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Dietary protein intake and 3-methylhistidine excretion in the rat

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1. Rats were fed for 14 d on diets containing 50, 150 or 250 g/kg casein as the protein source. The daily excretion of N^t-methylhistidine (His(τMe)), a non-re-utilized amino acid, was determined.

2. His(τMe) excretion/100 g body-weight appeared to be unaffected by increasing the concentration of dietary protein from 150 to 250 g/kg. Assuming no change in the proportion of muscle in the animals these results are indicative of no change in myofibrillar protein catabolic rate. The excretion rate/100 g body-weight of the animal given 50 g/kg casein was lower than the other two treatments, especially towards the end of the 14 d treatment period. Thus at this time the myofibrillar protein catabolic rate was lower than in the animals fed on the higher protein diet.

3. In the animals fed on the high protein diet there was a tendency for this (τMe) excretion rate/100 g body-weight to increase with age.

4. Nitrogen balance and creatinine excretion results are also presented.

The measurement of the rate of excretion of N^τ-methylhistidine (His(τMe)) provides a method for the continuous monitoring of the breakdown of myofibrillar proteins in muscle. Whilst the caution with which results obtained using this technique should be interpreted cannot be over emphasized, the technique would appear to be valid (for review see Ward & Buttery, 1978). Using this technique Young *et al.* (1973) demonstrated a decrease in myofibrillar protein breakdown in man during starvation for 20 d while Rao & Nagabhushan (1973) have demonstrated a similar reduction in catabolic rate in man during protein–energy malnutrition. Haverberg *et al.* (1975) have also reported similar results in protein–energy malnourished rats. Nishizawa *et al.* (1977) observed an increase in the breakdown of myofibrillar proteins following feeding high-protein diets to rats.

Since in these previously published studies only relative extremes of protein depletion and supplementation were investigated it seemed desirable to study the changes in urinary His (τMe) excretion that occur when animals are fed on diets containing a comparatively moderate excess or deficiency of protein and to relate these changes to the over-all nitrogen status of the animal. Such a study has particular relevance to comparable studies in large farm animals, particularly cattle where the His(τMe) technique would appear to be valid (Harris & Milne, 1978), as such animals are unlikely to experience such extreme variation in the composition of the diet as used in the previously reported studies with the rat referred to previously.

In the present report the results of the effects of diets containing 50, 150 or 250 g casein/kg on the His(τMe) excretion and N status of rats are reported.

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Table 1. *Composition (g/kg) of laboratory rodent diet and experimental diets with different casein concentrations*

Laboratory rodent diet	
Ground wheat	450
Ground oats	268
Dried skimmed milk	25
Dried yeast	25
White fish meal	150
Vitamin supplement	4·5
Mineral mix	9·7
Fat (tallow)	50
Sodium chloride	4·5
Cod liver oil	4·5
Bentonite	8·8

Experimental diet . . .	Low-protein	Adequate-protein	High-protein
Casein	50	150	250
Dextrin starch	300	300	300
Maize starch	537	437	337
Arachis oil	50	50	50
Mineral mix*	50	50	50
Vitamin mix*	11	11	11
DL-methionine	1·0	1·0	1·0
Vitamins (A, D, E; in oil)*	1·0	1·0	1·0
Gross energy (MJ/kg)	15·46	16·59	17·21
Protein (nitrogen × 6·25)	47·5	137·5	218·1

* For detailed composition of mineral mix, vitamin mix and fat soluble vitamins see Payne & Stewart (1972).

METHODS

Twelve male rats (Wistar strain, specific pathogen free) were weight-sorted into three groups. The rats were placed at random in stainless-steel metabolism cages. They were fed on a standard laboratory diet (Table 1) *ad lib.* and after 7 d each group of four rats was fed on one of the three experimental diets shown in Table 1. Each rat was allowed free access to the experimental diet and to water. Urine and faeces were collected separately, the urine being collected in approximately 0·5 ml 6 M-hydrochloric acid. At the same time each day the rats were weighed, the food intake for each rat recorded and the urine and faeces for each group of rats collected and pooled. The samples were stored at —15° before analysis.

Analytical procedures

Urine samples were desalted before amino acid analysis by the method of Armstrong (1966). The desalted urine samples were hydrolysed under reflux with excess 6 M-HCl at 110° for 22 h to convert any N-acetyl methylhistidine to methylhistidine (Young *et al.* 1972). The His(τMe) concentration in the pooled urine samples prepared as above was determined according to the method of Atkin & Ferdinand (1970) using either a Locarte (London) or an Evans Electroselenium model 294 (Halstead, Essex) amino acid analyser. The concentration of His(τMe) in tissue protein was determined from acid hydrolysates prepared from trichloroacetic acid-precipitated protein. The recovery of His(τMe) added to samples prepared by this procedure was 95–102 %. N in food and in the daily pooled urine and pooled faecal samples was determined by the Kjeldahl method in accordance with the recommendations of the Association of Official Analytical Chemists (1975). The concentration of creatinine in the pooled urine samples was determined according to the method of Owen *et al.* (1954).

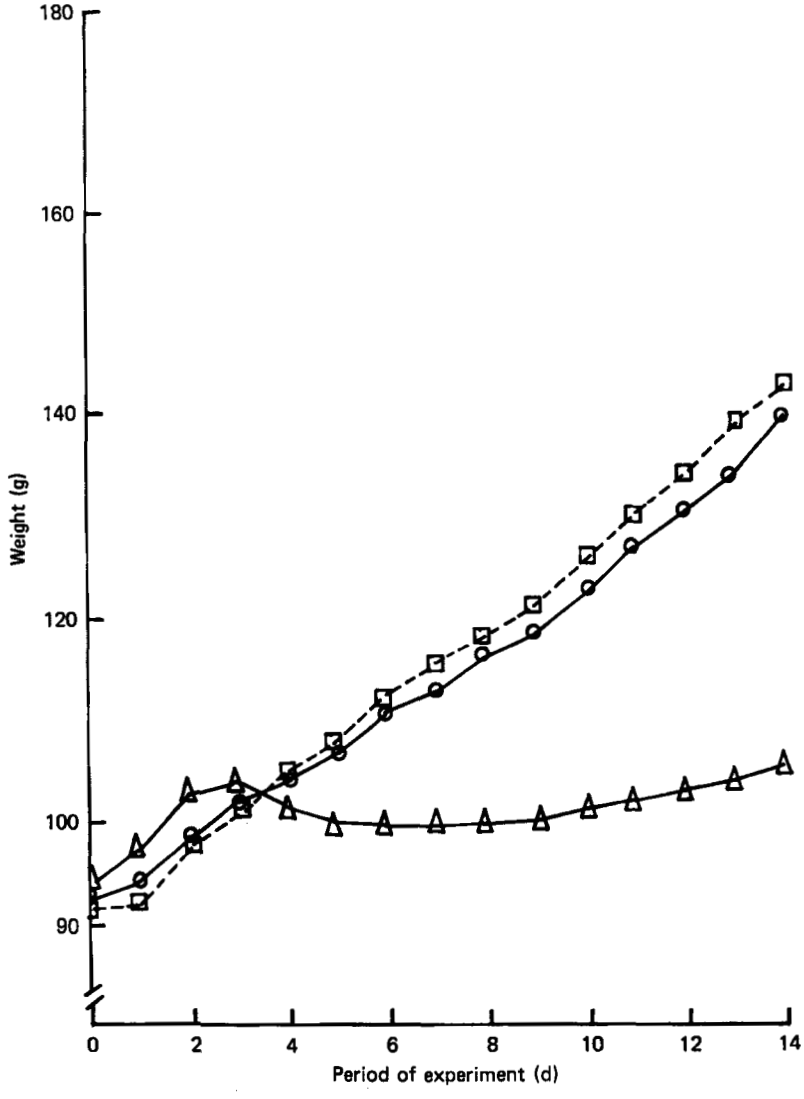

Fig. 1. Cumulative weight gain (g) of rats fed on diets containing three different concentrations of protein. Three groups of rats, four/group, were fed on diets containing 50 (Δ—— Δ), 150 (○——○) or 250 (□- - -□) g casein/kg. A standard laboratory diet (Table 1) was fed before feeding the experimental diets. The cumulative weight gain of the rats was determined daily.

Statistical analysis

Where applicable, the results are expressed as mean values with their standard errors. The significance of difference between the means was estimated using Student's '*t*' test (Snedecor & Cochran, 1967).

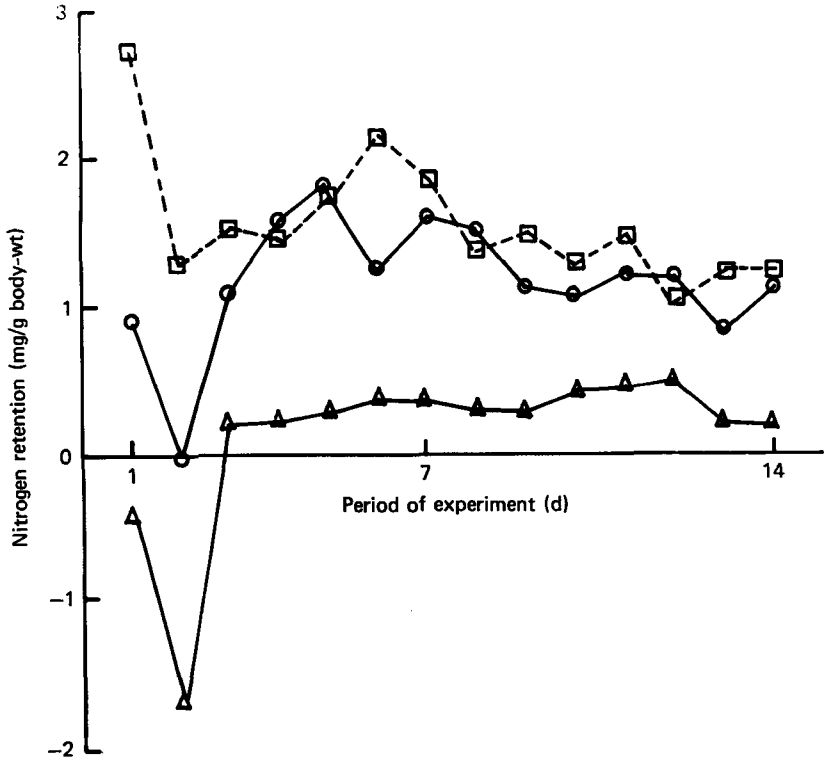

Fig. 2. Nitrogen retention (mg/g body-weight) by rats fed on diets containing three different concentrations of protein. Three groups of rats, four/group, were fed on diets containing 50 (Δ—— Δ), 150 (○——○) or 250 (□---□) g casein/kg. A standard laboratory diet (Table 1) was fed before feeding the experimental diets. N retention was calculated as described on p. 385. Values were determined from pooled samples for each group.

RESULTS

Changes in body-weight and food intake during feeding of the experimental diets

The rats fed on the diets containing 150 and 250 g casein/kg continued to gain weight during the experimental period, 3·4 and 3·7 g/d respectively (Fig. 1).

The rats receiving the diet containing 50 g casein/kg after 3 d lost weight and only started to regain weight (0·9 g/d) after receiving the experimental diet for 7 d. The differences in the mean body-weights between groups of rats before feeding the experimental diets was not significant. Rats fed on the diet containing 50 g casein/kg were generally lighter than those fed on diets containing 150 or 250 g casein/kg and this difference was significant ($P < 0.05$) on days 8 and 6 respectively. These differences were maintained ($P < 0.01$) at the end of the experiment, whereas the difference between the rats fed on the two higher-protein diets was not significant ($P > 0.05$).

There was no statistically significant difference over the 14 d feeding period in the mean daily food intake between the rats receiving the diets containing 150 or 250 g casein/kg (10·3 and 11·4 g/d respectively). The differences in daily food intake between rats receiving the diet containing 50 g casein/kg (9·3 g/d) and either those receiving the diet containing 150 g casein/kg or those receiving the diet containing 250 g casein/kg were statistically significant ($P < 0.05$) on days 11, 12, 13 and 14.

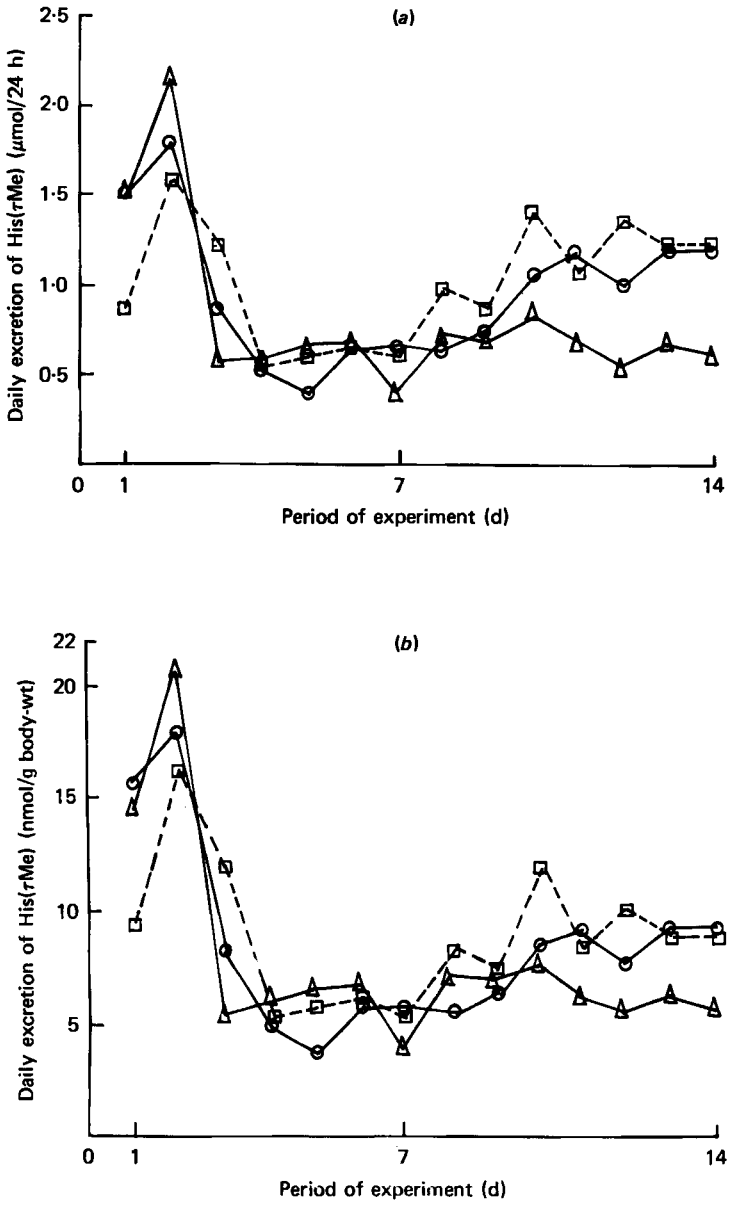

Fig. 3. Daily excretion (nmol (*a*), nmol/g body-weight (*b*)) of N^{τ}-methylhistidine (His (τMe)) by rats fed on diets containing three different concentrations of protein. Three groups of rats, four/group, were fed on diets containing 50 (Δ—— Δ), 150 (○——○) or 250 (□---□) g casein/kg. A standard laboratory diet (Table 1) was fed before feeding the experimental diets. Urinary His(τMe) was determined as described on p. 382. Values were determined from pooled samples for each group.

N retention during feeding of the experimental diets

N retention values for the three groups of rats are shown in Fig. 2. N retention has been expressed as mg N retained/g body-weight. N retention was calculated as the difference between N intake (food intake (g) × N concentration in the diet (mg/g)) and total N loss in

the faeces and urine. All calculations were made using the mean values for each group of rats. Except for the first 3 d all three groups of rats maintained a positive N retention. N retention of the rats receiving the diets containing 150 or 250 g casein/kg was similar and fell slightly during the experimental period. N retention of the rats receiving the diet containing 50 g casein/kg was constant during the experimental period and was 23 % of that of rats receiving the higher-protein diets.

The sharp depression in N retention after the start of the feeding trial may be a result of the adaptation to the change in diet. There was evidence of a check in the rate of weight gain (Fig. 1). The slight positive N balance without any apparent concomitant growth shown by the rats fed on the 50 g casein/kg diet probably reflects the inherent errors in the N balance technique but the possibility of a progressive change in body composition cannot be dismissed. Such a systematic change throughout the last 10 d of the feeding trial would, however, appear unlikely.

N^{τ}-methylhistidine and creatinine excretion during feeding of the experimental diets

Daily excretion of His(τMe) by the three groups of rats is shown in Fig. 3. The peak in excretion of His(τMe) may reflect some marked change in myofibrillar protein breakdown following adaptation to the experimental diets. It is relevant to note that on this day there was also a marked drop in N retention. This increase may also in part reflect excretion of His(τMe) originating from the diet the rats had been receiving before being fed on the experimental diets. His(τMe) has been found in fish muscle (Poulter *et al.* 1977) and is quantitatively excreted (Young *et al.* 1972). Fish meal was included in the standard laboratory diet and has been shown to contain His(τMe) at a concentration of 0·2 μmol/g dry weight (Ward, 1976). Since no His(τMe) was present in the experimental diets, excretion of His(τMe) during days 4–14 of the trial was considered to reflect endogenous His(τMe) excretion i.e. from muscle protein breakdown. The validity of this assumption has been discussed elsewhere (Ward & Buttery, 1978). Daily His(τMe) excretion was similar for the rats receiving the diets containing 150 or 250 g casein/kg and increased during the experimental period. His(τMe) excretion increased by approximately 130 % between day 4 and day 14 (Fig. 3*a*). This increased excretion of His(τMe) during the experimental period did not simply reflect the increased body-weight of the rats since His(τMe) excretion/g body-weight also increased (49 % greater on day 14 than on day 4) (Fig. 3*b*). The rats fed on the diet containing 50 g casein/kg excreted constant amounts of His(τMe) each 24 h (Fig. 3*a*) and excretion of His(τMe)/g body-weight also remained constant (Fig. 3*b*) at least during days 4–14 of the trial.

It is instructive to consider the ratio of His(τMe) to that of creatinine in the urine as an indicator of the rate of muscle protein degradation relative to muscle mass (Fig. 4). All groups of rats exhibited a decline in His(τMe) excretion per unit of creatinine between days 2 and 4 of the trial. Thereafter the excretion of His(τMe) relative to creatinine was constant in those rats receiving the low-protein diets indicating that protein depletion may be associated with parallel changes in muscle mass and His(τMe) excretion. In contrast whilst there was a large daily variation in this ratio there was evidence of an increase in His(τMe) excretion per unit of creatinine by rats fed on the diets containing either 150 or 250 g casein/kg (Fig. 4). Thus, in these groups of rats, this His(τMe) excretion appears to alter independently of creatinine excretion and thus presumably of muscle mass.

The concentration of His(τMe) in muscle protein measured at the end of the trial was not significantly altered ($P > 0{\cdot}05$) by the concentration of protein in the diet ($0{\cdot}74 \pm 0{\cdot}02(4)$, $0{\cdot}80 \pm 0{\cdot}30(4)$ and $0{\cdot}77 \pm 0{\cdot}03(4)$ μmol/g muscle wet weight for rats fed on diets containing 50, 150 and 250 g casein/kg respectively).

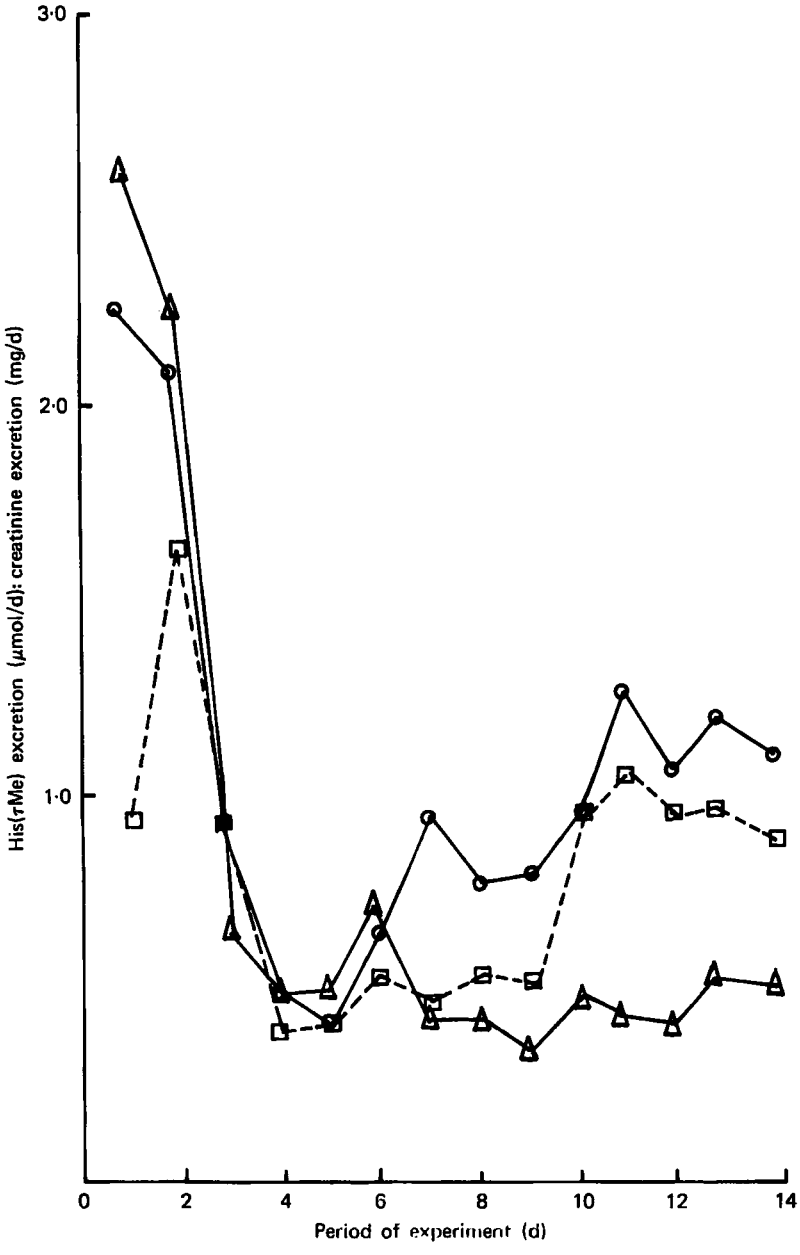

Fig. 4. Daily excretion of N^{τ}-methylhistine/unit creatinine by rats fed on diets containing three different concentrations of protein. Three groups of rats, four/group, were fed on diets containing 50 (Δ—— Δ), 150 (○——○) or 250 (□---□) g casein/kg. A standard laboratory diet (Table 1) was fed prior to feeding the experimental diets. Urinary His(τMe) and creatinine were determined as described in the text. Values were determined from pooled samples for each group.

DISCUSSION

The rate of His(τMe) excretion during protein deficiency has been determined by Haverberg *et al.* (1975). They observed a progressive decrease in urinary output of His(τMe) which greatly exceeded the decline in body-weight. This observation implies metabolic adaptation in order to conserve muscle protein from loss through breakdown. In the present study His(τMe) excretion was initially unaffected by protein deficiency and a constant excretion per unit body-weight was observed (Fig. 3*b*). The output of His(τMe) from the rats on the 50 g/kg treatment however became lower than that of the rats on the other two treatments as the trial progressed. Thus at the end of the trial period the myofibrillar catabolic rate was lower than in the rats fed on the higher dietary protein content. This pattern of change is contrary to the observations of Haverberg *et al.* (1975) noted previously. However, the results are not strictly comparable, since Haverberg *et al.* (1975) used a very severe protein depletion (5 g lactalbumin/kg diet), which caused a significant progressive decrease in body-weight compared with the less severe treatment (50 g casein/kg) used in the present study which caused no significant change in weight (Fig. 1).

The assumption that a constant rate of His(τMe) excretion during low protein feeding is indicative of a constant catabolic rate is valid only if ingestion of the diet does not affect His(τMe) excretion *per se* and that the concentration of His(τMe) in the myofibrillar protein pool is unchanged (Ward & Buttery, 1978). The results presented in this study indicate that the concentration of His(τMe) in the myofibrillar pool was not altered markedly by the protein concentration of diet. Haverberg *et al.* (1975), in their study, showed that the His(τMe) concentration in actin was 5 % lower in protein-deficient rats compared with well-fed controls. This difference was significant but insufficient to account for the low urinary output of His(τMe) by the protein-deficient rats.

In the present study, the feeding of a high-protein (250 g casein/kg) diet did not appear to alter His(τMe) output, either absolutely or per unit body-weight, when compared with an adequate (150 g casein/kg) diet. This is in contrast to the results of Nishizawa *et al.* (1977) who observed an increase in His(τMe) excretion concomitant with an increase in dietary protein intake. However, this increase in urinary His(τMe) was only apparent when protein concentration in the diet was greater than 400 g/kg. The creatinine:His(τMe) value also showed a tendency to increase with increasing protein intake. It would appear, therefore, that a moderate increase in protein intake does not change markedly His(τMe) excretion.

The small increase in His(τMe) excretion/unit body-weight with time in the adequately fed rats (150 and 250 g casein/kg diets) is difficult to explain since other evidence would indicate that the rate of muscle protein breakdown decreases with age (see for example Millward *et al.* 1975, who estimated the breakdown of the mixed proteins of rat muscle following determination of fractional synthetic and growth rates). Haverberg *et al.* (1975), observed in their control rats (i.e. those fed 180 g/kg lactalbumin) a general decline in the rate of His(τMe) histidine excretion/unit body-weight as this experiment progressed, especially during the latter part of their 28 d feeding trial. It is, however, interesting to note that during the first few days of their trial they noted a marked drop in His(τMe) excretion, presumably an adaption from the standard laboratory diet to the experimental diet. This was observed during the present study also. This marked drop in His(τMe) excretion was followed by a small rise in the excretion rate for approximately 10 d. Perhaps both in the study of Haverberg *et al.* (1975) and in the present work this observed rise in His(τMe) excretion was another consequence of the adaption to a different type of diet. Whether the surge of His(τMe) seen initially on changing the diet comes from the myofibrills of the skeletal muscle or from another source, either protein bound or free cannot be determined from the results presented here. It is interesting to note that balenine (β alanyl-His(τMe))

has been found in the rat, albeit in small quantities and that this peptide may be responsible for the erratic His(τMe) excretion patterns seen in the pig, (Harris & Milne, 1980).

The increase in His(τMe) excretion observed during the later part of the present experiments may have been a reflection of a change in body composition and an increase in the proportion of body mass represented by muscle. Creatinine excretion, however, did not provide conclusive evidence for this since His(τMe) excretion increased relative to that of creatinine. It is also possible that the release of His(τMe) from muscle is not a random process (see Ward & Buttery, 1979) and any slight change in His(τMe) concentration of muscle protein may have quite marked effects upon urinary excretion rates. Alternatively, if the suggestion of Bates *et al.* (1980) that a significant proportion of His(τMe) in the urine originates from tissues other than skeletal muscle is substantiated then the slight change in excretion rates may again not be directly related to changes in muscle breakdown. Further study of this possibility is required. The use of creatinine as an index of muscle mass is also open to criticism especially when studying the effects of nutritional and hormonal stimuli. Thus interpretations of results employing both these techniques must be made with extreme caution (see for example the comments of Tomas *et al.* 1979).

Table 2. *Calculated myofibrillar protein breakdown in rats fed on either a low-protein or an adequate-protein diet*

Dietary casein (g/kg) ...	50		150	
Day of experiment ...	4	14	4	14
Body-wt (g)	102	106	105	140
Muscle mass (g)*	38	40	39	56
Myofibrillar protein (g)†	4·5	4·8	4·7	6·7
Myofibrillar nitrogen (mg)‡	720	770	750	1070
Total muscle His(τMe) pool ‖ (μmol)	28·9	30	29·6	43
Daily excretion of His(τMe) (μmol)	0·52	0·51	0·56	1·17
Proportion of muscle His(τMe) pool excreted daily	0·018	0·017	0·019	0·027
Half-life for myofibrillar protein (d)§	38·5	40·8	36·5	25·7

* Estimated from the body-weight using the values of Miller (1969).
† Assuming protein content of muscle 200 mg/g and 600 mg/g of protein is myofibrillar protein (Wannemacher, 1975).
‡ Calculated assuming protein contains 16 % N.
‖ His(τMe) concentration in muscle 0·76 μmol/g.
§ First-order kinetics assumed (Ward & Buttery, 1978).

The daily excretion of His(τMe) per unit body-weight for rats weighing approximately 100 g observed in the present study was 5 nmol/g body-weight which is lower than the 12 nmol/g body-weight reported for similar rats by Haverberg *et al.* (1975). The value obtained in the present study for 150 g rats (8 nmol/g body-weight) was similar to that (7 nmol/g body-weight) obtained by Nishizawa *et al.* (1977) for 212 g rats fed on a 200 g casein/kg diet. Haverberg *et al.* (1975) reported a value of 8 nmol/g body-weight for rats weighing 278 g. These differences if of any significance are probably consequences of difference in experimental conditions rather than differences in analytical technique. The concentration of His(τMe) in muscle determined in this study was similar to that reported by Haverberg *et al.* (1975).

The fractional rates of myofibrillar breakdown calculated in this study (Table 2) are generally in good agreement with those observed by others, e.g. 0·044 (Millward, 1970) and

0·029/d (Gudbjarnason *et al.* 1964). Caution should be observed however, when making such comparisons since the rate of muscle protein breakdown varies considerably with age (see Millward *et al.* 1975), physiological state (see Trenkle, 1974), muscle type (Earl *et al.* 1975) and exercise (Goldberg *et al.* 1975). Swick & Song (1974) have compiled a table of published half-lives of myofibrillar proteins, which range from 20 to 145 d.

These values have been obtained using isotopic procedures and for these techniques the shortest experimental half-life is often assumed to approach more closely the true half-life of the protein (Swick & Song, 1974). Thus the better agreement between the values obtained in this study (Table 2) and the shorter half-lives determined using some isotopic techniques lends some confidence to the potential usefulness of the His(τMe) procedure for estimating rates of myofibrillar protein breakdown. Doubt must remain, however, as to the accuracy of such estimates immediately following abrupt changes in diet.

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