N^{τ}-Methylhistidine in human skeletal and smooth muscle proteins

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1. Fifteen pieces of rectus abdominus muscle and fifteen pieces of taenia muscle were removed from patients undergoing various surgical procedures.

2. The muscles were extracted, hydrolysed and the content of N^{τ}-methylhistidine was measured.

3. The rectus muscles contained $3{\cdot}13 \pm 0{\cdot}7$ μmols N^{τ}-methylhistidine/g fat-free dry solid and the taenia muscles $2{\cdot}4 \pm 0{\cdot}6$ μmols/g fat-free dry solid. There was a statistically significant difference ($P < 0{\cdot}05$) between these values using the Student's unpaired *t* test, although this could have been due to contamination of the taenia muscles with connective tissue.

4. The muscle content of N^{τ}-methylhistidine was at least 40 % higher than the only other reported value and therefore the method of calculating muscle protein breakdown based on N^{τ}-methylhistidine excretion requires revision.

N^{τ}-methylhistidine (3-methylhistidine) was first reported to be a normal component of muscle proteins of the rabbit and other species in 1967 (Asatoor & Armstrong, 1967; Johnson, Harris & Perry, 1967) and there was evidence to show that histidine residues are methylated after the formation of the peptide chains of actin and myosin (Asatoor & Armstrong, 1967). N^{τ}-methylhistidine is not re-utilized when muscle protein is broken down but is excreted in the urine because of the lack of a transfer RNA (Young, Baliga, Alexis & Munro, 1970; Young, Alexis, Baliga, Munro & Muecke, 1972). ^{14}C labelled N^{τ}-methylhistidine administered to humans has been shown to be almost completely excreted in urine within 48 h (Long, Haverberg, Young, Kinney, Munro & Geiger, 1975).

This evidence has led to the suggestion that the levels of N^{τ}-methylhistidine in urine may indicate the extent of muscle protein breakdown (Young, Haverberg, Bilmazes & Munro, 1973; Long *et al.* 1975; Haverberg, Deckelbaum, Bilmazes & Munro, 1975) and a number of workers have measured N^{τ}-methylhistidine excreted in urine by humans (Narasinga Rao & Nagabhushan, 1973; Young *et al.* 1973; Wannemacher, Dinterman, Pekarek, Bartelloni & Beisel, 1975; Williamson, Farrell, Kerr & Smith, 1977; Fitzpatrick, Meguid, Gitlitz & Brennan, 1977; Gross, Holbrook & Irving, 1978) and rats (Haverberg *et al.* 1975; Nishizawa, Funabiki & Hareyama, 1975; Funabiki, Watanabe, Nishizawa & Hareyama, 1976) for this purpose.

Two of these reports (Young *et al.* 1973; Williamson *et al.* 1977) have used the value of 0·027 % (1·76 μmols/g) for the N^{τ}-methylhistidine concentration in mixed proteins of human calf muscle (Asatoor & Armstrong, 1967) to calculate the amount of protein which had broken down.

The present work was carried out to verify this value and to determine the content of N^{τ}-methylhistidine in human smooth muscle protein as no reference has been found to this latter measurement having been made.

METHODS

Muscle biopsy specimens (approximately 400 mg) were obtained from thirty adult patients undergoing abdominal operations, such as cholecystectomy or elective colectomy. Rectus abdominus samples were obtained from eight male and seven female patients (age range

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43–83 years). The taenia muscles were obtained from five male and ten female patients (age range 49–83 years).

Pieces of rectus abdominus muscle were removed before diathermy, immediately snap-frozen in liquid nitrogen and stored at −20° until analysis. Smooth muscle was obtained by excising strips of the taenia from specimens of the colon immediately after excision. The specimens were treated similarly to the rectus samples. Smooth muscle specimens were taken from tissue which appeared normal as far from the carcinoma as possible. The specimens were freed by dissection from as much connective tissue as practical.

Each biopsy specimen was weighed and then homogenized by hand at 0° using 3 ml glass homogenizing-tubes. They were extracted as described by Haverberg, Omstedt, Munro & Young (1975) and finally dried in a dessicator. Duplicate portions of the fat-free dry solid were hydrolysed in 6 M-hydrochloric acid at 110° for 20 h under vacuum. The HCl was removed by rotary evaporation, the residue dissolved in 0·25 M-lithium citrate buffer, pH 2·2 and a portion containing the equivalent of approximately 2 mg of fat-free dry solid (5–7 nmol N^{τ}-methylhistidine) was analysed using a JEOL JLC 6AH amino acid analyser. The column (150 × 9 mm) of JEOL LCR-2 resin was eluted by sodium citrate buffer, pH 4·3, containing 0·38 M-sodium.

The peak heights were measured using a JEOL JLC-DK integrator. It was important not to overload the column as any trailing of the histidine peak interfered with the integration of the much smaller N^{τ}-methylhistidine peak. Measurements could be obtained without using the pyridine elution method to reduce levels of histidine in the hydrolysate (Haverberg, Munro & Young, 1974).

RESULTS

The results of the analyses are shown in Table 1. The level in smooth muscle was lower than in skeletal muscle and the values were found to be significantly different using an unpaired Student's t test ($P < 0{\cdot}05$).

DISCUSSION

The concentration of N^{τ}-methylhistidine was found to be considerably higher in skeletal muscle than had been previously reported (Asatoor & Armstrong, 1967), approaching the levels found in rat skeletal muscle (Haverberg *et al.* 1975).

The difference may be due to the preparation of the muscle. In the present study fat was extracted from the muscle protein before hydrolysis whereas Asatoor & Armstrong (1967) did not. This could explain why the values for rat skeletal muscle reported by Haverberg *et al.* (1975) are higher than those for rat thigh (Asatoor & Armstrong, 1967). In addition, the differences may be due to the type of muscle from which the specimens were obtained. We sampled from the rectus as it is exposed at many abdominal operations. The calf muscle is rarely available except in orthopaedic operations when a tourniquet is often used. However, in the present study two thigh muscles and one gluteus maximus muscle were also sampled and it was found that they had similar concentrations to the rectus muscle so the higher values were not a peculiarity of rectus alone. This lower level of N^{τ}-methylhistidine in smooth muscle compared to skeletal muscle was opposite to what was expected as myosin from the smooth muscle of the cow uterus has been shown to be richer in the N^{τ}-methylhistidine than myosin from red skeletal muscle (Johnson & Perry, 1970). The smooth muscle used in the present study was often difficult to separate from surrounding connective tissue and this contamination may have lowered the levels found.

Many of the patients from whom specimens were obtained were elderly, but no correlation between age and the amount of N^{τ}-methylhistidine was found so the values found are also likely to apply to a younger age group. Some patients may have been malnourished because

Table 1. *N^{τ}-methylhistidine in muscle protein from adult patients undergoing abdominal operations*

(Mean values and standard deviations)

	Concentration (μmols/g fat-free dry solid)	Residues (% fat-free dry solid)
Rectus	3·13±0·7	0·0472±0·011
Taenia	2·4±0·56	0·0363±0·008

of the nature of their disease and this would probably result in a lower level of N^{τ}-methylhistidine than in normally nourished adults (Haverberg *et al.* 1975).

If it is valid to use the N^{τ}-methylhistidine excreted in urine to calculate muscle protein breakdown then the values for skeletal muscle reported here show that after 3 and 20 d of starvation (Young *et al.* 1973), 91 g and 68 g (instead of 160 g and 106 g) of muscle were broken down/d respectively. Similarly Williamson *et al.* (1977) found after trauma the 'normoketonaemic-injury' group broke down 122 g (instead of 214 g) more muscle/d than the 'orthopaedic-surgery' group.

It will be interesting to compare estimations of muscle protein turnover measured by N^{τ}-methylhistidine excretion and with isotope studies to determine whether there is agreement between the two methods.

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