Myofibrillar Protein Turnover and Urinary N^{ϵ} -Methylhistidine Output

RESPONSE TO DIETARY SUPPLY OF PROTEIN AND ENERGY

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1. The urinary excretion of total N^{ϵ} -methylhistidine by the growing rat was measured to evaluate the effects of dietary protein and energy restriction on muscle protein turnover *in vivo*. 2. Young male rats (about 100 g initial wt.) were fed on one of three diets. Group I (controls) received an adequate 18% lactalbumin diet for 28 days, on which they sustained maximum growth. Group II (protein-depleted) was fed for 14 days on a 0.5% lactalbumin diet, which caused loss of weight; this was followed by repletion for 14 days with the control diet. Group III (protein-energy restricted) received a 1% lactalbumin diet at one-half the food intake of group II for 14 days, and this was also followed by 14 days of repletion with the control diet. 3. The controls showed a progressive rise in the daily urinary output of N^{ϵ} -methylhistidine, which was proportionally slightly less rapid than the body-weight increase. 4. The protein-depleted group II showed a marked and progressive decrease in N^{ϵ} -methylhistidine excretion, which was proportionally greater than the fall in body weight; during repletion, N^{ϵ} -methylhistidine output rose in parallel with body-weight increase, but it did not reach the value attained by the control group. 5. Group III, restricted in both dietary protein and energy, showed an initial small increase in daily N^{ϵ} -methylhistidine output, which contrasted with the sharp loss of body weight during this period. After 11 days on this restricted diet, group III then underwent a decrease in N^{ϵ} -methylhistidine output, which persisted into the first 4 days of the repletion period, after which output of the methylated amino acid became the same as for group II. 6. Creatinine output, used as an additional metabolic measure of muscle metabolism, showed a fairly constant relationship to body weight in groups I and II during depletion and repletion. However, rats with protein-energy deficiency (group III) underwent a marked increase in output of creatinine per unit of body weight, which also persisted into the repletion period before it fell to more normal values relative to body weight. 7. Analysis of the N^{ϵ} -methylhistidine content of actin isolated from a group of protein-depleted rats revealed a small (5%) but significant ($P < 0.02$) decrease relative to well-nourished controls. 8. Hence, the rate of muscle protein degradation, as indicated by changes in urinary N^{ϵ} -methylhistidine output, appears to respond sensitively and in opposite directions to insufficiency of protein and of energy in the diet.

Skeletal muscle represents a large reserve of protein that can be made available during periods of dietary stress. During prolonged protein-calorie malnutrition, skeletal muscle loses a greater percentage of its initial protein content and contributes a greater proportion of the total amount of body protein lost than does the liver (Young, 1970). Studies with rats have shown that the rates of muscle protein synthesis *in vivo* and *in vitro* are decreased by feeding with low-protein diets and protein-free diets (Young & Alexis, 1968; Waterlow & Stephen, 1968; Millward, 1970b; Von der Decken & Omstedt, 1970), and that short-term starvation increases the rate of protein breakdown in diaphragm (Fulks *et al.*, 1975) and leg muscle (Millward, 1970b).

However, the re-feeding of chronically malnourished rats with an adequate diet results in a temporary, marked decrease in the rate of breakdown of mixed muscle proteins during the early period of recovery (Young *et al.*, 1971). Hormones such as the corticosteroids (Goldberg, 1969b) and insulin (Fulks *et al.*, 1975; Rannels *et al.*, 1975) cause increased and decreased rates of muscle proteolysis respectively, and other factors such as exercise (Goldberg, 1969a), infection (Wannemacher *et al.*, 1975) and denervation (Goldberg, 1969b) also have been shown to modify the rate of muscle protein breakdown. Thus muscle protein content is regulated at the level of protein degradation as well as at control points associated with protein synthesis (e.g. Young, 1974).

Estimation of the rate of tissue protein breakdown in the intact organism, based on the decay of radioactivity after administration of the labelled amino acid, is complicated by the extensive inter- and intracellular reutilization of the label (Schimke, 1970), even for those compounds that have been considered to be minimally re-utilized, such as arginine (Nettleton & Hegsted, 1974) and aspartic acid and glutamic acid (Swick & Ip, 1974). Therefore it would be valuable to be able to develop a simple biochemical index specific for muscle protein turnover in the intact organism, which could be monitored daily and would be free from these errors. We have been exploring the use of urinary *N*^ε-methylhistidine excretion for this purpose.

In the rat, the principal source of urinary *N*^ε-methylhistidine appears to be the actin and myosin of skeletal muscle (Haverberg *et al.*, 1975). Further, administered *N*^ε-methylhistidine is quantitatively eliminated from the body via the kidney (Young *et al.*, 1972; Long *et al.*, 1975). In the rat, the amino acid does not undergo any conversion into metabolic products that are common to other amino acids, but a proportion is converted into the *N*-acetyl derivative (Young *et al.*, 1972). In human subjects *N*^ε-methylhistidine is also quantitatively eliminated, but there is less than 5% conversion into the *N*-acetyl form (Long *et al.*, 1975). Consequently, the output of total *N*^ε-methylhistidine in the urine should quantitatively reflect the rate of muscle protein breakdown.

Accordingly, we have performed experiments on rats to examine changes in urinary *N*^ε-methylhistidine output in response to decreased dietary protein and energy intake and then to nutritional rehabilitation. Also, as part of this investigation, an examination was made of the effect of dietary protein restriction on the content of *N*^ε-methylhistidine in the actin of skeletal muscle.

Materials and Methods

Studies on urinary N^ε-methylhistidine excretion

Male Sprague-Dawley rats (CD strain, Charles River Breeding Laboratories, Wilmington, Mass., U.S.A.), approx. 100g body wt., were housed in metabolic cages. After an initial adaptation period of 4 days, the rats were randomized into three experimental diet groups of six rats each, as follows: (I) controls, fed *ad libitum* on an 18% lactalbumin diet; (II) a low-protein group receiving *ad libitum* a 0.5% lactalbumin diet; (III) a protein-energy-restricted group, receiving a 1.0% lactalbumin diet and given one-half the total weight of food consumed by pair-fed mates receiving the 0.5% lactalbumin diet. The composition of the diets is given in Table 1. All groups were fed on the respective experimental

diets for 2 weeks and then groups II and III were repleted *ad libitum* with the 18% lactalbumin diet for an additional 2 weeks, whereas group I continued on the 18% lactalbumin diet as before.

Complete 24h urine collections (HCl being used as preservative) were obtained throughout, but analyses were performed on urine samples for selected days owing to a limitation in the number of samples that could be carried out. The urines were centrifuged after collection to remove food particles and occasional faecal pellets. Urinary *N*^ε-methylhistidine was determined with the aid of a Beckman 121 amino acid analyser as described (Haverberg *et al.*, 1974). Since some *N*^ε-methylhistidine is excreted in the *N*-acetylated form (Young *et al.*, 1972), urine was hydrolysed, before measurement, by heating in an equal volume of 12M-HCl at 110°C for 20h. The hydrolysed sample was desalted by using a minor modification of the method of Asatoor (1969). Urinary creatinine was determined by a modification of the Jaffe alkaline picrate reaction (Hawk *et al.*, 1954) adapted for automated analysis.

Content of N^ε-methylhistidine in actin

To aid the interpretation of dietary-induced changes in urinary *N*^ε-methylhistidine excretion, a study was undertaken to determine whether protein restriction altered the content of *N*^ε-methylhistidine in skeletal-muscle actin. One group (12 rats, weighing 250–300g) was killed before the start of the study to establish the *N*^ε-methylhistidine content of muscle of growing rats on a normal laboratory diet. A second group (16 rats, mean initial wt. 310g) was fed on the

Table 1. *Composition of diets used to study urinary N^ε-methylhistidine excretion in rats*

The 18 and 0.5% lactalbumin diets were offered *ad libitum* and the 1% diet was given to rats in one-half the amount of the 0.5% lactalbumin diet consumed by pair-fed mates. The mineral mix was purchased from General Biochemicals, Chagrin Falls, Ohio, U.S.A. [composition of mineral and vitamin mixtures described by Rogers & Harper (1965)]. Choline was added to the diet as an aqueous solution containing 1g of choline hydrochloride/5ml and the Wesson Oil was obtained from Wesson Oil Sales Co., Fullerton, Calif., U.S.A.

Ingredient	Content (% of diet)		
	18	0.5	1.0
Dextrin	44.2	55.9	51.7
Sucrose	22.1	27.9	25.8
Lactalbumin	18.0	0.5	1.0
Minerals	5.0	5.0	10.0
Vitamins	0.5	0.5	1.0
Choline	0.2	0.2	0.4
Wesson Oil	10.0	10.0	10.0

18% lactalbumin diet for 28 days to serve as dietary controls. A third group (32 rats, mean initial wt. 355 g) was given the 0.5% lactalbumin diet *ad libitum*, for 28 days. Rats in the latter two groups were killed at the end of 28 days and the hind-leg muscles removed and mixed. To obtain sufficient purified actin for N^5 -methylhistidine analysis, pooled samples of muscles from 8 to 12 rats were obtained as indicated in Table 3. Actin was extracted and purified from muscle samples and the N^5 -methylhistidine concentration determined as described (Haverberg *et al.*, 1974).

Statistical evaluation

The results were evaluated statistically by means of Student's *t* test.

Results

Body-weight changes

Group I (controls) gained weight at an average rate of 6.3 g/day (Fig. 1). In contrast, both the protein-restricted (group II) and protein-energy-restricted (group III) rats lost weight during the depletion period; group II underwent an average body-weight loss of 2.3 g per day. Repletion with the adequate diet caused a rapid body-weight gain for both groups during the 14 day repletion period; the protein-depleted group II showed a growth rate similar to the controls (6.6 g/day) whereas the rate of growth of the protein-energy-depleted group III was more rapid (7.9 g/day). On day 14 of repletion, both groups II and III had attained the same average body weight, but this was still only 62% of that of the group I rats of the same age.

Urinary N^5 -methylhistidine excretion

Because rats excrete a significant portion of total urinary N^5 -methylhistidine in the *N*-acetylated form (Young *et al.*, 1972), the urine was first treated with acid to hydrolyse the acetyl derivative before analysis for total N^5 -methylhistidine excretion. Table 2 summarizes data for urinary N^5 -methylhistidine output in relation to body weight and creatinine output and Fig. 2 displays graphically the pattern of change in excretion for the three dietary groups. In the control group, urinary N^5 -methylhistidine excretion increased from 1.3 μ mol/day initially to 2.2 μ mol/day after 28 days of adequate feeding. In contrast, animals given the low-protein diet (group II) exhibited an immediate decline in urinary N^5 -methylhistidine excretion, which progressively fell after 14 days on the deficient diet to about 20% of the initial output. As soon as an adequate content of dietary protein was restored (day 15), the output

of N^5 -methylhistidine rose progressively. Group III, receiving the protein-energy-deficient diet, maintained a normal or slightly increased output of N^5 -methylhistidine until day 11 of depletion and then it fell sharply continuing into the first 4 days of the repletion period. Thereafter N^5 -methylhistidine output started to rise again, so that the daily output paralleled that of group II.

Thus protein restriction and protein-energy restriction have profoundly different effects on N^5 -methylhistidine excretion in the rat. Protein deficiency caused a marked and immediate decrease in N^5 -methylhistidine excretion, whereas there was a maintenance of N^5 -methylhistidine excretion in protein-calorie deficiency for more than 1 week despite a rapid loss of body weight during this interval. This implies adaptive conservation of myofibrillar muscle protein during simple protein deficiency, but continued breakdown when energy intake is also insufficient.

Creatinine output

To provide another index of metabolism related specifically to muscle, total urinary creatinine output was monitored throughout the experiment (Fig. 3).

Fig. 1. Mean body weight of young rats fed on adequate (●) diet throughout or low-protein (○) or low-protein, low-energy (△) diets for 14 days and then an adequate diet during the subsequent 14 days of repletion

Each point is the mean value for six rats. The arrow represents day 1 of the repletion period. Body weights for groups II and III differed significantly ($P < 0.005$) on days 7, 11 and 14 of the depletion period.

Fig. 3. Mean urinary creatinine excretion by young rats fed on adequate (●) diet throughout or low-protein (○) or low-protein, low-energy (△) diets for 14 days of repletion

Each point is the mean value for six rats. The arrow represents day 1 of repletion period.

decreased output of N^5 -methylhistidine per g of body weight, which remained less than that of group I, despite the fact that animals in the control group were now larger than the rehabilitated animals and thus subject to a size-related decrease in output per unit body weight. In contrast, protein-energy depletion (group III) resulted in an increased N^5 -methylhistidine output while body weight was declining, so that the excretion of N^5 -methylhistidine per unit of body weight increased up to day 11. Thereafter there was a decline, which continued on re-feeding with an adequate diet until it had reached the same value as in group II. Thereafter this group, like the protein-depleted animals, continued to excrete a lower output of N^5 -methylhistidine per g of body weight than did the heavier, well-nourished controls.

Output of creatinine relative to body weight remained essentially constant in growing animals of group I receiving the control diet (Table 2). The protein-depleted group II showed no consistent trend during depletion or rehabilitation, suggesting that muscle mass and body weight changed in parallel. In contrast, the group receiving the protein-energy-deficient diet exhibited an increase in creatinine excretion relative to body weight during depletion and especially during early repletion, with a decline thereafter. These findings suggest that simple protein depletion and repletion (group II) is associated with an output of creatinine proportional to muscle mass,

whereas additional energy deficiency causes a disturbance in creatinine metabolism.

It appears that the output of creatinine relative to muscle mass is increased by energy restriction and therefore the measurement of creatinine excretion may not be an accurate index of muscle mass. Nevertheless, for comparative purposes, it is worthwhile to examine the N^5 -methylhistidine/creatinine ratio as an indication of the rate of muscle protein degradation relative to muscle mass for the various dietary groups (Table 2). The control group exhibited a definitive decline in N^5 -methylhistidine excretion per unit of creatinine during the first week of the study, with the ratio showing relatively little change thereafter. Between days 2 and 7 of the repletion period, N^5 -methylhistidine excretion by the protein-deficient group II, per unit of creatinine, decreased in parallel with that of the control group; however, the excretion of N^5 -methylhistidine per unit of creatinine in this group continued to decline significantly during the remainder of the depletion phase. In contrast, there was a significant increase in this ratio in the protein-energy-deficient group III relative to controls during the first 11 days of depletion and then a decline, which continued during the initial 4 days of repletion. Thereafter the ratio began to rise, so that by the end of the 14 day repletion period, all three groups exhibited a similar ratio of N^5 -methylhistidine/creatinine in the urine. The rise in the N^5 -methylhistidine/creatinine ratio may tend to underestimate the increased rate of methylhistidine release from muscle protein in group III, if the output of creatinine per unit of muscle mass is increased by energy restriction, in comparison with the protein-restricted group II.

Thus N^5 -methylhistidine output responds to diet independently of creatinine output in these groups of rats, in conformity with its different origin in muscle and its relationship to muscle protein breakdown.

Effect of diet on the N^5 -methylhistidine content of actin

To determine whether a decrease in the N^5 -methylhistidine content of muscle actin is an important factor in the low urinary output of this amino acid in protein deficiency, actin was extracted from the muscles of well-nourished rats and of animals after 28 days of dietary protein restriction.

Table 3 summarizes the concentration of N^5 -methylhistidine found in the purified actin from the various dietary groups. These data indicate that the N^5 -methylhistidine concentration of actin was about 5% lower in the depleted rats as compared with the controls. Although this quantitatively small difference was statistically significant ($P < 0.02$), the results suggest that the N^5 -methylhistidine in actin is maintained within a relatively narrow concentration limit, even in protein deficiency. Thus the marked

Table 3. *N*^ε-Methylhistidine concentration in actin extracted from mixed muscles of the hind leg of well-nourished and protein-restricted rats

The well-nourished and protein-restricted groups were fed *ad libitum* for 28 days with the 18 and 0.5% lactalbumin diets respectively. Body-weight values are mean $\pm$ s.d. *N*^ε-Methylhistidine content of actin was determined on a pooled muscle sample prepared from eight rats, except for one sub-set of the well-nourished rats, which was from 12 rats.

Dietary group	Body wt. (g)		<i>N</i> ^ε -Methylhistidine (mol/mol of actin)
	Initial	Final	
Well-nourished	—	250–300	0.93
	302 $\pm$ 8	427 $\pm$ 19	0.94
	318 $\pm$ 13	356 $\pm$ 36	0.92
	Mean $\pm$ s.d.		0.93 $\pm$ 0.01
Protein-restricted	341 $\pm$ 15	274 $\pm$ 13	0.86
	357 $\pm$ 14	272 $\pm$ 13	0.86
	364 $\pm$ 25	273 $\pm$ 16	0.90
	364 $\pm$ 29	269 $\pm$ 23	0.90
Mean $\pm$ s.d.			0.88 $\pm$ 0.02

changes in the urinary output of *N*^ε-methylhistidine discussed above cannot be accounted for, to any important extent, by changes in the *N*^ε-methylhistidine content of this major methylated muscle protein.

Discussion

Our experiments indicate that *N*^ε-methylhistidine excretion by the rat responds in different ways to dietary protein restriction and to protein-energy restriction. Protein deficiency caused an immediate and progressive decrease in the urinary output of this muscle protein metabolite, and this greatly exceeded the decline in body weight and creatinine output caused by dietary protein inadequacy. This observation implies metabolic adaptation in order to conserve muscle protein from loss through breakdown. In contrast, there was no decrease in *N*^ε-methylhistidine output by the animals over the first 11 days of restriction of both energy and protein intake, although loss of body weight was greater ($P < 0.005$) than was observed for animals restricted only in protein intake. Later, the output of *N*^ε-methylhistidine by the energy-restricted group fell. These findings suggest that, when energy intake is suboptimal, muscle protein breakdown does not undergo an initial adaptative decrease. Indeed, fractional catabolic rate is accelerated, since the same output of *N*^ε-methylhistidine comes from a diminishing muscle mass.

The progressive decrease in *N*^ε-methylhistidine output in protein depletion contradicts the observation by Millward (1970*b*) that breakdown of

pre-labelled myofibrillar protein is initially increased on a protein-free diet. However, Millward & Garlick (1972) subsequently studied turnover of mixed proteins in skeletal muscle of young rats during more prolonged protein deficiency and concluded from measuring the rate of synthesis and the change in total muscle protein that breakdown must have diminished after day 10 of feeding with a protein-free diet. It is possible that their use of a protein-free diet may have decreased the total food intake below that in our animals receiving the 0.5% lactalbumin diet and thus led to a state of both protein and energy deficiency. It is also relevant to record that Narasinga Rao & Nagabhushan (1973) observed a low output of urinary *N*^ε-methylhistidine in malnourished children, and they noted a marked increase in *N*^ε-methylhistidine output after 4 weeks of nutritional rehabilitation.

In the case of protein-energy deficiency, our evidence of increased muscle protein breakdown for the first 11 days is supported by evidence in starved rats of accelerated breakdown of both mixed protein from diaphragm and from leg muscle (Fulks *et al.*, 1975; Goldberg *et al.*, 1974) and of myofibrillar proteins from the leg muscle (Millward, 1970*b*). The subsequent decrease in breakdown that we observed after 11 days on the protein-energy-deficient diet may represent adaptation to utilization of body fat as the principal source of energy. In this connexion, Young *et al.* (1973) found that fasting obese humans undergo a progressive decrease in *N*^ε-methylhistidine excretion in parallel with the increased utilization of body fat. The latter presumably provides enough energy to inhibit the normal catabolic effect of dietary energy deficiency on muscle protein. A similar decrease in amino acid release from muscle has been noted in obese humans undergoing prolonged fasting (Pozefsky *et al.*, 1969). These adaptations could profitably be explored further in starved obese and normal rats.

Repleting the malnourished rats with an adequate diet brought about a rapid return to a normal growth rate and an increase in muscle mass, as shown by increased urinary creatinine output. The continued decline in *N*^ε-methylhistidine output during early re-feeding of the protein-energy-depleted group III agrees with the labelling studies of Young *et al.* (1971), who found a decreased rate of muscle protein catabolism during early re-feeding after chronic dietary protein restriction in rats. Goldberg *et al.* (1974) have summarized aspects of the importance of degradation of protein as a regulating component of protein metabolism in skeletal muscle, from which it can be speculated that the continued subnormal extent of protein breakdown during early rehabilitation may be due to the increased availability of branched-chain amino acids and a more favourable insulin balance, both of which are known to decrease

Table 4. *Estimation of muscle protein turnover from urinary N⁵-methylhistidine excretion for well-nourished rats at two stages during growth*

Muscle mass was taken to be 36 and 45% of body weight for the young and young adult rats respectively (Miller, 1969). Myofibrillar protein was taken as 60% of total muscle protein and actin and myosin were 60 and 30% respectively of myofibrillar protein (Young, 1970). Values for N⁵-methylhistidine content of actin (19 μ mol/g) and of myosin (2 μ mol/g) are taken from a previous report (Haverberg *et al.*, 1974).

	Young rat	Young adult rat
Body weight (g)	108	278
Muscle mass (g/rat)	39	125
Myofibrillar protein (g/rat)	4.0	12.8
Myosin (g/rat)	2.4	7.7
Actin (g/rat)	1.2	3.8
Total N ⁵ -methylhistidine (μ mol/rat) in:		
Actin	23	72.2
Myosin	4.8	15.4
Skeletal muscle	27.8	87.6
Daily excretion of N ⁵ -methylhistidine (μ mol/rat)	1.3	2.2
Daily proportion of N ⁵ -methylhistidine pool excreted (%)	4.7	2.5
Half-life for myofibrillar protein (days)	14.7	27.7

protein breakdown in muscle cells (Fulks *et al.*, 1975; Rannels *et al.*, 1975). In this connexion, decreased rate of catabolism, in addition to an increased rate of muscle protein synthesis (Young *et al.*, 1971), are factors responsible for bringing about a rapid restoration of muscle protein during the early re-feeding of malnourished rats. Our findings thus emphasize further that the content of protein in muscle is regulated at the level of protein degradation as well as through changes in the rate of muscle protein synthesis.

Our data on N⁵-methylhistidine output by the rats of group I which received an adequate diet throughout the experiment can be used to estimate changes in the fractional degradation rate of myofibrillar proteins as the animal grows from about 100 to 300 g body weight. This calculation (Table 4) is made on the assumption that, for all practical purposes, urinary N⁵-methylhistidine arises from skeletal muscle. The evidence for this has been presented by Haverberg *et al.* (1975). In Table 4, the daily output in the urine has been compared with the calculated protein-bound pool of N⁵-methylhistidine in skeletal-muscle myofibrils, from which the fractional rate of degradation can be calculated. Since there is some evidence (Swick & Song, 1974) that actin and myosin are degraded at slightly different rates, our computed fractional rates apply to the actin and myosin of the myofibril as a whole,

with emphasis on its actin component, which is richer in N⁵-methylhistidine. Our data show that approx. 4.7% of the actin-myosin pool is degraded daily ($t_{\frac{1}{2}} = 15$ days) in the 100g rat, compared with 2.5% ($t_{\frac{1}{2}} = 28$ days) in the 300g rat. By using ¹⁴CO₂ to label the glutamate and aspartate of myofibrils in the leg muscles of well-nourished 100g rats, Millward (1970*a,b*) obtained a degradation rate of 4.4% per day. Swick & Song (1974) applied the same isotopic procedure to adult female rats and obtained half-lives equivalent to a 2.0% fractional turnover for hind-leg myosin and 1.4% for actin, suggesting a combined myofibrillar value of approx. 1.6% per day. Hence, these values for the rate of loss of ¹⁴C from the myofibril show a 50% decrease in turnover rate between 100 and 300g body wt. and thus agree with a decrease of similar magnitude in the turnover rate as estimated from urinary N⁵-methylhistidine output. The agreement in absolute rate of turnover at these two stages of growth is also good, considering that only one muscle group was sampled for the ¹⁴CO₂ studies, whereas the N⁵-methylhistidine output involves the whole musculature. Published studies of degradation rates of mixed muscle proteins, or rates of mixed muscle protein synthesis from infused precursors, are not so easily compared because of the faster rate of turnover of the sarcoplasmic proteins. However, it is noteworthy that the studies of Waterlow & Stephen (1968) with continuous infusion of [¹⁴C]-lysine into rats show a synthesis rate for mixed muscle proteins that is nearly twice as rapid in 100g rats as in 300g rats. Finally, this decline in muscle protein turnover with increased growth and age is reflected in the decline in the intensity of total body protein turnover that occurs during growth and development in the mammalian organism (Waterlow & Stephen, 1967; Young *et al.*, 1975).

The present studies confirm the potential usefulness of N⁵-methylhistidine as a tool for studying muscle turnover in both animals and man. It has been demonstrated for both the rat (Young *et al.*, 1972) and man (Long *et al.*, 1975) that this metabolite released by myofibril breakdown is quantitatively excreted in urine either intact or as the N-acetyl derivative. Further, skeletal muscle appears to be the site of about 90% of the protein-bound N⁵-methylhistidine in the body (Haverberg *et al.*, 1975), so that the urinary excretion of this compound probably reflects myofibrillar turnover quantitatively.

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