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Quantitative importance of non-skeletal-muscle sources of N^{τ}-methylhistidine in urine

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Direct measurement of N^{τ}-methylhistidine turnover in skeletal muscle, skin and gastrointestinal muscle indicates that these three tissues contribute only 24.9, 6.8 and 9.8% of the total urinary excretion. Measurement of the decay rate of radioactively labelled N^{τ}-methylhistidine in urine indicates that skeletal muscle accounts for 74.5% of the urinary excretion and this is probably an overestimate. These results suggest that the common assumption, that N^{τ}-methylhistidine in urine originates almost entirely from skeletal muscle, may be wrong.

The urinary-excretion rate of N^{τ}-methylhistidine is widely used as an index of muscle-protein degradation (Young & Munro, 1978). The amino acid originates from the degradation of actin and some species of myosin heavy chains in which it is present after the post-translational methylation of specific histidine residues (Young & Munro, 1978). The calculation of muscle-protein-degradation rates from N^{τ}-methylhistidine excretion rates rests on two assumptions. First, N^{τ}-methylhistidine must be excreted quantitatively after liberation, and this seems to be the case in man (Long *et al.*, 1975) and rats (Young *et al.*, 1972), but not in all species (Harris & Milne, 1977; Milne & Harris, 1978). The second assumption is that contractile proteins in non-skeletal-muscle tissues contribute negligible amounts of N^{τ}-methylhistidine to the urine. This is assumed because the amount of N^{τ}-methylhistidine in tissues other than skeletal muscle is small. However, the contribution of each tissue source to N^{τ}-methylhistidine in urine depends on its turnover rate as well as its size. We report here measurements of protein-bound N^{τ}-methylhistidine-turnover rates in different tissues and in the whole body of the rat that indicate that non-muscle sources of N^{τ}-methylhistidine may account for a considerable proportion of urinary excretion of the amino acid.

We have conducted two separate experiments in rats. We have measured the methylation rate of protein-bound histidine in skeletal muscle, smooth muscle of the gastrointestinal serosa and in skin of rats *in vivo*. In the steady state the methylation rate (synthesis) should be equal to the rate of release of N^{τ}-methylhistidine (degradation). The contribution of each of the three tissues to urinary excretion can then be determined. We have also examined the kinetics of whole-body N^{τ}-methylhistidine turnover by monitoring the decay of the radioactivity in the urinary pool after labelling with [*methyl*-^{14}C]-methionine.

Experimental

Constant infusions

Turnover rates were measured by infusing [*methyl*-^{14}C]methionine (16.7μCi/ml; 0.5ml/h) for 6h into the tail veins of 250g female rats and measuring the specific radioactivities of *S*-adenosylmethionine and N^{τ}-methylhistidine in the skeletal muscle (mixed hind limb), skin and gastrointestinal serosa in the animals at the end of the infusion. *S*-Adenosylmethionine was isolated from the supernatant of the tissue as described previously (Grimble & Millward, 1977), after homogenization in 5% sulphosalicylic acid, by high-pressure liquid chromatography on a column (4.2cm × 0.6cm) of 3μm-bead-diameter cation-exchange resin with ammonium citrate [1.3M-NH_4^+ (pH4.2)/10% (v/v) ethanol/0.5% dithioglycol] as the eluting buffer and with u.v. monitoring at 254nm. Fractions corresponding to the *S*-adenosylmethionine peak were collected and counted for radioactivity in a liquid-

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scintillation counter. N^{τ}-Methylhistidine was isolated from the tissue proteins, after hydrolysis in 6M-HCl at 110°C for 20h, by preparative ion-exchange chromatography on a Locarte amino-acid analyser fitted with a stream-splitting device. Sodium citrate (1M, pH4.55) was the eluting buffer. The fractional rate of synthesis (k_s) of protein-bound N^{τ}-methylhistidine was calculated from the relative specific radioactivities of the *S*-adenosylmethionine (S_i) and N^{τ}-methylhistidine (S_B) according to the equation (Waterlow *et al.*, 1978):

$$\frac{S_B}{S_i} = \frac{\lambda_i(1 - e^{-k_s t})}{(\lambda_i - k_s)(1 - e^{-\lambda_i t})} - \frac{k_s}{\lambda_i - k_s}$$

where the values for λ_i, the rate constant for the rise to plateau of the *S*-adenosylmethionine were $37\,day^{-1}$ for skeletal muscle and $47.5\,day^{-1}$ for skin and gastrointestinal serosa. These are the values determined in separate experiments (G. K. Grimble, P. C. Bates & D. J. Millward unpublished work). Since these rats were not growing, k_s is assumed to be equal to k_d, the degradation rate.

Decay curve for N^{τ}-methylhistidine in urine

Rats were fed a meat-free purified diet (20% protein). Six rats (150g) were injected with 25μCi of [*methyl*-^{14}C]methionine and urine was collected continuously over the first 7 days. After that time collections were made at the times indicated for 24h periods. The total N^{τ}-methylhistidine in the urine was measured (after acid hydrolysis) on a Rank–Hilger Chromaspek amino-acid analyser. Specific radioactivities of labelled N^{τ}-methylhistidine were determined as described above. The analysis of the decay curve in Fig. 1 is based on the following arguments. The required value, P_U, the amount of the N^{τ}-methylhistidine in urine arising from each tissue pool, is related to P_T, the corresponding tissue pool, according to the equation:

$$P_U = kP_T \quad (1)$$

where k is the fractional degradation rate.

R_T, the amount of radioactivity in N^{τ}-methylhistidine in each tissue pool and its associated urinary-pool amount, R_U, are related according to the equation:

$$R_U = kR_T \quad (2)$$

However, the magnitude of the initial labelling of each tissue pool will be proportional to its turnover rate, k (i.e. $k = k_s = k_d$ in the steady state), i.e. $R_T \equiv kP_T$. It therefore follows from eqs. 1 and 2 that:

$$P_U \equiv \frac{R_U}{k} \quad (3)$$

Values for R_U and k can be obtained from the decay curve of total radioactivity in urine if it is assumed

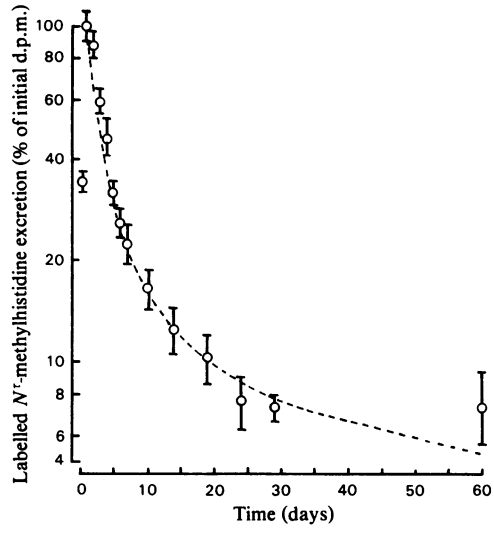

Fig. 1. *Decay of radioactivity in N^{τ}-methylhistidine in the urine of rats injected with [methyl-^{14}C]methionine*
Six rats (150g), fed a meat-free purified diet (20% protein), were injected with 25μCi [*methyl*-^{14}C]-methionine. Urine was collected continuously over the first 7 days, but after that time, 24h collections were made at the times indicated. Specific activities of the labelled N^{τ}-methylhistidine in acid-hydrolysed urine were determined as described in the text. Each point is the mean of three pairs of determinations and is the total daily radioactivity of N^{τ}-methylhistidine in urine normalized to an initial maximum value at day 1 of 100. The broken line is the composite curve of the three exponentials described in the text.

that protein degradation is random. In this case the decay of radioactivity will be a multi-exponential, i.e.:

$$\sum_{i=1}^{n} R_U(t)_i = \sum_{i=1}^{n} R_U(0)_i \cdot e^{-k_i t}$$

so that the values of k_i are equal to the rate constants (i.e. slopes) of each exponential resolved from the decay curve, and values of $R_U(0)_i$ are given by their intercepts.

The slope of the slowest exponential was assumed to be $0.0108\,day^{-1}$, the rate constant for muscle shown in Table 1, and this was assumed to be the only significant component at 29 days. Values for the intercept and for each time point were computed, subtracted from the values shown in Fig. 1, and the resultant curve drawn. This was further resolved into two components. The intercepts [$R_U(0)_i$] and rate constants (k_i) of the three exponentials were used to calculate the pool sizes of unlabelled N^{τ}-methylhistidine [$R_U(0)_i/k_i$].

Table 1. *Turnover rate of tissue pools of N^{τ}-methylhistidine and their contribution to urinary excretion in the rat*
Turnover rates were either measured as described in the text from the labelling of *S*-adenosylmethionine and N^{τ}-methylhistidine in the tissues at the end of a 6h constant intravenous infusion of [*methyl*-^{14}C]methionine (16.7 μCi/ml; 0.5 ml/h), or calculated from observed whole-body excretion rate and pool size and (for the remaining fraction) from excretion and body pool size unaccounted for by the three tissues. Tissue pool sizes are those reported by Nishizawa *et al.* (1977*a*,*b*). Whole-body N^{τ}-methylhistidine-excretion rates were measured as indicated in the text, whereas that arising from individual tissue pools was calculated from turnover rates and pool sizes. Unaccounted-for excretion was judged to have originated from the remaining fraction.

Pool	Turnover (%·day^{-1})	N^{τ}-Methylhistidine content (μmol/250mg rat)	N^{τ}-Methylhistidine excretion (μmol·day^{-1}) (%)
Whole body	3.25	68.8	2.24 (100)
Gastrointestine	9.57 ± 1.4	2.4	0.23 (9.8)
Skin	2.61 ± 0.20	5.8	0.15 (6.8)
Muscle	1.08 ± 0.30	51.6	0.56 (24.9)
Remainder	14.44	9.0	1.30 (58.6)

Results and discussion

The results of the first experiment are shown in Table 1. Since these measurements were made in fully grown female rats, the measured methylation rates can be considered to be equal to the degradation rates. The rates were 1.08, 2.61 and 9.57% · day^{-1} corresponding to half lives of 64, 27 and 7 days for N^{τ}-methylhistidine in skeletal muscle, skin and gastrointestinal serosa. Measurement of mixed-skeletal-muscle-protein-turnover rates made by the simultaneous infusion of [^{14}C]tyrosine in these animals indicate rates of 4% · day^{-1} as previously reported for adult rats (Millward, 1978). Thus actin, the major source of N^{τ}-methylhistidine, would appear to turn over more slowly than other proteins in muscle, as indicated by most other studies (see Millward, 1980). In serosal smooth muscle, measurements of the overall turnover rate of mixed proteins made in the Clinical Nutrition and Metabolism Unit indicates values of over 60% · day^{-1} (McNurlan & Garlick, 1979). Thus the actin-turnover rate would appear to be lower than the average rate in this smooth muscle as well as in skeletal muscle. We have no information on the overall rate of protein turnover in skin.

The only previous measurements of protein-bound-N^{τ}-methylhistidine turnover are those of Nishizawa *et al.* (1977*a*). They measured the decay of label in N^{τ}-methylhistidine in skeletal muscle, skin and gastrointestine after the injection of [*methyl*-^{14}C]methionine and because of the persistence of labelled methionine and *S*-adenosylmethionine in the tissues that we have observed in separate experiments (G. K. Grimble & D. J. Millward, unpublished work), such decay rates are not likely to be reliable indices of protein-turnover rates. Indeed, no decay of label in skeletal-muscle N^{τ}-methylhistidine was observed over a 21-day period, so that no turnover rate could be calculated. Nevertheless, turnover in skin and gastrointestinal muscle was obviously faster than in skeletal muscle.

In order to calculate the contribution of these three tissue pools to the excretion rate, an estimate of the tissue pool sizes must be made. The values shown in Table 1 are those of Nishizawa *et al.* (1977*a*,*b*), which are by and large similar to the values reported by Haverberg *et al.* (1975*b*). The remaining fraction, representing 13% of the total body N^{τ}-methylhistidine, includes a small amount (0.6% of the total) accounted for by various tissues, but the rest is unaccounted for. It may originate from the bone, adipose tissue, the tail and blood, since separate measurements were not made in these tissues.

In the two experiments reported here, N^{τ}-methylhistidine excretion was linearly related to body weight and equal to 1.06 ± 0.00474 μmol · day^{-1} · g body wt^{-1}. Thus in 250g rats it was 2.24 μmol · day^{-1}, a value similar to that reported by Haverberg *et al.* (1975*a*). This is equivalent to a whole-body N^{τ}-methylhistidine-turnover rate of 3.25% · day^{-1}. It would appear that the production rates from the three tissues, measured in the present study, account for less than half the observed excretion rate, with skeletal muscle accounting for only 24.9% of the total excretion. Thus it follows that the fraction unaccounted for, containing 13% of the body pool, turns over at 14.4% day^{-1}, and accounts for more than half the excretion.

As far as the accuracy of these measurements of N^{τ}-methylhistidine turnover are concerned, we have no reason to believe that these results can be underestimates of the real turnover rate. We have avoided any underestimation of the turnover rate due to selective losses of highly labelled contractile proteins during their preparation (Etlinger *et al.*, 1975) by making measurements on total tissue protein. In fact, since measurements were made on fed rats, the degradation rates could possibly have

been even less than the measured synthesis rates. Finally, since measurements were made on the total hind-limb skeletal muscles, it is unlikely that the turnover rate of the whole-body muscular pool is very different from the measured value.

The identity of rapidly-turning-over non-skeletal muscle-sources of N^{τ}-methylhistidine in the remaining fraction is not clear to us, but several possible sources are known (Clarke & Spudich, 1977). However, the presence of such pools is indicated by the rapid initial decay of radioactivity in N^{τ}-methylhistidine in the urine of rats after a single injection of [*methyl*-^{14}C]methionine (Fig. 1). In practice, as mentioned above, the decay of radioactivity in the tissue pools will be slowed by the persistent incorporation from labelled methionine. Thus the rate constants obtained by analysis of the curve in Fig. 1 will be underestimates. Indeed, since there was no observable decay between 30 and 60 days, the fitting of the slowest exponential was somewhat arbitrary. We chose a value of $k = 0.0108\,day^{-1}$, the turnover rate of skeletal muscle indicated in Table 1. This enabled the curve to be resolved into three exponentials from which values for k and P_U were obtained. This indicates that N^{τ}-methylhistidine in urine originates from three tissue pools turning over at 1.08, 12.2 and $55\% \cdot day^{-1}$ and accounting for 74.5, 15.3 and 10.2% of the urinary pool.

If the true decay curve were steeper than indicated in Fig. 1, which we believe it should be, were it not for persistence of labelled methionine, then the size of the slow pool (i.e. originating from muscle) would be smaller than the calculated value of 74.5%, but by how much we cannot yet estimate.

This analysis can only be made with the assumption that protein degradation is random and first-order. Non-random degradation with the selective degradation of recently-synthesized highly labelled N^{τ}-methylhistidine would give a steep decay curve, and this is the interpretation given by Ward & Buttery (1979) when explaining similar results. However, if this occurs at all, it does not occur in skeletal muscle, skin or gastrointestinal muscle, since in none of these tissues was the decay rate of labelled N^{τ}-methylhistidine sufficiently rapid to account for the urinary decay curve (Nishizawa *et al.*, 1977*a*).

Each of these two experiments raises serious doubts about the common assumptions that skeletal muscle is the source of most of the N^{τ}-methylhistidine in the urine of the rat. Without identification of the source of all N^{τ}-methylhistidine in urine, we cannot say whether the problem is as severe in man. However, even without the rapidly-turning-over component, if the relative turnover rates of skin, gut and muscle are the same in man and rats, then non-muscle sources will still be significant, especially in subjects with low muscle mass, such as infants, and patients with muscle-wasting diseases. Nevertheless, with few exceptions, reports on N^{τ}-methylhistidine excretion that have assumed skeletal muscle to be the origin of N^{τ}-methylhistidine indicate apparent changes in muscle-protein degradation that are generally in accord with what is known about protein degradation in muscle, and this is puzzling if non-muscle pools are the source of the excreted amino acid. For this reason we feel that the problem deserves more attention if appropriate methods can be designed. In the meantime it would appear that the common assumption that skeletal muscle is the source of excreted N^{τ}-methylhistidine is not supported by the available experimental evidence.

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