major route of nitrogen loss. This means that nitrogen requirements are closely linked to the losses of NDFN which are in turn closely linked to the level of dry matter intake (see above).

Comparisons of digestion in frugivorous mammals and birds

One major difference between non-volant mammals and frugivorous birds relates to the rate at which digesta pass through the gut. Frugivorous birds tend to have very short passage times but do not compensate for this with rapid rates of glucose or amino acid transport (Karasov and Levey 1990; Martinez del Rio and Karasov 1990). Rapid passage of fruit pulp through the gut may preclude the total hydrolysis of disaccharides such as sucrose even in those species that possess substantial sucrase activity (Karasov and Levey 1990).

In contrast, the mean retention times of the solute marker Cr-EDTA in the woolly opossums was 13–21 h which is substantially longer than in frugivorous birds. The reasons why frugivorous birds have short passage times are not clear (Karasov and Levey 1990) but involve both characteristics of the fruit and the gut. The most likely explanation for the longer passage times in the woolly opossums studied here is the greater development of the hindgut, especially the caecum; digesta are assumed to be mixed here and subjected to microbial fermentation.

A moderate degree of caecal development is found in most frugivorous mammals (Chivers and Hladik 1980) but others have much simpler guts and in these a fast passage of fruit pulp could occur. These include megachiropterans (mean retention time of a fruit pulp diet of the order of 20 min; Tedman and Hall 1985) and those members of the Carnivora which are frugivorous. For example the gut of the kinkajou (*Potos flavus*) lacks any offset regions (Chivers and Hladik 1980) such as a caecum, and by analogy with the giant panda (Dierenfeld et al. 1982), could be expected to have a very rapid passage of fruit. Data collected opportunistically by Julien-Laferriere (1993) suggest that transit times (i.e. time of first appearance of the food in the faeces) are as short as 45 min. Clearly, studies of frugivorous Carnivora such as the kinkajou would help in understanding whether gut structure constrains absorption of sugars in fruit pulps eaten by mammals.

Another area where the digestive physiology of frugivorous birds and mammals may differ is in their response to the fibre fraction of fruits. Martinez del Rio and Restrepo (1993) have suggested that the traditional division between cell walls and cell contents that is applied in studies of forages may be inadequate for studying the nutrition of fruit-eating animals. Although we agree that this simple division is not an adequate description of the likely nutritional quality of fruits, our results show that the nitrogen requirements of our woolly opossums were affected substantially by losses of non-dietary N in the faeces since NDFN was between 62% and 85% of total faecal N. Provided that tannins are not present (Izhaki and Safriel 1989), much of this extra nitrogen can be attributed to the loss of microbial nitrogen and mucosal cells that are abraded from the gut wall by food (Mason 1969). Both the amount and nature of the fibre will influence the magnitude of these N losses. This aspect was not addressed in our study but we believe that the fibre content of fruits may well be an important determinant of nutritional quality for frugivorous mammals.

The morphology of the digestive tract of frugivorous mammals varies more widely than does the gut of frugivorous birds. Future studies with frugivorous mammals should examine species with a range of different digestive morphologies to determine how digestive physiology influences fruit choice, seed survival and subsequent seed dispersal.

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