EFFECTS OF DIETARY PROTEIN LEVEL ON CATABOLIC RATES OF MYOSIN AND ACTIN IN THE RAT REVEALED BY URINARY EXCRETION OF 3-METHYLHISTIDINE

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Myosin and actin contain 3-methylhistidine (1, 2) which is synthesized after the formation of the peptide chain and is not reutilized for the protein synthesis after it is released by the breakdown of the protein (3). Since the main part of 3-methylhistidine in urine is due to the breakdown of myosin and actin (4), we can estimate the catabolic rates of these proteins by measuring the urinary excretion of 3-methylhistidine. Asatoor and Armstrong suggested the usefulness of urinary 3-methylhistidine as an index of muscle protein turnover (1) and YOUNG *et al.* (5) used this method to estimate the catabolic rate of human muscle protein during starvation.

In the present investigation, we measured urinary 3-methylhistidine in the rat at various levels of dietary protein intake, since few works have been published on the catabolic rates of myofibrillar proteins related to dietary protein intake.

Male rats of the Wistar strain, weighing 200 to 260 g were used. During the preliminary period, rats were fed 20% casein diet. After 7 days, rats were divided into 4 groups of 6 each, and fed 10%, 20%, 40% and 60% casein diet respectively with controlled feeding of 12 g per day per rat for 4 weeks. Each diet contained the above mentioned percentage of casein, 5% of soybean oil, 4% of salt mixture (6), 0.85% of vitamin mixture (6), 0.15% of choline chloride and corn starch with which the diet was made up to 100%.

The diet did not contain 3-methylhistidine. Urine samples were collected during the last 6 days of the test period and were hydrolyzed in 6 N HCl for 2 hr at 110°C to convert *n*-acetyl-3-methylhistidine to 3-methylhistidine (3). The highest value of 3-methylhistidine was obtained after hydrolysis for 2 hr. Analyses of 3-methylhistidine in the hydrolyzates were performed on a JEOL-5AH amino acid analyzer. Chromatography was carried out on a column (0.8×26 cm) of JEOL LC-R-2 resin. The buffer and starting column temperature were 0.38 N sodium

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citrate, pH 4.26 and 30°C, respectively. At 150 min the temperature was increased to 57°C. Under these conditions, histidine and 3-methylhistidine were completely

Table 1. Urinary 3-methylhistidine of rats fed diets containing various levels of protein.

Diet	3-Methylhistidine in urine (mg/100 g body weight/24 hr)
10% casein	0.118±0.020 ¹
20% casein	0.117±0.025
40% casein	0.133±0.014 ^a
60% casein	0.137±0.015 ^b

¹ Mean ± s. e. m. of 5 to 6 rats.

^a $P < 0.2$ compared to 20% casein.

^b $P < 0.1$ compared to 20% casein.

separated. Duplicate determinations showed the accuracy of this method to be within 2.8%.

Table 1 shows that the urinary excretion of 3-methylhistidine per day per 100 g body weight tends to increase with increasing dietary protein level. When dietary protein level raises from 20 to 40% and 20 to 60%, urinary excretion of 3-methylhistidine increases 13 and 16%, respectively. But no difference is detected between 10 and 20% dietary protein level.

If the breakdown of myosin and actin obeys to first order kinetics, and this is very probable (7-10), the amount of urinary excretion of 3-methylhistidine should be proportional to the amount of myosin and actin in skeletal muscle. To cancel such an effect of different amount of muscle protein which has correlation with body weight (11), on excretion of 3-methylhistidine, the data are presented as per 100 g body weight. So the data (Table 1) represent the quantities proportional to fractional catabolic rates of myosin and actin rather than the catabolic rate themselves.

Taking into account an assumption, namely, that myosin and actin have the same fractional turnover rate (12) and also no change occurs on 3-methylhistidine content in myosin and actin under this experimental condition, the results presented here support the conclusion that higher protein intake tends to bring about increase in fractional catabolic rate of myosin and actin in skeletal muscle.

The same but statistically significant results (13) in rats fed diets containing ovalbumin at various levels encourage us to persist in the conclusion mentioned above, though we fail to clarify the difference among the dietary groups at highly significant levels in this experiment. The mechanism that controls these changes is beyond the scope of this experiment.

We thank Drs. T. Noguchi and V. R. Young for their helpful discussions.

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