Fractional flux rates of N^{τ}-methylhistidine in skin and gastrointestine: the contribution of these tissues to urinary excretion of N^{τ}-methylhistidine in the rat

BY N. NISHIZAWA, T. NOGUCHI AND S. HAREYAMA
Department of Agricultural Chemistry, Iwate University, Morioka, Iwate, Japan

AND R. FUNABIKI
Department of Agricultural Chemistry, Tokyo Noko University, Fuchu-shi, Tokyo, Japan

(*Received* 11 *March* 1977 – *Accepted* 1 *April* 1977)

1. Fractional flux rates of N^{τ}-methylhistidine (3-methylhistidine; Me-His) of skin and gastrointestine were measured by administering [*methyl*-$_3$H]methionine to rats.
2. The results showed that the contribution of these tissues to urinary excretion of Me-His was at least 16·6 %. This means that when fractional catabolic rates of myosin and actin were estimated from urinary excretion of Me-His, the part of Me-His derived from skin and gastrointestine should not be neglected.

The method to measure the fractional catabolic rate of myosin and actin in skeletal muscle by urinary excretion of N^{τ}-methylhistidine (3-methylhistidine; Me-His) has been shown to be valid by several investigators (Young, Alexis, Baliga, Munro & Muecke, 1972; Haverberg, Omstedt, Munro & Young, 1975; Long, Haverberg, Young, Kinney, Munro & Geiger, 1975; Nishizawa, Shimbo, Hareyama & Funabiki, 1977). Analysis of Me-His in the proteins of various organs and tissues revealed that approximately 90 % of the total amount of Me-His in the body was recovered from skeletal muscle. However, approximately 10 % of the total amount of Me-His in the body was found in skin and gastrointestine (Nishizawa *et al.* 1977). If the flux rates of Me-His in these tissues are greater than that in skeletal muscle, we have to take into account the amount of urinary Me-His released by these two tissues.

The present work was undertaken to determine the fractional flux rates of Me-His in skin, gastrointestine and skeletal muscle by administering [*methyl*-^{3}H]methionine to rats. Results showed the significant contribution of skin and gastrointestine to the excretion of Me-His into the urine.

METHODS

Sixteen Wistar male rats weighing 197–260 g were individually housed in stainless-steel cages in an air-conditioned room maintained at 22 ± 1°. They were given a diet with 200 g casein, 50 g soya-bean oil, 40 g salt mixture, 8·5 g vitamin mixture, 1·5 g choline chloride and 700 g maize starch/kg. The salt and vitamin mixtures were prepared according to Harper (1959). Food intake was restricted to 12 g/d per rat as in a previous paper (Nishizawa *et al.* 1977). After 7 d the animals were administered intraperitoneally 26 μCi L-[*methyl*-^{3}H]methionine (specific radioactivity 200 mCi, New England Nuclear, Boston) per kg body-weight. This radioactive amino acid was dissolved in saline (9 g NaCl/l). On the first, third, seventh, fourteenth and twenty-first days after the administration of the radioactive methionine, the animals were lightly anaesthetized with diethyl ether and killed by exsanguination. The hind limb muscles and gastrointestinal tract were removed

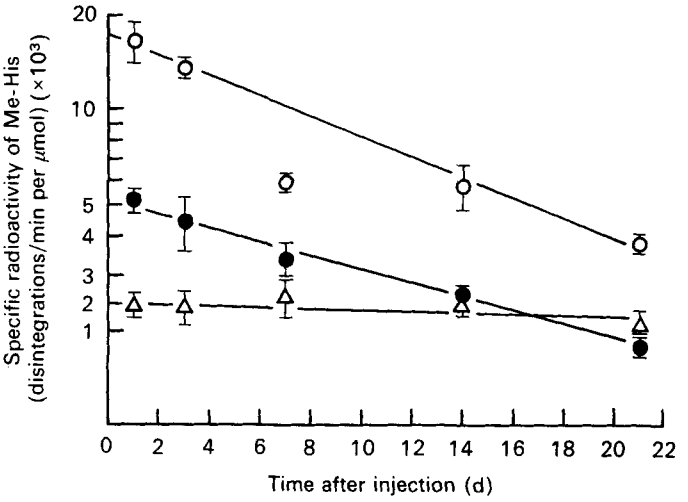

Fig. 1. Changes in specific radioactivity of N^{τ}-methylhistidine (3-methylhistidine; Me-His) in gastrointestine (○), skin (●) and skeletal muscle (△) of adult rats after injection of L-[*methyl*-^{3}H]-methionine. Points for the first, seventh, and twenty-first days of skin and skeletal muscle are mean values with their standard errors for four rats and those of third and fourteenth days are mean values with their ranges for two rats. All points for gastrointestine are mean values with their ranges for two rats. The regression lines were calculated by the method of least squares of points. However, in the case of gastrointestine, the point of the seventh day was omitted in calculating the line.

immediately, weighed and stored at −80° until they were analysed. The skin was removed, shaved with a shaver for small animals, weighed and stored at −80°. Approximately 1 g of fresh muscle or defatted skin or 2 g of fresh gastrointestine were hydrolysed with 80 ml 6 M-HCl at 110° for 24 h under reflux. The hydrolysates were cooled in ice and filtered through a sintered glass filter. To remove HCl, the filtrates were reduced to dryness using a rotary evaporator and redissolved in water. This procedure was repeated four times and the dried hydrolysates were finally dissolved in 5 ml 0·2 M-pyridine. These samples were applied to a column of Dowex 50 × 8 of 200–400 mesh (12 × 122 mm, 15 ml bed volume). The column had been previously equilibrated with 0·2 M-pyridine. The acid and neutral amino acids and creatinine were eluted with 150 ml 0·2 M-pyridine. Me-His was then eluted with 55 ml 1 M-pyridine. The Me-His fraction was dried in a rotary evaporator, and dissolved in 5 ml 0·2 M-pyridine. Histidine, lysine, 1-methylhistidine, methyllysines and methylarginines were eluted after applying 60 ml 1 M-pyridine. This procedure to isolate Me-His was repeated in order to reduce quenching in the liquid-scintillation spectrometer. Me-His thus obtained was dissolved in 2 ml 0·01 M-HCl and an aliquot of 1·5 ml was used to measure the radioactivity. The rest of the sample was used to confirm that the fraction contained only Me-His by amino acid analysis according to the procedure of Nishizawa *et al.* (1977). Duplicate determinations showed the recovery of Me-His to be 95–100 %.

The details of the methods for the isolation of Me-His will be published elsewhere. The radioactivity was measured on a liquid-scintillation spectrometer (Packard Model 3320 Automatic Tricarb, Packard Instruments Ltd, Wembley, Middx.), using toluene-based scintillation fluid (Kawakami & Shimura, 1974). The counting efficiency was approximately 14·8 % and the relative standard error was less than 1·5 %. The counts were corrected for quenching by using an external standard.

Table 1. *Fractional flux rates of N^{τ}-methylhistidine (3-methylhistidine; Me-His) in skin and gastrointestine and the contribution of these tissues to urinary excretion of Me-His in the rat*

	Total tissue Me-His (mg/g fresh tissue per kg body-wt)		Fractional flux rate of tissue Me-His*	Calculated urinary Me-His from tissue†	Me-His from tissue relative to urinary Me-His‡
	Mean	SE	(%/d)	(mg/d)	(%)
Skin	2·40	0·09§	5·0	0·120	10·4
Gastrointestine	0·99	0·05‖	7·3	0·072	6·2
Total	—	—	—	—	16·6

* The regression lines in Fig. 1 were used to calculate the fractional flux rates (exponential regression of specific radioactivity of Me-His *v.* time after injection: $100 \times k$(%/d), where k is the rate-constant from the regression equation).

† Total tissue Me-His (mg/g fresh tissue per kg body-wt) times fractional flux rate of tissue Me-His (%/d ÷ 100).

‡ Amount of urinary Me-His from tissue expressed as percentage of daily excretion of Me-His per kg body-wt (1·15 mg ± 0·03; mean ± SE). Daily excretion of Me-His is the mean value of fifty-four rats given a diet with 200 g casein/kg (Nishizawa, Shimbo, Noguchi, Hareyama & Funabiki, unpublished results).

§ Value from sixteen rats.

‖ Total amount of stomach and intestine of ten rats.

RESULTS AND DISCUSSION

Changes in specific radioactivity of Me-His in three tissues are shown in Fig. 1. One d after the administration of radioactive methionine to rats, Me-His in gastrointestine showed the maximum specific radioactivity. This value was the highest among the tissues examined. The specific radioactivity *v.* time curve of gastrointestine may suggest that this organ is composed of two different metabolic components. The changes in specific radioactivity of skin Me-His showed an exponential curve and suggested that this tissue is composed of a single metabolic component as far as Me-His metabolism concerns. The specific radioactivity of Me-His in skeletal muscle showed little decrease during 21 d of the experimental period. The plateau of specific radioactivity of Me-His in this tissue may suggest that radioactive methionine, after release by degradation of the protein, is greatly re-utilized for methylation of histidine in myosin and actin.

From these results, the fractional flux rate of Me-His of skin was calculated to be 5·0 %/d (half-life of 13 d) and that of gastrointestine, 7·3 %/d (half-life of 9 d), when calculated as a single component (Table 1). The amount of urinary Me-His originating from these two tissues was calculated from these flux rates and the Me-His content of these tissues (Table 1). As given in Table 1, at least 16·6 % of urinary Me-His is released from skin and gastrointestine. Therefore, it was concluded that the amount of Me-His derived from these two tissues should not be neglected when calculating the fractional catabolic rate of myosin and actin from the urinary excretion of Me-His.

REFERENCES

Harper, A. E. (1959). *J. Nutr.* **68**, 405.
Haverberg, L. N., Omstedt, P. T., Munro, H. N. & Young, V. R. (1975). *Biochim. biophys. Acta* **405**, 67.
Kawakami, M. & Shimura, K. (1974). *Radio-isotopes, Tokyo* **23**, 81.
Long, C. L., Haverberg, L. N., Young, V. R., Kinney, J. M., Munro, H. N. & Geiger, J. M. (1975). *Metabolism* **24**, 929.
Nishizawa, N., Shimbo, M., Hareyama, S. & Funabiki, R. (1977). *Br. J. Nutr.* **37**, 345.
Young, V. R., Alexis, S. D., Baliga, B. S., Munro, H. N. & Muecke, W. (1972). *J. biol. Chem.* **247**, 3592.

Printed in Great Britain