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Quantitative Urinary Excretion of Unmetabolised N^{τ}-[Me-^{14}C]Methylhistidine by the Common Ringtail Possum (*Pseudocheirus peregrinus*) Marsupialia

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ABSTRACT. Six common ringtail possums (*Pseudocheirus peregrinus*) were intravenously injected with a standard dose of radioactive 3-Methylhistidine (N^{τ}-[Me-^{14}C]MeH). The dose was rapidly and quantitatively excreted by the possums. More than 90% of radioactivity was recovered within 3 days. Thin layer chromatography and mass spectroscopy showed that 97% of recovered radioactivity was associated with unmetabolised N^{τ}-[Me-^{14}C]MeH. These data satisfy two key requirements for the validity of urinary 3-Methylhistidine (N^{τ}3MeH) excretion as an index of muscle protein catabolism. in *P. peregrinus*. COMP BIOCHEM PHYSIOL 115A;1:53–55, 1996

KEY WORDS. 3-Methylhistidine, N^{τ}-[Me-^{14}C]MeH, muscle protein catabolism, common ringtail possum, *Pseudocheirus peregrinus*, arboreal folivore, marsupial, *eucalyptus*

INTRODUCTION

Representing some 80% of the body's total protein stores, skeletal muscle has a central role in accounting for whole body protein metabolism, and as a protein reserve (12). Under specific conditions such as extreme nutritional stress, trauma and injury, and endocrinological disturbance, substantial changes in body protein metabolism can occur. In turn this may influence the rate of muscle protein accretion and catabolism (9).

The urinary excretion of the amino acid 3-methylhistidine (N^{τ}3-MeH), a product of post-translational methylation of specific histidine residues in the contractile proteins actin and myosin (13), has been used as a quantitative measure of the rate of muscle protein catabolism in clinical research (for review see 9) and animal production (10). The method is subject to several assumptions and limitations which are extensively discussed in literature (2,5,6,9). One such limitation is the metabolism of N^{τ}3-MeH to metabolites common to other biosynthetic pathways, for example, 1-methylimidazole-4-acetic acid by female white mice (7); or its retention in the body as the dipeptide balenine (3). In such cases the urinary excretion of the amino acid will not provide a reliable index of the rate of muscle protein catabolism.

This study presents evidence for the quantitative urinary excretion of unmetabolised N^{τ}-[Me-^{14}C]MeH in the common ringtail possum, a folivorous marsupial known to feed extensively on *Eucalyptus* foliage. Chemical defences present in *Eucalyptus* have been implicated as promotors of muscle protein catabolism, the processes of detoxification and excretion imposing a cost to the animal in terms of body protein reserves (1).

METHODS

This study conforms with the Australian Code of Practice for the care and use of animals for scientific purposes, and was approved by the Experimental Ethics Committee of James Cook University.

Six adult common ringtail possums, 3 males and 3 nonlactating females, were held in individual metabolism cages fitted with urine-faeces separators. The possums were fed an artificial diet based on fruit and cereal. The composition of the diet is as described in Foley (1) with the exception that it contained 10% dry weight unprocessed bran, and vitamin-mineral supplements were omitted. Food and tap water were provided ad libitum.

N^{τ}-[Me-^{14}C]MeH (specific activity 4.2×10^{-13} Bq/mmol) was obtained from Amersham International plc, Buckinghamshire, UK. Removal of nonspecific radioactivity and verification of the purity of the fraction chosen for injection was carried out as described in Nishizawa *et al.* (8).

The possums were given a standard dose of 8.1×10^{-14}

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Bq N$^{\tau}$-[Me-^{14}C]MeH, injected as a solution in 1.5 ml sterile saline (0.15 M) via a brachial vein. Consecutive 24-hr urine collections were made over a period of 7 nights. Urine was collected from stainless steel collection trays into sample bottles contained in insulated flasks of dry ice. Samples were collected frozen and stored at −4°C until analysed. The gridded floor of the cages, the separators, and collection trays were washed with warm distilled water and the washings collected for analysis.

Total radioactivity was measured by liquid scintillation spectrophotometry, using 100 μl of urine or washings mixed with 4 ml scintillation cocktail (Ecolite™ ICN Biomedicals). To test for possible metabolites of N$^{\tau}$-[Me-^{14}C]MeH in urine, samples were acetylated and put onto TLC plates (silica gel 60 F-254 0.2 mm depth on 20 × 20 cm aluminum sheets). The plates were eluted with methanol/chloroform/water (60:20:20) and the zones corresponding to an acetylated N$^{\tau}$3-MeH standard were extracted in methanol/water (1:1). Recovery of radioactivity from the TLC plates and the identity of the radioactive compound were confirmed by scintillation counting and mass spectroscopy, respectively.

RESULTS

The injected dose of N$^{\tau}$-[Me-^{14}C]MeH was rapidly excreted (Fig. 1). Variation in dose recovery was evident, with 2 animals giving a total recovery of less than 90% (82.7% and 78%) as compared to 97 ± 1.6% (mean + SD) for the other 4 possums. Urine volume had no apparent effect on recoveries of radioactivity and there was no loss of urine during the experiment. TLC and mass spectroscopy confirmed that for all animals, an average of 96 ± 3.8% of the recovered radioactivity was associated with unmetabolised N$^{\tau}$-[Me-^{14}C]MeH.

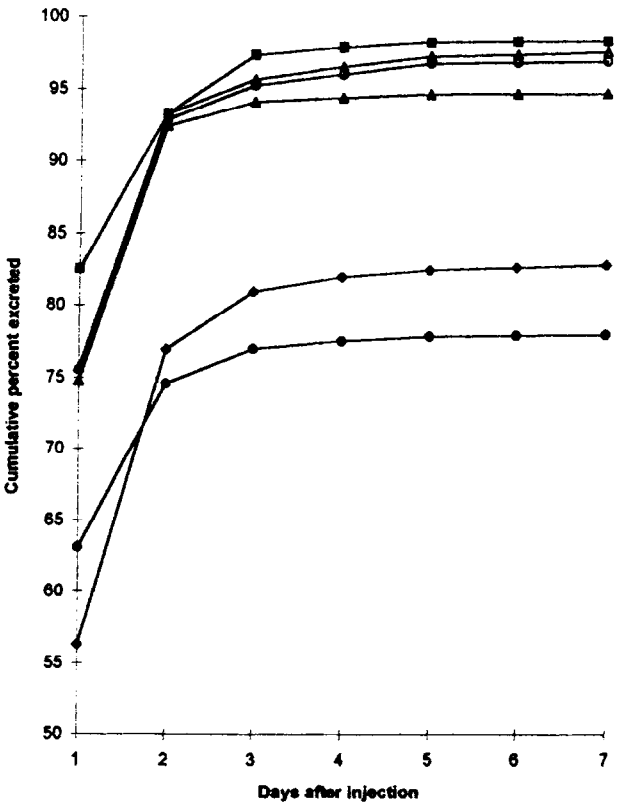

FIG. 1. Cumulative excretion of radioactivity in urine after dosing with L-3-[^{14}C]methylhistidine in 6 adult *P. peregrinus*. Points show cumulative percentage of dose. Each line corresponds to data from a single possum.

DISCUSSION

The rate of excretion of N$^{\tau}$-[Me-^{14}C]MeH by *P. peregrinus* is comparable with that of rats (13) and humans (4) and the recovered dose was present predominantly as unmetabolised N$^{\tau}$-[Me-^{14}C]MeH, satisfying two of the criteria established by Young *et al.* (13). It was not possible at the time to test for correlations between N$^{\tau}$3-MeH turnover and muscle protein catabolism, or that N$^{\tau}$3-MeH in the blood came entirely from skeletal muscle protein. Relative contributions of smooth muscle tissue of skin and intestine to total N$^{\tau}$3-MeH excretion (11,5) are at present uninvestigated for *P. peregrinus*.

Several suggestions can be made to explain the low total recoveries of 2 possums, a female and a male whose ages could not be precisely determined. The first is that some of the injected dose went into muscle or fatty tissue when the animal struggled, resulting in a slower rate of release into the bloodstream. With the exception of Young *et al.* (13) who found no difference in total recovery or excretory rate of labelled N$^{\tau}$3-MeH in rats dosed orally or intravenously, the available literature refers exclusively to arterial or intravenous injection. Otherwise, the effect of dose route has not been examined.

Secondly, the presence of the dipeptide balenine in muscle tissue of *P. peregrinus* could be associated with retention of N$^{\tau}$3-MeH if present in quantities exceeding 5 μMol/g of wet tissue (3), but as carcass analysis was not permissable at the time this could not be verified. The concentration of balenine in *P. peregrinus* muscle is likely to be quite low considering the rapid overall excretion of the labelled dose, and the absence of the metabolite on the TLC plates.

This study demonstrates that N$^{\tau}$3-MeH is excreted rapidly and quantitatively by *P. peregrinus*. It should be noted that the factors controlling N$^{\tau}$3-MeH, for example age (3), in these animals are unknown, as are the relative contributions of sources other than skeletal muscle to total N$^{\tau}$3-MeH excretion.

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