N^{τ}-Methylhistidine content of organs and tissues of cattle and an attempt to estimate fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth by measuring urinary output of N^{τ}-methylhistidine

BY N. NISHIZAWA, Y. TOYODA, T. NOGUCHI* AND S. HAREYAMA

Department of Agricultural Chemistry, Iwate University, Morioka, Iwate, Japan

AND H. ITABASHI†

Tohoku National Agricultural Experiment Station, Morioka, Iwate, Japan

AND R. FUNABIKI

Department of Agricultural Chemistry, Tokyo Noko University, Fuchu-shi, Tokyo, Japan

(*Received* 15 *January* 1979 – *Accepted* 27 *February* 1979)

1. Distribution of N^{τ}-methylhistidine (3-methylhistidine; Me-His) among organs and tissues in cattle was determined. From the amount of Me-His in skeletal muscle protein and daily urinary output of Me-His, fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth were calculated.

2. More than 93·4% of the total Me-His (35·6 mg/kg body-weight) in the analysed cattle tissues occurred in skeletal muscle protein. The amount of Me-His in tissues other than skeletal muscle was relatively small. Daily urinary excretion of Me-His in the cattle which were fed on hay and concentrate was 135 mg at a growing stage of 217 kg body-weight and 145 mg at a stage of 312 kg. The Me-His content of foodstuffs was also carefully checked in the present investigation. Assuming that absorbed dietary Me-His is quantitatively excreted in the urine without delay, the contribution of Me-His in foodstuffs was calculated to be approximately 30% of the urinary Me-His. Rumen protozoa and bacteria contained little Me-His.

3. From these results, fractional catabolic and synthetic rates of myofibrillar proteins of the cattle at a growing stage of 217 kg body-weight were calculated to be 1·22%/d (half-life 56 d) and 2·73%/d, while these rates at a stage of 312 kg body-weight were 1·02%/d (half-life 67 d) and 1·51%/d respectively. These values were calculated on the same assumptions as those for the rat (Young *et al.* 1972; Funabiki *et al.* 1976). Of the Me-His present in foodstuffs 94% was tentatively assumed to be excreted into urine.

Estimation of synthetic and catabolic rates of skeletal muscle proteins of domestic animals is very important for studying whole-body protein metabolism.

Perry (1974) estimated synthetic and catabolic rates of muscle proteins of piglet. Buttery *et al.* (1975) showed that the fractional turnover rate of muscle protein in sheep is 1·7%/d (half-life 40·7 d). However, there have been no reports of studies of the turnover rates of myofibrillar proteins of skeletal muscle in cattle.

The turnover rate of myofibrillar proteins is often calculated from urinary excretion of N^{τ}-methylhistidine (3-methylhistidine; Me-His) in the instance of small animals, as well as man (Young *et al.* 1972; Long *et al.* 1975; Haverberg, Deckelbaum *et al.* 1975; Funabiki *et al.* 1976; Nishizawa *et al.* 1977; Nishizawa, Shimbo *et al.* 1978; Young & Munro, 1978).

In order to apply this method to cattle, Me-His distribution among proteins of various organs or tissues in cattle has been determined in the present investigation, together with the amount of Me-His in excreta and food.

* Present address: Department of Agricultural Chemistry, The University of Tokyo, Tokyo, Japan.

† Present address: National Institute of Animal Industry, Chiba, Japan.

0007-1145/79/3271-1501 $01.00

METHODS

Animals and feeding methods

Five Holstein steers and one male Holstein of 8–9 months of age, weighing approximately 217 kg, were used. The animals were penned individually and given *ad lib.* cocksfoot (*Dactylis glomerata*) hay and mixed concentrate for 65 d. The concentrate was composed of (g/kg): 400 maize, 300 barley, 160 bran, 60 rice bran, 60 soya-bean meal, 20 salt mixture. The mean food intake during the experimental period was 1·58 kg/d for hay and 5·79 kg/d for concentrate. Urine samples (24 h) and faeces were collected every 21 or 23 d during the experimental period. Urine samples were collected in plastic vessels containing 500 ml 3 M-hydrochloric acid and 10 ml toluene. They were stored at −20° until required for analysis. Faeces were sprayed with 3 M-HCl and dried at 50° under an air-stream. At the end of the experimental period, the animals were slaughtered. Viscera were removed quickly and weighed. After removal of the viscera, the skin was removed and weighed. The halves of the dressed carcasses were weighed and the skeletal muscles were excised carefully with a dissecting knife and weighed. A sample of the organs or tissues was retained for analysis of Me-His and stored at −20°.

Rumen protozoa and bacteria were collected according to the method described previously (Itabashi & Kandatsu, 1975).

Tissue proteins were prepared as described in a previous paper (Nishizawa *et al.* 1977).

Analytical procedures

Nitrogen and creatinine in urine samples and N in faeces were measured by the method of a previous paper (Nishizawa *et al.* 1977).

The Me-His content in samples was measured as follows. Me-His was isolated from acid-hydrolysates corresponding to 15–30 mg tissue proteins or rumen microbes by the method described in a previous paper (Nishizawa, Noguchi *et al.* 1978). Samples (0·2–0·5 g) of food, faeces, rumen contents, defatted skin and rumen-reticulum were used for the isolation of Me-His which was determined using an amino acid analyser (Nishizawa *et al.* 1979). The Me-His content in urine acid-hydrolysates was analysed by the method described previously (Nishizawa *et al.* 1977).

RESULTS

Me-His contents of tissue proteins

Table 1 shows the organ and tissue weights and the protein contents, together with the Me-His contents of the corresponding proteins. The skeletal muscle in the dressed carcass constituted 356 g/kg live weight. Weights of skin, reticulo-rumen and intestine were 80, 21 and 32 g/kg live weight respectively. These values are consistent with previously reported values (Waldman *et al.* 1971; Takeshita *et al.* 1972; Takeshita *et al.* 1975).

The Me-His content of protein in skeletal muscle was 594 μg/g, the highest value found in the various tissues. Furthermore, heart and spleen proteins contained 313 and 292 μg Me-His/g respectively. The corresponding values for intestine, lung and kidney were 211, 172 and 106 μg/g respectively. As a whole, the Me-His contents of tissue proteins of cattle were similar to those found in rats (Haverberg, Omstedt *et al.* 1975; Nishizawa *et al.* 1977), although heart, spleen or kidney showed slightly higher values in the cattle.

The distribution of Me-His among organs or tissues is shown in Table 1: skeletal muscle contained 93·4% of the total body content. The digestive tract and other tissues contained 3·5 and 3·1% of the total body Me-His respectively.

Table 1. N$^{\tau}$-*Methylhistidine* (*3-methylhistidine; Me-His*) *content in mixed proteins of organs and tissues of cattle*

(Mean values with their standard errors for six animals weighing 274–341 kg)

	Tissue				Me-His content in:				
	Fresh wt (g/kg body-wt)		Protein content (mg/g fresh tissue)		Mixed proteins (μg/g protein)		Total organ* (mg/g fresh tissue per kg body-wt)		Tissue Me-His relative to total organ Me-His† (%)
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Skeletal muscle	356	9	157	2·8	594	24	33·29	1·55	93·4
Reticulo-rumen	21·0	0·9					0·817	0·111	2·2
Intestine	32·7	1·7	62·6	4·4	211	38	0·432	0·085	1·2
Skin and hair	80·7	1·8					0·314	0·029	0·9
Lung	16·3	0·3	112	2·2	172	17	0·321	0·035	0·9
Heart	5·0	0·1	138	1·9	313	14	0·215	0·009	0·6
Liver	14·3	0·8	155	6·4	47	6	0·104	0·015	0·2
Spleen	2·7	0·2	134	4·8	292	22	0·106	0·011	0·2
Kidney	2·9	0·1	116	3·6	106	8	0·036	0·003	0·1
Omasum and abomasum	15·7	1·0							
Others‡	258	1							
Total	805						35·63		

* (g fresh tissue/kg body-wt) × (protein content ÷ 1000) × (μg Me-His/g protein ÷ 1000).
† Tissue Me-His (mg/g fresh tissue per kg body-wt) ÷ total organ Me-His (mg/kg body-wt).
‡ Head, tail, bones, adipose tissues and lower limbs including nails.

Me-His in food, faeces, rumen contents and rumen microbes

Me-His in the food, faeces, rumen contents and rumen microbes was analysed. Hay and concentrate contained 2·40 and 7·73 mg/kg (mean values of five determinations) respectively. This suggests that animals obtained approximately 49 mg Me-His/d from their food. On the other hand, rumen protozoa contained trace amounts of Me-His, but it was not present in rumen bacteria. Rumen contents contained approximately 0·131 mg Me-His/kg fresh material. Faeces contained 1·51 ± 0·14 mg Me-His/kg dry matter (mean ± SE, n 6). These results suggest that animals excreted 2·49 mg Me-His/d into faeces at a stage of 312 kg body-weight. From food intake and faecal excretion of Me-His, apparent digestibility of Me-His in the food was calculated to be 0·94. This suggests that almost 30% of the urinary Me-His output is contributed by Me-His in the food, if Me-His from food is neither degraded nor metabolized in the digestive tracts of the cattle. The details of Me-His contents in various feedstuffs will be published elsewhere.

Changes in body-weight and urinary output of Me-His, creatinine and N during growth

Table 2 shows the changes in body-weight and urinary output of Me-His, creatinine and N during the experimental period. Animals grew at a mean rate of 1·5 kg/d during this period. The urinary output of Me-His was 135 mg/d at a growing stage of 217 kg body-weight and it increased as animals grew. The Me-His output per kg-body weight was 622 μg/d at this stage. At a stage of 312 kg body-weight, animals excreted 145 mg Me-His/d into the urine (465 μg/d per kg body-weight). The Me-His output per unit body-weight fell almost linearly as animals grew.

Creatinine output per kg body-weight was almost constant at 31 mg/d. These values for creatinine output were similar to those for rats (Haverberg, Deckelbaum *et al.* 1975; Nishizawa *et al.* 1977; Nishizawa, Shimbo *et al.* 1978).

Table 2. *Changes in body-weight and daily urinary output of* N^{τ}*-methylhistidine (3-methylhistidine; Me-His), creatinine and nitrogen during experimental period in cattle*

(Mean values with their standard errors for six animals)

Period of experiment (d)	Body-wt (kg)		Urinary Me-His: Total amount (mg)		Urinary Me-His: Amount relative to body-wt (μg/kg body-wt)		Urinary creatinine: Total amount (g)		Urinary creatinine: Amount relative to body-wt (mg/kg body-wt)		Urinary N: Total amount (g)		Urinary N: Amount relative to body-wt (mg/kg body-wt)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	217	7	135	6	622	23	6·62	0·29	30·5	1·7	40·2	3·1	185	9
21	255	9	154	5	605	3	8·69	0·28	34·0	0·8	63·8	2·3	250	6*
42	289	10	160	4	555	28	9·27	0·38	32·0	1·1	56·2	3·1	194	9
65	312	10	145	5	465	15*	8·64	0·32	27·7	0·9	51·3	1·6	164	6

For creatinine, differences between means were not significant statistically.
* Significant differences from day 0 of the experiment: $P < 0{\cdot}05$.

DISCUSSION

Of the total Me-His in the tissues and organs of the cattle analysed in the present study 93·4% was found in skeletal muscle (Table 1). Taking into account the amount of skeletal muscle remaining on the carcass, most of the Me-His in the body was found in skeletal muscle and the amount of Me-His in tissues other than skeletal muscle was relatively small. These results provide evidence of the validity of the method which uses urinary output of Me-His to measure the rate of myofibrillar proteins of skeletal muscle in the cattle. The present study also indicated the presence of Me-His in food, suggesting that the Me-His content in food should be determined before estimating catabolic rates of skeletal-muscle protein of domestic animals from urinary excretion of Me-His. We attempted to calculate fractional turnover rates of myofibrillar proteins in the cattle from the present values for the Me-His content of skeletal muscle and urinary output of Me-His. In order to calculate these values by the method of Funabiki *et al.* (1976), the following assumptions must be valid: Me-His released by catabolism of muscle proteins and ingested from food is neither degraded nor re-utilized for protein synthesis and excreted into urine quantitatively; the contribution of Me-His from tissues other than skeletal muscle to daily urinary output of Me-His is negligible. If these are valid in the instance of cattle, fractional catabolic and synthetic rates of myofibrillar proteins at a growing stage of 217 kg body-weight would be calculated to be 1·22%/d (half-life 56 d) and 2·73%/d, while the corresponding values at a stage of 312 kg body-weight would be 1·02%/d (half-life 67 d) and 1·51%/d respectively. These values are the mean of twelve determinations (six animals × 2 d) at each stage of growth. To calculate these rates, 94% of dietary Me-His was assumed to be absorbed and excreted into urine.

It is very difficult to estimate the turnover rates of muscle proteins in large animals such as cattle. A method for measuring the rates of catabolism and synthesis of myofibrillar proteins by estimating urinary output of Me-His would have considerable advantages when studying the regulation of muscle-protein mass in the cattle.

Recently, Harris & Milne (1977) have shown that, in sheep, recovery of intravenously administered [$^{14}CH_3$]Me-His in the urine was incomplete even after 7 d. They suggested that small fluctuations in the pool size of bound-form Me-His in the non-protein fraction in muscle dramatically alter excretion rates of Me-His. They claim that urinary Me-His output in sheep is not a reliable measure of muscle-protein degradation. In the pig, using [$^{14}CH_3$]Me-His, they have reported similar results (Milne & Harris, 1978). For this reason metabolism of Me-His in cattle should be studied carefully before concluding the validity of the calculation of catabolic rate from urinary output of Me-His.

The authors thank Dr M. Kandatsu, Professor Emeritus of The University of Tokyo, for his helpful discussion and critical review of the manuscript, and Dr T. Matsukawa of Tohoku National Agricultural Experiment Station for his generous support in the conduct of this work. They also thank the students of the authors' laboratory for their helpful technical assistance.

REFERENCES

Buttery, P. J., Beckerton, A., Mitchell, R. M., Davies, K. & Annison, E. F. (1975). *Proc. Nutr. Soc.* **34**, 91A.
Funabiki, R., Watanabe, Y., Nishizawa, N. & Hareyama, S. (1976). *Biochim. biophys. Acta* **451**, 143.
Harris, C. I. & Milne, G. (1977). *Proc. Nutr. Soc.* **36**, 138A.
Haverberg, L. N., Deckelbaum, L., Bilmazes, C., Munro, H. N. & Young, V. R. (1975). *Biochem. J.* **152**, 503.
Haverberg, L. N., Omstedt, P. T., Munro, H. N. & Young, V. R. (1975). *Biochim. biophys. Acta* **405**, 67.
Itabashi, H. & Kandatsu, M. (1975). *Jap. J. Zootech. Sci.* **46**, 409.

Long, C. L., Haverberg, L. N., Young, V. R., Kinney, J. M., Munro, H. N. & Geiger, J. M. (1975). *Metabolism* **24**, 929.
Milne, G. & Harris, C. I. (1978). *Proc. Nutr. Soc.* **37**, 18A.
Nishizawa, N., Noguchi, T., Hareyama, S. & Funabiki, R. (1978). *J. chromat.* **151**, 424.
Nishizawa, N., Noguchi, T., Hareyama, S. & Funabiki, R. (1979). *Agric. biol. Chem. Tokyo* **43**, 177.
Nishizawa, N., Shimbo, M., Hareyama, S. & Funabiki, R. (1977). *Br. J. Nutr.* **37**, 345.
Nishizawa, N., Shimbo, M., Noguchi, T., Hareyama, S. & Funabiki, R. (1978). *Agric. biol. Chem. Tokyo* **42**, 2083.
Perry, B. N. (1974). *Br. J. Nutr.* **31**, 35.
Takeshita, K. Tanaka, S. & Yoshida, S. (1972). *Bull. Tohoku Natl agric. Exp. Stn, Morioka* **43**, 209.
Takeshita, K., Yoshida, S., Tanaka, S. & Nishimura, K. (1975). *Bull. Tohoku Natl agric. Exp. Stn, Morioka* **50**, 99.
Waldman, R. C., Tyler, W. J. & Brungardt, V. H. (1971). *J. Anim. Sci.* **32**, 611.
Young, V. R., Alexis, S. D., Baliga, B. S., Munro, H. N. & Muecke, W. (1972). *J. biol. Chem.* **247**, 3592.
Young, V. R. & Munro, H. N. (1978). *Fedn Proc. Fedn Am. Socs exp. Biol.* **37**, 2291.

Printed in Great Britain