The urinary excretion of N^{τ}-methyl histidine by cattle: validation as an index of muscle protein breakdown

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1. The recoveries of radioactivity in cattle urine following the intravenous administration of N^{τ}-[$^{14}CH_3$]methyl histidine were essentially quantitative in 5–7 d in non-lactating cows, bulls and steers and did not change with age.

2. The N^{τ}-methyl histidine was excreted unchanged in urine.

3. N^{τ}-methyl histidine occurred in muscle extracts both in the free form and as a perchloric acid-soluble, acid-labile form which accounted for approximately 85% of the total non-protein-bound N^{τ}-methyl histidine in muscle and appeared identical to a similar component identified in muscle extracts of sheep and pigs.

4. There was probably an age-related decrease in the concentration of the acid-labile component in muscle but which did not produce a measurable change in recovery of radioactivity in urine.

5. The daily excretion of N^{τ}-methyl histidine (E, μmol) by male cattle was highly correlated with live weight (W, kg) by the equation:

$$E = 50{\cdot}4 + 3{\cdot}536\ (\pm 0{\cdot}044)W \quad (r\ 0{\cdot}997).$$

The excretions progressively decreased from 4·04 μmol/d per kg at 100 kg weight to 3·62 μmol/d per kg at 600 kg.

6. By the criterion of the rate of clearance of labelled N^{τ}-methyl histidine from the body, the excretion of N^{τ}-methyl histidine in urine appears to be a valid index of muscle protein breakdown in cattle.

Although skeletal muscle protein constitutes the major protein deposit of the animal body and can function as a protein reserve in protein metabolism (Daniel *et al.* 1977), such a role remains poorly characterized in quantitative terms in farm animals. The accumulation or mobilization of muscle protein is determined by the relative contributions of protein synthesis and breakdown, the description of both processes being essential for the optimization of the economic and biological efficiency of the production of meat protein. To this end, a number of reports of the measurement of protein synthesis in species of agricultural significance have appeared, such as Buttery *et al.* (1975) for sheep, Garlick *et al.* (1976), Edmunds & Buttery (1978) and Simon *et al.* (1978) for pigs and Lobley *et al.* (1980) for cattle. However no direct measurements of muscle protein breakdown have been reported in these species.

The urinary excretion of N^{τ}-methyl histidine (N^{τ}-MH) from rats and man has been accepted as a measure of muscle protein breakdown in vivo following the demonstration that (1) more than 90% of whole body N^{τ}-MH in rats occurs in skeletal muscle (Haverberg, Omstedt *et al.* 1975; Nishizawa *et al.* 1977) and (2) N^{τ}-MH was not re-utilized in the body but was rapidly and quantitatively excreted in urine (Young *et al.* 1972; Long *et al.* 1975). The procedure was later extended to rabbits (Harris *et al.* 1977) and cattle (Harris & Milne, 1979) but does not appear to be a valid index of muscle protein breakdown in sheep (Harris & Milne, 1977, 1980*a*) or pigs (Milne & Harris, 1978; Harris & Milne, 1981). The attraction of the method is that it is non-destructive, permitting repeated measurements from the same animal and, once validated in a species, does not require the use of radioisotopes, thus retaining the commercial value of the carcass if applied in a farm species.

This paper describes the validation of urinary N^{τ}-MH excretion in cattle (non-lactating cows, bulls and steers) as a measure of muscle protein breakdown using the criterion of validity employed in the instance of rats, man and rabbits, i.e. the rapid and quantitative

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recovery in urine of an intravenously-administered dose of labelled N^{τ}-MH. A preliminary report of this work has appeared (Harris & Milne, 1979).

MATERIALS AND METHODS

Animals

Pure-bred Friesian cattle were used in all experiments. Females were not lactating at the time of experiment and two males, nos. 868 and 869, castrated at 32 weeks of age, were allowed to recover for 19 weeks before further experimentation. Several muscle biopsies of m. longissimus dorsi were taken from these two male animals under general anaesthesia, sampling on alternate sides anteriorly from the first site at the last complete rib. Animals were maintained on a cereal-based diet, details of which have been reported (Harris & Milne, 1980*a*). Female animals were offered twice daily 2 kg diet and 1 kg hay. Male animals were fed 300 g Volac Easimix milk replacer (Volac Ltd, Crayden Old Farm, Wendy, Royston, Herts) in 1200 ml water twice daily at 4 weeks of age and thereafter fed the cereal-based diet *ad lib.* until weights reached 350 kg. Subsequently, the diet was restricted to 3·75 kg and hay to 1 kg twice daily. Muscle samples were also obtained at slaughter from two Friesian steers, nos. 820 and 843, used as controls in a separate experiment and fed *ad lib.* on a semi-synthetic diet devoid of animal meat products.

Material

Details of the preparation of N^{τ}-[$^{14}CH_3$]methyl histidine have been described (Harris & Milne, 1980*a*). The purity of the preparation of labelled N^{τ}-MH varied between 84 and 88% during the period of experimentation and was determined by chromatography in the presence of carrier N^{τ}-MH in the system used for distribution of radioactivity in urine. A solution of the labelled N^{τ}-MH in sterile saline (9 g sodium chloride/l) was administered intravenously into the jugular vein by direct injection or by way of an indwelling catheter.

Collection of urine samples

Urine was obtained from female animals using Foley type, two-way bladder catheters, FG 24, with 30 ml balloon (Warne Surgical Products Ltd, Andover, Hants). A similar but larger catheter, FG 30, 70–100 ml balloon (Eschmann Bros & Walsh Ltd, Peter Road, Lancing, Sussex) was found necessary in larger, mature cows. Urine from male animals up to 140 kg body-weight was collected in metabolic cages and thereafter using harness and a penile cup to which negative pressure was applied. Urine was collected under toluene or toluene was added to the sample when taken.

Sample preparation and analysis

Muscle biopsies and tissues obtained at slaughter were removed as quickly as possible, frozen in liquid nitrogen and stored at −20° until deproteinized with perchloric acid and analysed for N^{τ}-MH as previously described (Harris & Milne, 1980*a*). Blood samples, usually taken from the jugular vein, were processed immediately. Urine samples were desalted on 9 × 50 mm beds of Dowex 50 × 8, 100–200 mesh resin, H^+ form and eluted with 3 M-ammonium hydroxide. After freeze-drying, the sample was dissolved in 0·1 M-hydrochloric acid before analysis for distribution of radioactivity on a 6 × 230 mm bed of Beckman M72 resin (Beckman Instruments Ltd, Glenrothes, Fife) maintained at 28° in sodium citrate buffer, 0·38 M in Na^+, pH 4·18 and operated at 30 ml/h. Fractions (3 ml) of column effluent were collected and 0·5 ml portions of each fraction counted for radioactivity. The N^{τ}-MH-containing dipeptide, balenine, identified in muscle extracts, was demonstrated analytically after chromatography at 15 ml/h on a 6 × 350 mm bed of L16 resin (The Locarte Co., Wendell Road, London) maintained at 50° in sodium citrate buffer, 0·38 M in Na^+, pH 4·07 to which *n*-propanol (20 ml/l) was added. Under these conditions

Table 1. *Recoveries and distribution of radioactivity in urine following intravenous injection of labelled N^{τ}-methyl histidine (N^{τ}-MH)*

Animal no.	Sex	Age	Wt at injection (kg)	Recovery		% of daily urinary radioactivity as N^{τ}-MH*			
				% of dose	Period (d)	1†	2	3	4
439	♀	21 weeks	123	82·4	5				
		30 weeks	190	87·1	6				
		36 weeks	204	90·7	5				
		40 weeks	242	86·0	6	86·4 (81·9)‡	86·2	91·2	91·2
440	♀	20 weeks	127	82·8	4				
		35 weeks	210	93·7	6				
		43 weeks	263	93·8	6	82·2 (89·4)‡	89·6	84·8	89·7
200	♀	22 months	376	92·2	6	85·2	89·1	87·3	79·0
		22·5 months	390	93·6	6	(85·1)‡			
981	♀	95 months	590	91·7	6	85·9	95·1	94·9	89·5
		98 months	620	99·0	6	(80·0)‡			

* Percentage of radioactivity as N^{τ}-MH in injected solutions of labelled N^{τ}-MH 85·9 and 87·7, mean 86·8. Samples desalted before chromatography.

† Days of recovery.

‡ Proportion of radioactivity as N^{τ}-MH in undesalted sample.

anserine, balenine and carnosine were eluted at 9·25, 11 and 12·1 h respectively. For preparative isolation the column effluent was collected at the rate of ten fractions/h and the balenine was obtained from pooled fractions identified by their position relative to carnosine which was detected after reaction with ninhydrin (Rosen, 1957).

Urine samples were analysed for N^{τ}-MH after pretreatment to remove ammonia. Typically, 1 ml urine was adjusted to pH 10·5 with 1 M sodium hydroxide and maintained in vacuo for 30 min over conc. sulphuric acid. The solution was made up to 5 ml with a solution of 5-sulphosalicylic acid (60 mg/l; BDH Ltd, Poole, Dorset or Pierce & Warriner (UK) Ltd, 44 Upper Northgate, Chester) and 1·5–2·0 ml of the solution analysed on a 6 × 470 mm bed of M72 resin (Beckman Instruments Ltd, Glenrothes, Fife) maintained at 28° in sodium citrate buffer, 0·38 M in Na^{+}, pH 4·12 and operated at 30 ml/h.

RESULTS

Recovery and distribution of radioactivity in urine following intravenous administration of N^{τ}-[$^{14}CH_3$]methyl histidine

The cumulative recoveries of radioactivity in urine from four female, non-lactating cows over periods of 5–6 d are shown in Table 1. At least two recovery experiments were carried out on each animal, the ages of which ranged from 21 weeks to 8 years. The results of the last two experiments in each animal show a recovery of greater than 90% of the total radioactivity in all four animals with the exception of the last recovery from animal no. 440. The earlier recoveries from animals nos. 439 and 400 were between 80 and 90%, and may result from either an age-related increase in the proportion of dose recovered in unit time or incomplete recoveries of urine due to leakage around the catheters.

Since urinary recoveries of radioactivity in 5–6 d were usually in excess of 90%, it was assumed that the C of N^{τ}-MH was not excreted by any other route such as faeces or in expired gases.

In view of the possible age-related increase in the proportion of dose recovered in the

Table 2. *Recovery of radioactivity in the urine of male cattle following intravenous injection of labelled N^{τ}-methyl histidine at intervals of 6–27 weeks*

Animal no. ...	868 *	Recovery			869*	Recovery	
Age (weeks)	Wt at injection (kg)	% of dose	Period (d)	Age (weeks)	Wt at injection (kg)	% of dose	Period (d)
4	39	88·9	5	3	46	94·3	6
10	67	87·6	7	10	70	95·1	3
16	109	90·1	7	16	108·5	90·9	6
27	232	83·0	7	30	243	94·0	7
54	418	91·9	5	51	401	92·7	7
71	489	94·6	3	73	521	90·3	3

* Castrated at 32 weeks.

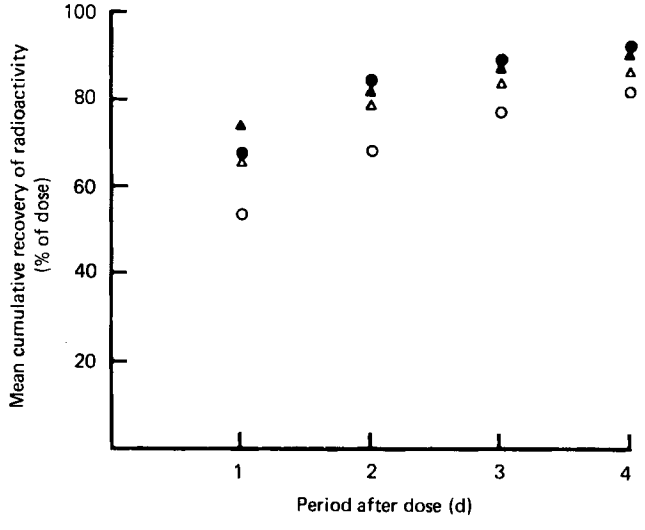

Fig. 1. Mean cumulative recoveries of radioactivity (% dose) in cattle urine. (○), Animal no. 439. (●), animal no. 440. (△), animal no. 868. (▲), animal no. 869. The points are the mean values of three recoveries from animal no. 440 and of four recoveries from each of the other three animals.

urine from young animals nos. 439 and 400, recoveries from two further animals (males) were monitored at intervals from before weaning to over 1 year of age and did not show a consistent trend (Table 2). Castration did not appear to influence either the rate or amount of radioactivity recovered in urine (Table 2). The mean recoveries of radioactivity were 89·3 and 92·9% for animals nos. 868 and 869 respectively. Although calculated from different times of recovery, this difference in mean recovery remained if calculated from the shorter recovery time at each age (mean 89·0 and 92·5% for animals nos. 868 and 869) or if calculated from the recovery in 3 d at all ages (mean 85·2 and 90·1% for animals nos. 868 and 869) and provides documentation of a difference in the rate of excretion of radioactivity between two animals. This information is presented in Fig. 1 as the mean daily cumulative excretion of radioactivity calculated from four collections, together with similar values from animals nos. 439 and 440, and show that within a group of four animals, the mean daily recovery of radioactivity may differ by 20% on day 1 and by 7% on day 2.

The distribution of radioactivity in daily urine samples was also determined and showed

Table 3. *Changes in the proportion of radioactivity associated with N^{τ}-methyl histidine in individual urine samples from cattle following intravenous administration of labelled N^{τ}-methyl histidine*

Animal no. ...		868		869
Sample	Time (min)	% of radioactivity as N^{τ}-MH	Time (min)	% of radioactivity as N^{τ}-MH
1	19	63·6	10	63·9
2	90	74·2	36	68·9
3	215	77·3	96	70·8
4	327	80·4	180	69·2
5	372	80·7	227	74·4
6	—	—	271	80·5
Pooled 24 h urine		80·5		79·3

that the proportion of the radioactivity eluting in the position of N^{τ}-MH was very similar to that in the injected solution (Table 1), suggesting that metabolism of N^{τ}-MH in cattle was insignificant or absent. That the distribution was not altered by the desalting procedure employed was established by analysing untreated urine samples from the first day of each recovery period (Table 1). The impurities present in the preparation of labelled N^{τ}-MH were also excreted in urine since recoveries of radioactivity were usually greater than 86·8%, the mean proportion of radioactivity associated with N^{τ}-MH in the injected solution (Table 1). The impurities appear to be cleared from the body faster than the labelled N^{τ}-MH since the values in Table 1 show that the proportion of radioactivity associated with N^{τ}-MH increased with time and often exceeded that in the injected solution on days 2–4. This differential clearance of N^{τ}-MH and radio-active impurities in the preparation is further supported by the progressive increase in the proportion of radioactivity associated with N^{τ}-MH in individual urinations from animals nos. 868 and 869 following intravenous injection of labelled N^{τ}-MH (Table 3).

The concentration of non-protein-bound N^{τ}-MH in muscle and blood

Analysis of deproteinized extracts of muscle (m. longissimus dorsi) and blood for N^{τ}-MH showed that the concentration of free N^{τ}-MH in unhydrolysed extracts of both tissues was usually very low and was often difficult to quantitate reliably (Tables 4 and 5). Extracts from muscle showed marked increases on acid-hydrolysis in 6 M-HCl (Table 4), suggesting that N^{τ}-MH was present in both the free form and an acid-labile form of low molecular weight which contained on average 84·8% of the total non-protein-bound N^{τ}-MH in muscle (calculated excluding the values for calves nos. 1 and 2 in Table 4). The limited information from muscle biopsies of animals nos. 868 and 869 (Table 4) may suggest an age-related decrease in the total concentration of non-protein-bound N^{τ}-MH in muscle. The concentration of free N^{τ}-MH in blood was also very low and showed little change after acid-hydrolysis (Table 5).

The acid-labile, N^{τ}-MH containing component was identified as the dipeptide, balenine, (β-alanyl-N^{τ}-MH) by chromatographic elution position and by amino acid composition and sequence (Harris & Milne, 1980*b*). Analyses of serial muscle biopsy samples showed the presence of the N^{τ}-MH containing dipeptide in all samples taken from animals nos. 868 and 869 between 5 and 51 weeks of age (Table 4). The dipeptide component was also identified by elution position in muscle samples (m. longissimus dorsi) from a young Friesian calf and a further two adult Friesian steers used as controls in separate experiments.

Table 4. *Concentration of non-protein-bound N^{τ}-methyl histidine (N^{τ}-MH in m. longissimus dorsi)*

Animal no.	Age	Wt (kg)	Non-protein bound N^{τ}-MH (nmol/g musle)		Percentage of N^{τ}-MH in acid labile form
			Free	Total	
439	41 weeks	236	—	141·1	—
440	46 weeks	263	9·6	63·9	85·0
200	23 months	403	—	87·8	—
981	98 months	627	3·9	103·7	96·2
868†	5 weeks	45	•	314·1	—
(biopsy samples)					
	12 weeks	75	•	142·0	—
	19 weeks	115	5·5	73·0	92·5
	30 weeks	250	6·1	17·2	64·5
	51 weeks	398	8·0	41·4	80·7
869†	5 weeks	50	•	232·0	—
(biopsy samples)					
	12 weeks	77	•	59·9	—
	19 weeks	115	3·9	76·0	94·9
	30 weeks	230	8·2	77·3	89·4
	51 weeks	385	10·0	40·0	75·0
Calf 1†	Stillborn	—	78·3	206·0	62·0
2†	6 hours	—	23·8	89·5	73·4

• N^{τ}-MH present but too low to quantitate.
† Samples uncorrected for losses during processing; typical recovery 70%.

Table 5. *Concentration of N^{τ}-methyl histidine (N^{τ}-MH) in cattle blood*

Animal no.	Age	Non-protein-bound N^{τ}-MH (μmol/ml blood)	
		Free	Total
439	21 weeks	1·9	1·5
	37 weeks	5·8	ND
440	24 weeks	3·1	4·8
	35 weeks	2·9	ND
	39 weeks	3·2	ND
200	22·5 months	3·1	ND
	23 months	4·4	ND
981	92 months	2·6	ND
	95 months	5·7	ND
868	5 weeks	2·7	4·3
869	5 weeks	4·7	3·6

ND, not determined.

The excretion of N^{τ}-methyl histidine in urine

The mean daily excretions of N^{τ}-MH in urine of cattle are shown in Fig. 2 for animals between 39–620 kg weight. Each value is the mean excretion from at least three consecutive days. Most of the results were obtained from two male animals before and after castration at 250 kg. The daily excretion of N^{τ}-MH (E, μmol) calculated from the regression on live weight (W, kg) for male animals (n 10) is:

$$E = 50{\cdot}4 + 3{\cdot}536\,(\pm 0{\cdot}044)W \quad (r\ 0{\cdot}997)$$

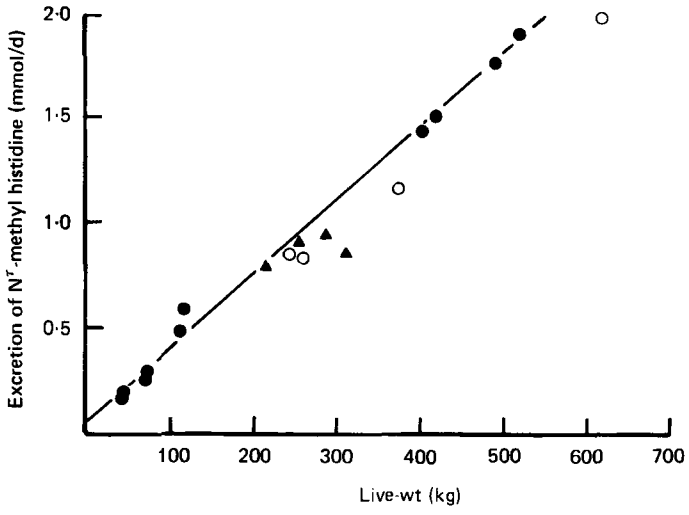

Fig. 2. Mean daily urinary excretion of N^{τ}-methyl histidine (mmol/d) from cattle. (●), Bulls and steers; (○), dry cows; (▲), values for steers from Nishizawa *et al.* 1979.

The excretion from female animals (Fig. 2) followed a similar trend but was significantly lower ($P < 0{\cdot}05$) than that from male animals. The recently published results of N^{τ}-MH excretion by Holstein steers (Nishizawa *et al.* 1979) are included for comparison (Fig. 2).

DISCUSSION

The apparent simplicity of obtaining the rate of muscle protein breakdown in vivo from measurements of the rate of excretion of N^{τ}-MH in urine makes such a procedure highly attractive, since both short-term changes in the rate of protein breakdown and the contribution of muscle protein turnover to that of the whole body can be assessed in whole-body protein turnover is measured simultaneously (reviewed in Young & Munro, 1978).

The main assumptions and limitations in the use of urinary N^{τ}-MH as an index of muscle protein breakdown have been pointed out by Harris & Milne (1977) and subsequently reviewed in more detail (Young & Munro, 1978; Ward & Buttery, 1978). These are that (1) potential sources of N^{τ}-MH should be avoided in the diet, (2) urinary N^{τ}-MH originates largely in skeletal muscle, (3) muscle protein methylation remains constant and (4) urinary N^{τ}-MH is not metabolized. The further assumptions that have been little considered are (5) that N^{τ}-MH released from protein breakdown is rapidly eliminated from the body and the implications of this for the size of the body pool of non-protein-bound N^{τ}-MH and (6) that actin and myosin have similar rates of breakdown in vivo. This follows since N^{τ}-MH from actin cannot be distinguished analytically from that of myosin. Hence, urinary excretion of N^{τ}-MH is thus a measure of the breakdown of these two proteins only. Its use as a measure of myofibrillar or total muscle protein breakdown involves a further assumption that the average rate of breakdown of myofibrillar or mixed muscle proteins are similar to those of actin and myosin.

In this study the diet was N^{τ}-MH-free, assumptions 2, 3 and 6 were accepted as valid and assumptions 4 and 5 were tested by experiment.

Validation of the urinary excretion of N^{τ}-MH as a measure of muscle protein breakdown in vivo in cattle

In agreement with assumption 5, the rapid recoveries of radioactivity in cattle urine (Tables 1 and 2) suggest that the urinary excretion of N^{τ}-MH is a satisfactory index of muscle protein breakdown in cattle. The urinary recoveries of radioactivity range from 82·4 to 99·0% of dose in a total of twenty-three recovery measurements from six animals aged between 3 weeks and 8 years (Tables 1 and 2). Only seven of these recoveries were less than 90% of dose in 4–7 d and three of these measurements were in young female animals (Table 1). The 86% recovery from heifer no. 439 at 40 weeks of age is thought to be due to losses of urine during collection as are those recoveries less than 90% from bull calf no. 868 (Table 2). These values may be compared with recoveries of 100% of dose in 3 d from rats (Young *et al.* 1972), 95% in 2 d from adult humans (Long *et al.* 1975) and 90–97% in 7d from adult rabbits (Harris *et al.* 1977). In contrast, sheep (Harris & Milne, 1980*a*) and pigs (Harris & Milne, 1981) only slowly excrete labelled N^{τ}-MH in urine. The slightly lower recoveries observed in young female cattle at 20 weeks of age (Table 1) may (1) reflect a genuine sex difference, since recoveries from young males (Table 2) did not show such differences, (2) be indicative of an age-related increase in the recovery of radioactivity in urine as also occurs in sheep (Harris & Milne, 1980*a*) or (3) be due to methodological problems associated with the use of bladder catheters in female animals. The last explanation is favoured in view of the difficulties experienced in achieving a completely leak-free system when catheters were used in young animals although the variation between animals in the acceptance of the catheter was quite marked. In addition, later work showed that an appreciable proportion of the dose was excreted in relatively small volumes of urine in the first few hours of the experiment (C. I. Harris and G. Milne, unpublished observations). Hence, significant losses of radioactivity in small volumes of urine could occur by leakage. However, it is difficult to reconcile the low recoveries observed in young female animals with the volumes of urine that would have to be lost by leakage in order to obtain recoveries 5–10% lower than the highest values obtained in the same animals (Table 1).

Table 2 and Fig. 1 demonstrate that recoveries between animals vary appreciably as illustrated by the mean recoveries from animals nos. 868 (mean 89·3%, *n* 6) and 869 (mean 92·9%, *n* 6) and that such differences remain when adjusted for similar periods of collection. The reasons for such differences have not been documented in this study but may be due to differences either in the magnitude of the body pool of non-protein-bound N^{τ}-MH or in the rate-constants of one or more of the processes involved in the elimination of N^{τ}-MH from the body. The very limited information from muscle biopsies (Table 4) suggests that, while the concentration of non-protein-bound N^{τ}-MH in muscle may decrease with age, this change did not appear to influence the proportion of dose recovered in 5–7 d (Table 2).

The other essential requirement for the validation of the urinary N^{τ}-MH as a measure of muscle protein breakdown is the absence of metabolic degradation of the N^{τ}-MH excreted in urine (assumption 4) as might occur in the kidney. This requirement is clearly met by the distribution values in Table 1 which show that the mean proportion of radioactivity excreted daily as N^{τ}-MH was 88·0% (mean of four consecutive daily collections in each of four animals) compared with a mean distribution of 86·8% of the radioactivity as N^{τ}-MH in the labelled preparation of N^{τ}-MH. Although the labelled N^{τ}-MH contained up to 14% of the radioactivity associated with compounds other than N^{τ}-MH, these impurities also must have been eliminated in urine since total recoveries in excess of 87% were often obtained (Tables 1 and 2). However, the rate of elimination of the impurities may differ from that for N^{τ}-MH and probably accounts for the increased proportion of

radioactivity associated with N^{τ}-MH as observed on days 2, 3 and 4 in several instances (Table 1) and in successive urinations (Table 3). The results also suggest that N^{τ}-MH is not metabolized as reported to occur in the rat (Young *et al.* 1972) and man (Long *et al.* 1975).

As a result of the rapid recoveries of a dose of labelled N^{τ}-MH in urine from dry cows, bulls and steers (Tables 1 and 2) and the apparent absence of metabolic products of N^{τ}-MH in urine (Table 1), it is proposed that cattle may now be included in the group of animals for which urinary excretion of N^{τ}-MH is a valid index of muscle protein breakdown in vivo. This validation in the non-lactating cow, bull and steer is timely in view of the growing interest in the separate measurements of protein synthesis and breakdown in muscle tissue relative to growth, the contribution of muscle protein metabolism to that of the whole body and the energetics of protein accretion. The validation has also been completed in the lactating dairy cow and will be reported separately (Harris *et al.* 1980).

The concentration and distribution of non-protein-bound N^{τ}-MH in blood and muscle

As suggested in assumption 5, the rapid elimination in urine of a dose of labelled N^{τ}-MH implies a relatively small body pool of non-protein-bound N^{τ}-MH. The concentration of free N^{τ}-MH in muscle was very low (Table 4) and usually less than approximately 6 nmol/g muscle or /ml blood. The increase in the concentration of N^{τ}-MH in muscle extracts on hydrolysis indicated the presence of an acid-labile, N^{τ}-MH containing component of low molecular weight which contained on average 84·8% of the non-protein bound N^{τ}-MH in muscle (Table 4). The magnitude of the increase in N^{τ}-MH on hydrolysis (Table 4) is thus similar to that occurring in sheep (Harris & Milne, 1980*a*) although the absolute values appear to be lower in cattle samples. In contrast at least 90% of the non-protein-bound N^{τ}-MH in pig muscle was present in the acid-labile component which increased to 99·8% of the total N^{τ}-MH as the concentration of non-protein-bound N^{τ}-MH increased with age (Harris & Milne, 1981). The absolute concentration of non-protein-bound N^{τ}-MH in pig muscle was up to fifty times greater than the highest values found in cattle muscle (Table 4).

Blood samples also showed very low concentrations of free N^{τ}-MH (Table 5) which did not appear to change on hydrolysis. This suggests that the acid-labile component found in muscle extracts was either absent or present in very low concentrations in blood and contrasts with the situation in sheep blood or plasma where the levels of free N^{τ}-MH are relatively elevated (Leibholz, 1968; Wolff *et al.* 1972; Harris & Milne, 1980*a*) and where an appreciable increase in N^{τ}-MH occurred on hydrolysis (Harris & Milne, 1980*a*). The low concentrations of free N^{τ}-MH in cattle blood are similar to those reported in the plasma of steers (Oltzen *et al.* 1967), man (Stein & Moore, 1954; Cusworth & Dent, 1960; Delaporte *et al.* 1978), the rabbit (Stave & Armstrong, 1972) and in rat serum (Haverberg, Omstedt *et al.* 1975), all species which show rapid clearances of labelled N^{τ}-MH. The concentrations of free N^{τ}-MH in muscle appear to reflect the levels in blood and are correspondingly higher in sheep (Harris & Milne, 1980*a*) than in the rabbit (Stave & Armstrong, 1973), man (Delaporte *et al.* 1978) and cattle (Tables 4 and 5).

The acid-labile component containing N^{τ}-MH in extracts of cattle muscle appeared identical by chromatographic elution position to the dipeptide, balenine, also found in muscle extracts from sheep and pigs. The identity has now been confirmed by amino acid composition and sequence (Harris & Milne, 1980*b*). Thus, the occurrence of the peptide in muscle is not simply related to the rate at which labelled N^{τ}-MH is eliminated from the body since it has also been identified in the muscle of rats and rabbits (Harris & Milne, 1980*b*), species which also show rapid clearances of labelled N^{τ}-MH, as well as in the muscle of sheep and pigs, species which slowly excrete labelled N^{τ}-MH in urine (Harris & Milne, 1981).

The excretion of N^{τ}-methyl histidine in urine

The linear relationship between the mean daily excretion of N^{τ}-MH in urine and body-weight in Friesian cattle (Fig. 1) was derived from ten periods of urine collection from two male animals at weights between 39 kg (before weaning) and 520 kg. The high correlation of excretion of N^{τ}-MH on body-weight (r 0·997) over such a wide weight range suggests that the excretion is determined by a body component which contributes a constant fraction of body mass and a constant proportion of which is degraded over the weight range. This component is likely to be muscle tissue (or strictly, the N^{τ}-MH containing proteins actin and myosin of muscle tissue), not only because more than 90% of whole-body N^{τ}-MH occurs in that tissue (Haverberg, Omstedt *et al.* 1975; Nishizawa *et al.* 1977; Nishizawa *et al.* 1979) but also because the contribution of muscle tissue to body-weight (Munro, 1969) and the proportion of total muscle protein contributed by actin and myosin remain constant (as suggested by the contribution of myofibrillar to total muscle protein (Young, 1970)). The recent measurements of N^{τ}-MH excreted by Holstein steers (Nishizawa *et al.* 1979) are in close agreement with the results of this study (Fig. 1).

Female animals excrete less N^{τ}-MH daily than male cattle of the same body-weight and similar dietary intakes, e.g. at 620 kg the female excreted 88·6% of that calculated for the male. A similar sex difference in excretion of N^{τ}-MH has been reported in man (Munro & Young, 1978; Tomas *et al.* 1979). The larger mean daily excretions of N^{τ}-MH by male animals (Fig. 2) suggest that males have a greater proportion of body-weight contributed by muscle tissue, a higher fractional breakdown rate for muscle protein or a higher protein-bound content of N^{τ}-MH or both than found in females at the same weight. Although the urinary excretion of N^{τ}-MH has also been validated in lactating cattle as judged by the rapid recovery in urine of a subcutaneously-administered dose of labelled N^{τ}-MH, the daily excretion of N^{τ}-MH is considerably lower than in non-lactating females of the same weight (Harris *et al.* 1980).

The daily excretion of N^{τ}-MH by cattle (Fig. 1) calculated from the regression equation, divided by the respective live weights to give the N^{τ}-MH excreted/d per kg body-weight, results in a curvilinear graph when plotted *v.* live weight, decreasing from 4·04 μmol N^{τ}-MH/d per kg at 100 kg to 3·62 μmol/d per kg at 600 kg. The greatest change in slope occurred at low weights and the relationship became almost linear at weights above 400 kg. Such a decrease in N^{τ}-MH excretion per unit body-weight was also noted in cattle by Nishizawa *et al.* (1979) but the almost linear decrease in N^{τ}-MH excreted/d per kg claimed by those authors in the range 200–300 kg may well be associated with the narrow range in weight and the small number of animals on which their measurements were made.

Similar decreases in the mean excretion of N^{τ}-MH/d per kg with age have been demonstrated in man (Munro & Young, 1978; Tomas *et al.* 1979) and the same trend with age is apparent in the excretion of N^{τ}-MH by rats (Haverberg, Deckelbaum *et al.* 1975) although the values of 12·04 μmol N^{τ}-MH/d per kg at 110 g body-weight and 7·91 μmol/d per kg at 280 g weight are substantially higher than were found in this study and by Nishizawa *et al.* (1979) for cattle. These elevated values for the rat are supported by similar results reported by Omstedt *et al.* (1978) and Bohley *et al.* (1979) where excretions of 9·2 μmol/d per kg at 130 g weight and 8·5 μmol/d per kg at approximately 100 g respectively were found. This difference between species is not due to a higher protein-bound content of N^{τ}-MH in rat muscle (Haverberg, Omstedt *et al.* 1975; Nishizawa *et al.* 1977) than in cattle muscle (Nishizawa *et al.* 1979) but probably results from the higher fractional rates of protein breakdown found in small animals such as the rat (Waterlow *et al.* 1978). The difference may also be influenced in part by comparisons between species at different stages of development since fully-mature rats (450 g) and rabbits (3600 g) excrete 7·1

Table 6. *The contribution of muscle protein turnover to whole-body protein breakdown in adult female cattle*

Animal no. ...	439	440	200	981
Age (years)	0·9	0·9	1·9	8·0
Live wt (kg)	236	263	376	628
N^{τ}-MH excretion (μmol/d)	852·0	828·2	1166·9	1988·2
Muscle protein degraded (g/d)*	243·4	236·7	333·4	568·1
Fractional breakdown rate (%/d)	1·41	1·37	1·23	1·18

N^{τ}-MH, N^{τ}-methyl histidine.
* Calculated using a protein-bound content of 3·5 μmol N^{τ}-MH/g muscle protein (Nishizawa *et al.* 1979). See also assumptions on p. 417.

(D. J. Millward, personal communication) and 5·3 (Harris *et al.* 1977) μmol N^{τ}-MH/d per kg. Hence, the excretion of N^{τ}-MH/d per kg body-weight appears to decrease not only with age within a species, but also with increasing body-weight between small laboratory animals and larger mammals at the same stage of development.

As suggested by the values in Fig. 1, the mean excretion of 3·32 μmol N^{τ}-MH/d per kg body-weight by steers at 200–300 kg (Nishizawa *et al.* 1979) are in close agreement with the value of 3·74 μmol/d per kg calculated at 250 kg weight from the regression equation and may be compared with the values of 2·92 (Bilmazes *et al.* 1978), 3·2 (Munro & Young, 1978), 3·93 (Long *et al.* 1977) and 4·3 (Tomas *et al.* 1979) reported for young adult men. The reason for the wide range of values from studies in man is not clear. Since total excretions of N^{τ}-MH were lower in female cattle than from males of the same weight (Fig. 1), the daily excretion per kg body-weight will also be lower for female animals. This is consistant with observations in man (Munro & Young, 1978; Tomas *et al.* 1979).

The amount of protein broken down each day in muscle of adult cattle can be calculated (Table 6) from the excretion of N^{τ}-MH by four female animals (Fig. 1) using a protein-bound content of 3·5 μmol N^{τ}-MH/g muscle protein (Nishizawa *et al.* 1979). The fractional breakdown rate (FBR) range from 1·41 at 230 kg to 1·18 at 620 kg (Table 6), values which are in close agreement with the FBR determined for muscle protein in adult man from the urinary excretion of N^{τ}-MH (Tomas *et al.* 1979).

The FBR of muscle protein determined from N^{τ}-MH excretion for female animals (Table 6) were very similar to the fractional rates of protein synthesis reported for these animals by Lobley *et al.* (1980) as measured from the incorporation of radioactive amino acids. Since these animals were either non-growing or were changing weight only slowly, the similarity of the fractional rates of protein synthesis and breakdown calculated by unrelated methods suggests that urinary N^{τ}-MH originates largely in muscle and that non-muscle sources contribute at most only a small proportion of the N^{τ}-MH excreted in urine.

It has been shown that intravenously administered N^{τ}-[$^{14}CH_3$]methyl histidine was rapidly and quantitatively excreted by cattle in urine, demonstrating that N^{τ}-MH released by protein breakdown was quickly removed from the body. It does not prove that urinary N^{τ}-MH originates only in muscle proteins. Since such rapid excretions in other species have been accepted as validation of the urinary N^{τ}-MH as an index of muscle protein breakdown, it is proposed that cattle be added to the species in which the method has been established.

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