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An Automatic Method for Determination of Urinary and Myofibrillar 3-Methylhistidine: Fractional Rate of Myofibrillar Protein Breakdown in Rats Fed on Casein as Source of Protein

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J. A. MARTINEZ and J. LARRALDE. *An Automatic Method for Determination of Urinary and Myofibrillar 3-Methylhistidine: Fractional Rate of Myofibrillar Protein Breakdown in Rats Fed on Casein as Source of Protein.* Rev. esp. Fisiol., **40**, 103-108. 1984.

An automatic, more rapid and simplified analytical system for determination of 3-methylhistidine in urine and skeletal muscle is described, which may be applied to more extensive studies of large number of samples within a reasonable period of time and constitutes a powerful tool in understanding the dynamics of protein metabolism in the intact organism.

This procedure allows the analysis of 3-Methylhistidine by ion-exchange chromatography in 140 ± 2.5 min using a single column system. The mean urinary 3-Methylhistidine output of rats weighing about 200 g fed on an adequate diet of casein was 0.84 ± 0.02 μM/100 g BW. and the mean values for skeletal muscle in these animals were 0.74 ± 0.03 μM/g tissue. The fractional rate of myofibrillar protein breakdown assessed by the urinary 3-Methylhistidine was 0.028 ± 0.002 %.

Key words: 3-Methylhistiden, Myofibrillar protein, Breakdown.

The aminoacid 3-Methylhistidine (3-Mehis) is a component of actin and myosin of skeletal muscle (1, 17) which is not reutilized for protein synthesis or other metabolic events (24, 26), but quantitatively excreted in the urine of animals (25) and man (9). Therefore the measurement of the urinary 3-Methylhistidine has been proposed as an index for determining the rate of myofibrillar protein breakdown (2, 27) and has become a very useful and non invasive tool to evaluate the protein turnover under various nutritional (13, 22) hormonal (19) and pathological (4) conditions. However, detailed investigations and clinical studies have been hampered by the lack of a routine and manageable

method capable of rapid and specific determination of 3-Methylhistidine in numerous samples.

In the present communication a simplified system for automatic analysis of 3-Methylhistidine is described which is sufficiently sensitive for clinical purposes as well as for research work. Also we present data from a pilot study on 3-Methylhistidine muscle content and urinary excretion by rats fed on casein as source of protein, which allows the evaluation of the rate of myofibrillar breakdown.

Material and Methods

Intact young Wistar male rats (90 g initial weight) were housed in metabolic cages and fed on an adequate diet containing 12.4 % casein described previously (10) for seven weeks.

Samples of urine were collected and centrifugated for 10 min at 2,000 rpm and the clean urine was frozen at —20° C for further determination of 3-Methylhistidine.

On the last day of the experimental period all the rats were killed by decapitation. Immediately a sample of gastrocnemius muscle was excised by careful dissection.

Apparatus. Analyses were performed with a Hitachi KLA-5 amino acid analyzer consisting of a double column system packed with Hitachi-2613 resin. The absorbance of the column eluent, after reaction with ninhydrin, was monitored at 440 and 570 nm.

Chemicals. Amino acid standard calibration mixture was purchased from Sigma. Other reagents were obtained from Merck.

Ninhydrin. Ninhydrin (Merck) 80 g is dissolved in 3 liters of Methylcelosolve (Merck) and 1 liter of 4N sodium acetate buffer (2,730 g of sodium acetate and 500 ml of concentrated acetic acid in 5 liters of distilled water). The reagent solution is stirred under nitrogen for 10 min. The reducing agent was ascorbic acid (7 g). The reagent is stored at least 24 h before use in a dark bottle.

Buffers. Four citrate buffers are used for acidic, neutral and basic amino acid analysis (11). The determination of 3-Methylhistidine content in urine and skeletal muscle by the recommended procedure required only a lithium citrate buffer. The elution programme and the buffer composition are illustrated in table I.

Samples preparation. Urine was deammoniated by the method of BENSON and PATERSON (2). In the rat, some of urinary 3-Methylhistidine exists as the N-Acetylderivative which by hydrolysis is converted into 3-Methylhistidine. Five ml of urine are mixed with an equal volume of HCl conc. and hydrolyzed at 110° C for 3 h in a sealed bubble. The hydrolyzate was reduced to dryness in a rotary evaporator. Later this residue was dissolved in 2 ml of 0.01 N HCl to obtain a pH about 2.2. This solution was millepored (1 μM) before the analysis.

For measurement ot 3-Methylhistidine content in myofibrillar protein, the gastrocnemius muscle (~350 mg) was minced with scissors, later was fractionated by the method of RIKIMARU *et al.* (18) and hydrolyzed with HCl conc. at 110° C for 24 h. An aliquot of 3-Methylhistidine was measured in the automatic amino acid analyzer following a procedure similar to above.

Results and Discussion

The described method presents a good obtainable resolution for the amino acid 3-Methylhistidine under the conditions given in table I. A chromatogram of a

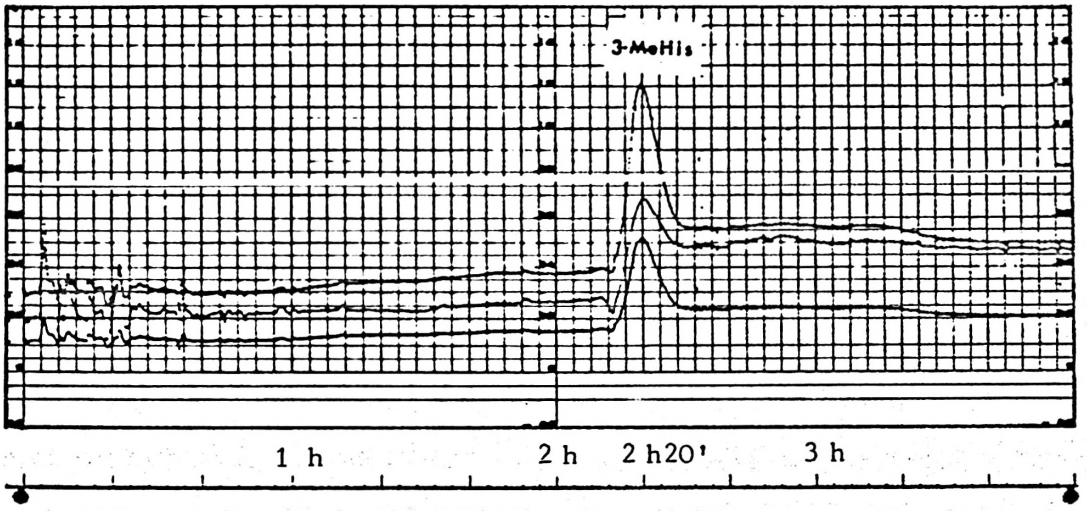

Fig. 1. *Chromatogramme of the 3-methylhistidine amino acid according to the proposed technique.*

standard sample is illustrated in fig. 1. The quantitation of this amino acid was performed by the peak area (5).

Our improved procedure required a single column system and involved only a lithium citrate buffer while previous methods (3, 11) used a double column system and two or three different buffers to eluate basic amino acids. Also 3-Methylhistidine is measured without need a previous desalting or separation of the basic amino acids from the acidic and neutral ones; moreover with this modifications, the retention time for the 3-Methylhistidine is 140 ± 2.5 min which is lower than the reported by those autors. The factor causing this reduction seems to be the increased concentration of Cl^-. The pH of the eluting buffer must be controlled to within ±0.05 because the sep-

Table I. *Elution Programme*

TIME:	0:00	0 36	2 46
BUFFER	pH: 4.10		
COLUMN (550 MM)	RESIN SIZE 11.5 UM		
TEMPERATURE	45° C	55°	

BUFFER COMPOSITION

CITRIC ACID (g)	LITHIUM CHLORIDE (g)	LITHIUM HYDROXIDE (g)	HCl CONC (ml)	BRIJ-35 (25%) (ml)	THIODIGLYCOL (ml)	N-CAPRYLIC ACID (ml)	FINAL VOLUME (l)	FINAL pH
200.0	143.7	118.1	275.0	35.0	20.0	1.0	10.0	4.10

Table II. *Urinary 3-Mehis excretion (μM/day and μM/100 g BW.), skeletal muscle content (μM/g tissue) and values of the rate of myofibrillar protein breakdown in young male rats fed on an adequate control diet (Casein 12.4 %) for seven weeks.*
Entries are mean values (± SEM) from five rats.

Diet	3-Mehis μM/day	3-Mehis μM/100 g BW.	3-Mehis μM/g tissue	Fractional* rate (%)
Casein 12.4%	1.42±0.04	0.84±0.02	0.74±0.03	0.028±0.002

* Assuming data of WARD and BUTTERY (22) for muscle mass, protein content and myofibrillar fraction.

aration of the amino acids is sensitive to small differences in pH. The color yield and the recovery of the samples were checked by comparing the reaction of guanidobutyric acid used as an internal standart allowing an increased accuracy of analysis (5).

Table II summarizes the results of urinary 3-Methylhistidine excreted by rats fed on casein as source of protein for seven weeks is 1.42 ± 0.04 μM/day or 0.84 ± 0.02 μM/100 g BW respectively which are in agreement with published evidence (8, 20). The 3-Methylhistidine output seems to be affected by the amount and quality of protein (6, 12).

Studies made showed (16, 23) that the skeletal muscle is the major source of urinary 3-Methylhistidine excretion but only 10 % of 3-Methylhistidine is recovered from the skin and the intestine in the rat (14). The total 3-Methylhistidine content in skeletal muscle measured with our procedure is 0.74 ± 0.03 μM/g tissue. This value is compatible with those reported by HAVERBERG *et al.* (7) and WARD and BUTTERY (23), but higher than those given by FUNABIKI *et al.* (13). This difference may be technical owing to the difficulty of measuring 3-Methylhistidine in muscle. Results presented by WARD and BUTTERY (23) indicate that the concentration of 3-Methylhistidine in the myofibrillar pool was not altered significantly by the protein concentration of diet.

The rate of catabolism of myofibrillar protein (C) was calculated from the urinary excretion of 3-Methylhistidine (U-3MH) and the concentration of 3-Methylhistidine in the myofibrillar protein (M-3MH) using the following equation:

$$C = 0.8\,(U\text{-}3MH) / (M\text{-}3MH)$$

The coefficient of 0.8 was chosen to represent the contribution of muscle 3-Methylhistidine to total urinary 3-Methylhistidine (14).

The fractional rate of myofibrillar breakdown calculated in this study (table II) is generally in good agreement with those observed by others, e.g. 0.027 (22) and 0.026 (18). Caution should be observed however, when making such comparisons since the rate of muscle protein breakdown varies considerably with age, physiological state, etc. (27).

The use of 3-Methylhistidine as an index of muscle protein degradation —following measurement of both parameters urinary and myofibrillar 3-Methylhistidine— is becoming increasingly useful. The present communication reports an adequate method to determine 3-Methylhistidine and stablish the value of fractional rate of myofibrillar breakdown in rats weighing about 200 g fed for seven weeks, on casein 12.4 % as source of protein, being this quality and concentration widely applied in animal nutrition.

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Resumen

Se describe un método analítico en 140 ± 2,5 min, por cromatografía de intercambio iónico, de la 3-Metilhistidina presente en orina o tejido muscular.

Los valores de 3-Metilhistidina eliminada en orina por ratas de aproximadamente 200 g alimentadas con una dieta adecuada de caseína son 0,84 ± 0,02 μM/100 g PC, mientras que el contenido de este aminoácido en el músculo esquelético es 0,74 ± 0,03 μM/g tejido. La velocidad de degradación proteica miofibrilar calculada a partir de estos parámetros es 0,028 ± 0,002 %.

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