Measurement of 3-methylhistidine production in lambs by using compartmental-kinetic analysis*

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The kinetics of 3-methylhistidine (3MH) metabolism in four crossbred lambs were studied. Each lamb was injected with an intravenous dose of 3-[$^2H_3$]methylhistidine (d_3-3MH) and the stable isotope disappearance in plasma and appearance in both urine and muscle were measured. Immediately after the administration of tracer there was a phase of rapid disappearance of tracer from the plasma, which was followed by a more gradual decrease in d_3-3MH from the plasma during the last 4 d of the experiment. A minimum of three exponentials was required to describe the plasma decay curve adequately. The kinetic model of 3MH in the whole animal was constructed by using the SAAM/CONSAM computer modelling program. Two different configurations of a three-compartment model are described: (1) a simple three-pool model, in which plasma kinetics were entered into pool 1 out of which they had one undefinable exit; (2) a plasma–urinary three-pool model with two exits, in which the urinary kinetics were entered as an exit out of pool 1 and required a second exit out of pool 3 to produce an adequate fit. In addition, muscle kinetics from biopsies of the *longissimus dorsi* were entered into either pool 2 or 3 using the plasma–urinary model. Steady-state mass and transport rate values were obtained for each model construct described, and a *de novo* production rate corresponding to a fractional breakdown rate of myofibrillar protein of approximately 5%/d was also calculated. The model predicted that only 15% of 3MH was excreted in urine as free 3MH, which is consistent with current knowledge of 3MH excretion in sheep. The simple three-pool plasma kinetic model, therefore, could be used to estimate, by a relatively simple injection–sampling technique, the extent of muscle protein turnover in lambs.

3-Methylhistidine: Kinetic model: Lamb

The primary sequence of actin and of the fast-twitch white myosin in skeletal muscle contains the unique amino acid 3-methylhistidine (N^τ-methylhistidine; 3MH; Johnson *et al.* 1967). During degradation of these muscle proteins free 3MH is released, but because 3MH does not have a specific tRNA it is not reutilized for protein synthesis (Young *et al.* 1972). Instead of being used for protein synthesis, 3MH is quantitatively excreted in the urine of man, rat, cattle, and rabbit (Young *et al.* 1972, 1973; Harris *et al.* 1977; Harris & Milne, 1981) and, therefore, is thought to be a marker of skeletal muscle protein breakdown. This method depends on quantitative urine collection and accurate measurement of urinary 3MH and on the assumption that no metabolism of 3MH occurs *in vivo*. In most species no metabolism occurs, but in sheep (Harris & Milne, 1980) a proportion of 3MH is thought to be retained in muscle as a dipeptide (balenine) of 3MH and β-alanine (Harris & Milne, 1987). In sheep, therefore, urine 3MH cannot be used to estimate muscle protein breakdown because 3MH production from muscle is not equal to urinary 3MH production.

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To date, no attempts at integrating tracer and tracee findings into a comprehensive whole-animal kinetic model of 3MH metabolism in sheep or any other species have been reported. The initial studies showing the inadequacy of 3MH as an index of muscle protein breakdown in sheep required the intravenous administration of a dose of labelled 3MH, but the decay curve of [^{14}C]3MH in plasma was not characterized (Harris & Milne, 1980). The development of the SAAM/CONSAM computer modelling program (Berman & Weiss, 1978) has made possible the detailed analysis of tracer observations at multiple sites in the body and has facilitated the interpretation of findings in the context of a physiologically identifiable kinetic model.

The objective of the present study was to characterize the kinetic behaviour of intravenously administered 3-[$^{2}H_3$]methylhistidine (d_3-3MH) in growing lambs and to develop a kinetic model of 3MH metabolism based on the analysis of tracer response of plasma, urine excreta, and muscle. This kinetic model will also offer an alternative method for quantifying the *de novo* 3MH production rate *in vivo*, which could be used to estimate the fractional breakdown rate of muscle. The design strategy emphasized detailed observations over time and serves as a reference for comparison with results obtained in other investigations in which the turnover of muscle protein may be altered.

MATERIALS AND METHODS

Materials

d_3-3MH was purchased from MSD Isotopes, Merck Chemical Division, St Louis, MO, USA (99·5% isotopic purity). 3MH, 1-methylhistidine (1MH), Jack bean (*Canavalia ensiformis* DC.) Type IX urease (*EC* 3.5.1.5) (61000 units/g), and Dowex-50W in the hydrogen form were obtained from Sigma Chemical Co. (St Louis, MO). Acetonitrile and N-methyl-N-(*t*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) were purchased from Regis Chemical Co., Morton Grove, IL, USA. Ammonium hydroxide, perchloric acid, hydrochloric acid and filter columns were procured from Fisher Scientific, Fair Lawn, NJ, USA. Plastic microinjection sample vials were obtained from Sun Brokers, Inc., Wilmington, NC, USA.

1-Methyl[1,1-^{18}O]histidine ($^{18}O_2$-1MH) was prepared by exchanging the carboxyl O in $H_2{}^{18}O$ at low pH. To 50 mg dry crystalline 1MH, 1 ml $H_2{}^{18}O$ was added (96% ^{18}O; Cambridge Isotopes, Woburn, MA, USA). HCl gas was bubbled through the mixture for 1–2 min. The sample was capped and incubated at 100° for 30 min, after which it was neutralized with 3 M-NaOH. The resulting neutral amino acid solution was diluted to 100 ml, with the final product being 86% $^{18}O_2$-1MH as assessed by GC-MS.

The cation-exchange columns were prepared by adding 3 ml Dowex-50W cation exchange resin (50:50, v/v) in the hydrogen form to filtering columns and washing with four 1 ml portions of 0·01 M-HCl.

Animals and experimental protocol

Four crossbred (Dorset × Suffolk) lambs (three wethers and one ewe) weighing 25 (SE 0·5) kg were obtained from a herd at Iowa State University. They were approximately 3–4 months of age. The lambs were fed twice daily on a pelleted diet (Table 1) and had free access to nipple drinkers. At 9 d before the initiation of the experiment the lambs were transferred to stainless-steel metabolism cages designed for the collection of urine. On the morning of study a catheter was placed into each jugular vein to facilitate tracer injection and withdrawal of blood for each lamb. Approximately 2 h later a bolus dose of d_3-3MH, approximately 52 μmol in 10 ml sterile saline (9 g NaCl/l), was injected into one catheter and flushed with saline. Blood (10 ml) was collected from the other jugular vein and

Table 1. *Composition of the diet (g/kg total mix, as-fed)*

Ingredient		
Maize, grain cracked		450·7
Oats, grade 2 ground		200·1
Soya-bean meal (expeller)		200·1
Dehydrated lucerne		100·1
Molasses (sugar cane)		30·0
Limestone		7·5
Dicalcium phosphate		2·5
Salt		2·5
Vitamin premix		5·0
Pell-aid*		1·5
	Requirement	Supplied
Daily intake (kg)†	1·58	1·58
Metabolizable energy (MJ)†	16·73	16·85
(Mcal)	4·00	4·03
Crude protein (N × 6·25; kg)†	0·22	0·27
Calcium (g)†	7·2	8·5
Phosphorus (g)†	3·4	6·1

* Lignotech, Greenwich, CT, USA
† Calculated from National Research Council (1985) values.

transferred to EDTA-coated tubes at 2, 7, 10, 15, 45, 90, 150, 210, 270, 720, 1440, 2880, 4320, 5760 and 7200 min post injection. Blood samples were placed directly in an ice bath until they could be centrifuged and plasma collected and stored at −70°. After the injection of tracer, muscle was taken by needle biopsy (60–70 mg) at 12, 60, 300, 1440 and 4320 min from the *longissimus dorsi* after the area 5 cm from midline was treated with local anaesthesia. The muscle sample was immediately placed on ice and stored at −70°. During the first 5 d of the tracer experiment, consecutive 24 h total urine outputs were collected. Portions of each urine collection were kept frozen at −20°. Additional baseline blood, muscle, and urine samples were collected before injection to subtract background enrichment from the experimental samples.

Internal standards

Because of its structural similarity to 3MH and its undetectability in the plasma of sheep, 1MH was used as an internal standard (50 μl 3540 μM stock solution) in the analysis of 3MH concentration in plasma. But $^{18}O_2$-1MH (40 μl 2955 μM stock solution) was used as the internal standard for the quantification of 3MH in urine because a significant quantity of 1MH accumulates in urine.

Analysis of plasma 3MH

Plasma (1 ml) and 50 μl 1MH internal standard (3·5 mM) were added to a plastic sample tube (12 ml). Perchloric acid (PCA; 1·5 M; 3 ml) was added, and the tube contents stirred using a vortex mixer and centrifuged at 2300 *g* for 15 min at 5°. The supernatant fraction was poured onto a prepared cation-exchange column. After draining through the column the supernatant fraction was rinsed with four 1 ml portions of 0·01 M-HCl and the washings were discarded. Then, 3MH and internal standard (1MH) were eluted from the column

with four 1 ml washes of NH_4OH (250 ml/l) into a 20 ml scintillation vial. The eluant was heated to 65° in a block and dried under a stream of N_2. When the eluant seemed dry, any remaining water was removed by the sequential addition of two 100 μl portions of methylene chloride, drying off samples between each addition. A derivative of the dried sample was prepared for GLC–MS analysis by adding 100 μl acetonitrile and 100 μl MTBSTFA and incubating overnight at room temperature.

The derivatized sample was transferred into an injection vial and capped. GLC–MS was accomplished by means of a Hewlett-Packard gas chromatograph–mass selective detector (model 5890/5970B). The column used was a 25 m × 0·22 mm i.d. × 0·11 μm film thickness, cross-linked methyl silicone gum phase capillary column (HP-1; Hewlett-Packard, Avondale, PA, USA). The injector and the transfer line were set at 285° and the initial oven temperature at 50°. After splitless injection, the oven was programmed to hold for 0·5 min and increased (ramp 1) to 240° at 50°/min which was held for 4·1 min, followed by an increase (ramp 2) to 300° at 50°/min, which was held for 1 min. The retention times were between 6·5 and 7·5 min for 3MH and 7·0 and 8·0 min for 1MH, depending on column age and exact length. The major ion fragments for 3MH and d_3-3MH were monitored by means of selective ion monitoring. 3MH was monitored at 238 m/z and its stable isotope, d_3-3MH, at 241 m/z, whereas 1MH was detected at 340 m/z. 3MH in plasma was quantified from a linear peak height standard curve. When d_3-3MH was used as a tracer, as will be described, it was quantified in plasma in the same manner, that is by subtracting the natural background enrichment d_3-3MH/3MH from detected d_3-3MH.

Standards were prepared by pipetting known quantities of 3MH from a 50 μM stock solution into plastic sample tubes (12 ml) along with 50 μl 1MH internal standard. Double-deionized water (1 ml) was added to each standard tube. Because the neutralizing capacity of standards was small compared with that of plasma, the standards were acidified with only 0·25 ml 1·5 M-perchloric acid and treated the same as was the plasma sample.

Analysis of urine 3MH

Unlike plasma, urine contains substantial amounts of 1MH, making 1MH an impractical internal standard; therefore, $^{18}O_2$-1MH was used as the internal standard with which to quantify 3MH in urine. Standards were prepared as outlined except that 40 μl $^{18}O_2$-1MH was used as the internal standard. A urine sample was prepared by transferring 1 ml to a microfuge tube and removing particulate matter by spinning for 3 min. From this tube, 100 μl urine was pipetted into a plastic sample tube (12 ml) with 40 μl internal standard. In addition, 1 ml double-deionized water was added to the tube and the contents were acidified with 5 μl 3 M-HCl. The urine sample was stirred with a vortex mixer, poured over a cation-exchange column (prepared as outlined) and allowed to drain. The column was rinsed with four 1 ml washes of 0·01 M-HCl and eluted with four 1 ml washes of NH_4OH (250 ml/l), which were collected in a scintillation vial and dried with a stream of N_2 on a heating block at 65°. Once dry, the urea in the sample was hydrolysed with 1 ml urease solution (35 mg/l) by incubating the sample on a heating block for 2 h at 37°. The sample was again dried on the heating block, derivatized with 100 μl acetonitrile and 100 μl MTBSTFA and incubated overnight at room temperature. The derivatized sample was transferred into an injection vial and injected in a Hewlett-Packard gas–liquid chromatograph by using the same conditions and ramps as described previously. The retention time for 3MH was the same in urine as in plasma and exhibited the same mass spectra. $^{18}O_2$-1MH had a retention time similar to that of natural 1MH, but major ions from the mass spectra were 4 m/z heavier. 3MH and d_3-3MH were quantified by peak height as explained for plasma analysis except that $^{18}O_2$-1MH replaced 1MH in the calculations.

Analysis of free 3MH in muscle

Approximately 70 mg muscle was transferred into a plastic sample tube (12 ml) with 40 μl internal standard. In addition, 1·5 ml double-deionized water was added to the tube, and the contents were homogenized by using a Polytron tissue homogenizer with a 15 mm stator generator. The homogenization was performed at 15000 rev./min for 15 s. The blades of the homogenizer were washed with 0·5 ml double-deionized water and added to the sample tube. PCA (1·5 M; 1 ml) was added, and the tube contents were stirred with a vortex mixer and centrifuged at 2300 ***g*** for 15 min at 5°. The supernatant fraction was poured onto a prepared cation-exchange column. The protein pellet was resuspended twice in 0·5 M-PCA and recentrifuged at 2300 ***g*** for 15 min at 5°. On both occasions, the supernant fraction was poured over a cation-exchange column and allowed to drain. The column was rinsed with four 1 ml washes of 0·01 M-HCl and eluted with four 1 ml washes of NH_4OH (250 ml/l), which were collected in a scintillation vial and then dried with a stream of N_2 on a heating block at 65°. A derivative of the sample was prepared and subjected to GLC–MS as described previously.

Kinetic modelling

Modelling of 3MH kinetics was done with the SAAM/CONSAM computer program (Boston *et al.* 1981). Exponential equations were initially fitted to the plasma concentration of d_3-3MH time curves to characterize the complexity of the response and to allow for comparisons with other studies. The first step in constructing a compartmental model was to determine the minimum number of compartments and interconnections suitable for the data. Thus, only the plasma kinetic data were used, which were expressed as a proportion of dose/ml. These findings were used in addition to previous knowledge of 3MH metabolism in sheep (Harris & Milne, 1980). Second, this basic model was expanded to include a urinary exit in which values were expressed as a proportion of the dose. The connections between compartments were adjusted to optimize the fit and eventually included a second exit from the model. Finally, the muscle enrichment of 3MH from the muscle biopsies was incorporated into the model and all values were simultaneously fitted. In addition, steady-state masses and transport rates were calculated for each model, and the *de novo* production rate calculated could be used to calculate a fractional degradation rate for the myofibrillar proteins. The nomenclature and illustrations used in describing the model are the same as in the SAAM (simulation analysis and modelling) manual (Berman & Weiss, 1978). Brief definitions of the terms used are given: L(I, J) is the proportion of material transported from compartment J to I per unit time (proportion/min), R(I, J) is transport of tracee (natural 3MH) from compartment J to I per unit time (nmol/min) under steady-state conditions, U(I) is entry of tracee into compartment I from outside the model per unit time (nmol/min) (the *de novo* production of unlabelled 3MH from breakdown of actin and myosin proteins into intracellular muscle pool under steady-state conditions), M(I) is mass of tracee (unlabelled 3MH) in compartment I, K(I) is proportionality constant associated with compartment I. CONSAM assumes that a component number is specified under an H DATa header statement in the input file, and it will automatically multiply the category associated with this component by K(I) to calculate QC(I). QC(I) is CONSAM's notation for calculated values. Data associated with a component I are called QO(I). K(I) represented the inverse of the space of distribution. The number of compartments in the model is arbitrary and reflects simply the choices of compartment numbers made during the development of the model.

RESULTS

Tracer response profiles

The mean tracer enrichment in plasma and muscle and the percentage recovery of tracer in urine are presented in Table 2. The d_3-3MH values exhibited a typical disappearance pattern (Fig. 1). After the injection of tracer, d_3-3MH was lost rapidly from the plasma during the first 150–180 min, followed by a transition period through 720 min in which the decrease in tracer concentration seemed to level off. The final phase was characterized by a gradual decline in tracer during the remaining 5 d of the kinetic study.

During the preliminary fitting of exponential equations to the plasma kinetic data we found that a minimum of three exponential terms was required to achieve an adequate fit. The exponential equations for individual animals are given below, where $c(t)$ is the concentration of d_3-3MH in plasma at time t (min post injection):

$$\text{lamb no. 9173: } c(t) = 1{\cdot}36\exp^{0{\cdot}000133t} + 4{\cdot}02\exp^{0{\cdot}133t} + 0{\cdot}757\exp^{0{\cdot}00490t},$$

$$\text{lamb no. 9183: } c(t) = 1{\cdot}47\exp^{0{\cdot}000197t} + 4{\cdot}44\exp^{0{\cdot}126t} + 0{\cdot}914\exp^{0{\cdot}00422t},$$

$$\text{lamb no. 9186: } c(t) = 1{\cdot}57\exp^{0{\cdot}000204t} + 4{\cdot}13\exp^{0{\cdot}183t} + 1{\cdot}61\exp^{0{\cdot}00638t},$$

$$\text{lamb no. 9169: } c(t) = 1{\cdot}45\exp^{0{\cdot}000143t} + 5{\cdot}84\exp^{0{\cdot}273t} + 1{\cdot}77\exp^{0{\cdot}00517t}.$$

Model development

The model developed used linear first-order differential equations; therefore, it is based on the assumption of steady-state kinetics in the animals over the duration of the experiment. No significant differences were seen in individual lamb plasma levels or in urinary production of 3MH during the duration of the experiment, so the lambs were assumed to be in steady-state. No animal by-products were included in the diet so it was assumed that the only source of natural 3MH was from the degradation of actin and myosin.

The stages of model development are illustrated in Figs. 2–4 to describe the sequential addition of different metabolic pools; the model configuration is included in an inset in each figure. The first step (Fig. 2) was to describe the plasma kinetic data in terms of a compartmental model. This was a simple three-compartment model in which the plasma data were entered into compartment 1 as a fraction of injected dose/ml. Compartment 1 was connected in a series with compartments 2 and 3, and there was one exit from the system from compartment 1. This plasma model required a minimum of three compartments to produce an adequate fit between the values calculated by the model and the observed data points. Model variables and steady-state masses and transport rates are shown in Table 3. The plasma curve is characterized by a sharp decline in tracer during the first phase and a more gradual decline during the last 5 d. Steady-state transport rates indicate that there is rapid exchange of 3MH (nmol/min) between pools 2 and 3, compared with a slowly turning over pool 3. The average *de novo* 3MH production rate (U(2)) for four lambs was 205 (SE 3·6) nmol/min. The fractional breakdown rate of myofibrillar protein could be calculated in the same manner as Harris & Milne (1980), assuming that muscle was 35% of body weight and that protein-bound 3MH was 0·6 μmol/g muscle. The average *de novo* production rate into compartment 2 corresponded to an average fractional breakdown rate of 5·4%/d.

The next stage in the model development was to incorporate the urine kinetic data. We first attempted to include the accumulative urine recovery of d_3-3MH tracer as the only exit from compartment 1. This model was inadequate to describe both the plasma and urine kinetic data, inasmuch as we saw a significant decrease in the plasma sum of squares. This would be expected because only a proportion of the radioactivity was recovered in the urine of sheep after an intravenous dose of [^{14}C]3MH, whereas a large proportion was retained

Table 2. *Mean tracer response profiles in plasma, muscle and urine of four lambs injected with 52·6 μmol 3-[$^2H_3$]methylhistidine (d_3-3MH)**

(Mean values with their standard errors)

Period after dose (min)	Plasma enrichment†		Muscle enrichment‡		Percentage tracer recovery in urine§	
	Mean	SE	Mean	SE	Mean	SE
2	0·181	0·0206				
7	0·111	0·0128				
10	0·104	0·0084				
12			0·020	0·0069		
15	0·091	0·0070				
45	0·081	0·0007				
60			0·029	0·0088		
90	0·063	0·0049				
150	0·055	0·0048				
210	0·052	0·0035				
270	0·050	0·0045				
300			0·024	0·0029		
720	0·039	0·0033				
1440	0·033	0·0020	0·034	0·0074	3·41	1·01
2880	0·025	0·0018			5·89	1·45
4320	0·019	0·0009	0·018	0·0031	7·96	1·66
5760	0·015	0·0003			9·41	1·85
7200	0·011	0·0008			10·80	1·88

* For details of procedures, see pp. 744–747.

† Calculated as the background corrected d_3-3MH/3-methylhistidine (3MH) peak height abundance (ion 241/238).

‡ Enrichment of tracer in *longissimus dorsi* biopsies was calculated as the background corrected d_3-3MH/3MH peak height abundance (ion 241/238).

§ The percentage d_3-3MH accumulating in the urine.

(Harris & Milne, 1980). We next modified this plasma–urinary model (Fig. 3) to include a second exit from compartment 3, an exit meant to represent an exit into a balenine (Harris & Milne, 1980, 1987) 'sink' that turns over very slowly or not at all during the time frame of the study. This model, illustrated in Fig. 3, was constructed by including the plasma kinetic data in compartment 1, and the urinary kinetic data were described by an exit from compartment 1 (L(10,1)). With this model we saw close agreement between observed and calculated values for describing both plasma and urinary data simultaneously (Fig. 3). The kinetic urinary 3MH curve shown in Fig. 3 was similar to the accumulative recovery of radiolabelled-3MH described by Harris & Milne (1980), in whose work the majority of tracer is excreted during the first 24 h and excretion is gradual thereafter. Model variables and steady-state masses and transport rates were obtained and are presented in Table 3. The steady-state values obtained for this model were very similar to those of the previous plasma model, except that the 3MH leaving the system was partitioned into two exits: a small urinary exit averaging 29·4 (SE 5·1) nmol/min and a much larger exit from pool 3 averaging 172·1 (SE 4·4) nmol/min. This urinary exit accounted for only 15% of the total 3MH leaving the system. The *de novo* production rate of 3MH into compartment 3 was 201·6 (SE 6·0) nmol/min, which corresponded to an average fractional breakdown rate of 5·4%/d.

The final phase in the development of the 3MH kinetic model was to relate the developed kinetic models to 3MH production in muscle by using the enrichment of 3MH from muscle

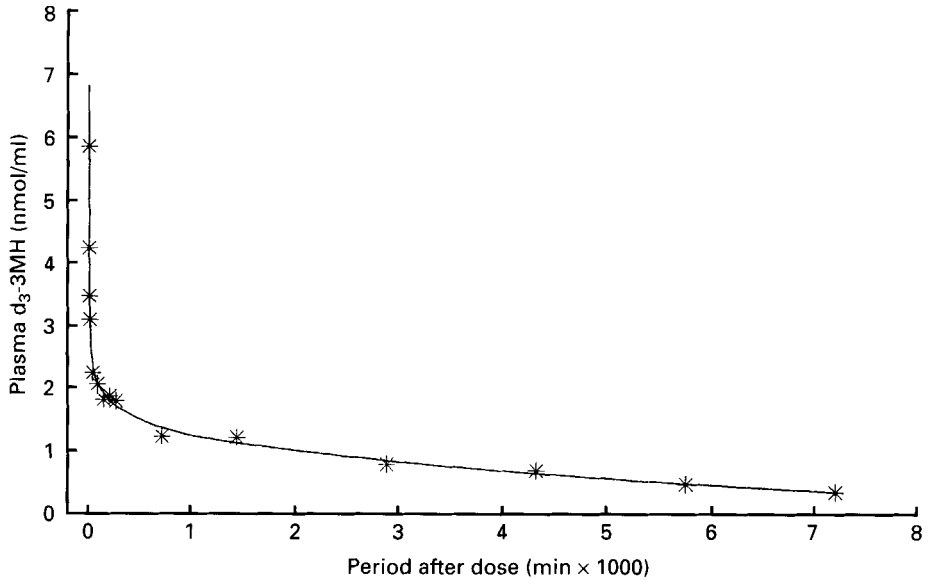

Fig. 1. The fitting of the time course of plasma 3-[$^2H_3$]methylhistidine (d_3-3MH) disappearance from plasma of lamb no. 9183 is presented. (*), Observed values; (——), values calculated by SAAM (Boston *et al.* 1981), with a three exponential fit given. The residual sums of squares for a two and three exponential fit were: 3 exp 0·026 ($P < 0·005$), 2 exp 0·122. $c(T)$ is the concentration of d_3-3MH (nmol/ml plasma) at time T (min post injection) given by $c(T) = 1·5\exp(0·0002T) + 4·4\exp(0·13T) + 0·91\exp(0·004T)$. For details of procedures, see p. 747.

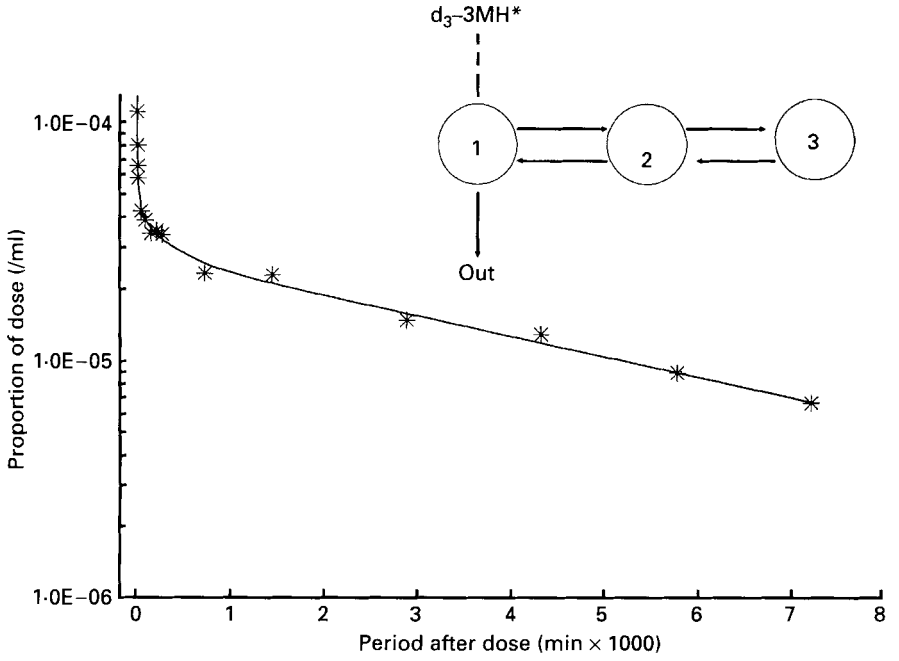

Fig. 2. Plasma 3-[$^2H_3$]methylhistidine (d_3-3MH) for lamb no. 9183 following an injection. The tracer d_3-3MH was converted to proportion of dose/ml by dividing by the injected dose as depicted on the y-axis. (*), Actual measurements; (——), model prediction. Also depicted is a graphical representation of the kinetic model (model A) used to describe this curve. d_3-3MH was injected into pool 1, and an exit was through the same pool. For details of procedures, see p. 747.

Table 3. *Kinetic variables of 3-methylhistidine (3MH) metabolism with steady-state 3MH masses and transport rates for two different compartmental models in four lambs**

(Estimated variable and steady-state value means with their standard errors. Each mean is composed of four values generated by SAAM/CONSAM (Boston *et al.* 1981) during the final fit of the data)

Model...	Simple plasma (A†)		Plasma + urine (B‡)	
	Mean	SE	Mean	SE
K(1) (ml)	0·000138	0·0000123	0·00013	0·00001
Space distribution (ml)	7380	607	7620	649
L(2,1) (min)	0·113	0·0235	0·104	0·0224
L(1,2) (min)	0·0661	0·0150	0·0598	0·0144
L(3,2) (min)	0·00340	0·000977	0·00331	0·00116
L(2,3) (min)	0·00303	0·000241	0·00217	0·000422
L(0,1) (min)	0·000804	0·0000738	NA	
L(10,1) (min)	NA		0·000114	0·0000256
L(0,3) (min)	NA		0·000337	0·0000415
M(1) (μmol)§	261	24·6	271	26·8
M(2) (μmol)	478	86·3	503	96·0
M(3) (μmol)	473	83·0	543	90·9
U(2) (μmol/min)	0·205	0·00360	0·201	0·00600
R(2,1) (μmol/min)	28·8	5·14	27·3	5·03
R(1,2) (μmol/min)	29·0	5·14	27·3	5·03
R(3,2) (μmol/min)	1·42	0·224	1·39	0·331
R(2,3) (μmol/min)	1·42	0·224	1·21	0·327
R(0,1) (μmol/min)	0·205	0·00360	NA	
R(0,3) (μmol/min)	NA		0·172	0·00440
R(10,1) (μmol/min)	NA		0·029	0·00510

* For details of procedures, see p. 747.

† Model A is a simple three-compartmental plasma model with one exit from the system from compartment 1(L(0,1)); see Fig. 2.

‡ Model B, which is a plasma–urinary model with two exits from the system, is a modification of the simple plasma model in which the accumulative urinary 3-methylhistidine kinetic data were included as an exit from compartment 1 (L(10,1)), and a second exit from compartment 3 (L(0,3)) was included; see Fig. 3.

§ L(I, J), Proportion of material transported from compartment J to I per unit time (proportion/min); R(I, J), transport of tracee (natural 3MH) from compartment J to I per unit time (nmol/min) under steady-state conditions; U(I), entry of tracee into compartment I from outside of the model per unit time (nmol/min). The *de novo* production of unlabelled 3MH from breakdown of actin and myosin proteins into intracellular muscle pool under steady-state conditions; M(I), mass of tracee (unlabelled 3MH) in compartment I; K(I), proportionality constant associated with compartment I. CONSAM assumes that a component number is specified under an H DATa header statement in the input file, and it will automatically multiply the category associated with this component by K(I) to calculate QC(I). QC(I) is CONSAM's notation for calculated values. Data associated with a component, I, are called QO(I); K(I) represented the inverse of the space of distribution. The number of compartments in the model is arbitrary and reflects simply the choices of compartment numbers made during the development of the model; NA, not applicable.

Steady-state values were initiated by multiplying the average plasma concentration of 3MH by the space of distribution.

biopsies of the *longissimus dorsi*. The kinetic data from muscle were entered into either compartment 2 or compartment 3 by using the plasma–urinary kinetic model with two exits (Fig. 4). When the muscle kinetic data were entered into compartment 2, the values at 12, 60 and 300 min calculated by the model overestimated the observed values at these same times and produced identical values at 1440 and 4320 min. When the data were entered into compartment 3, however, the model underestimated the early time-points and slightly overestimated the latter time-points.

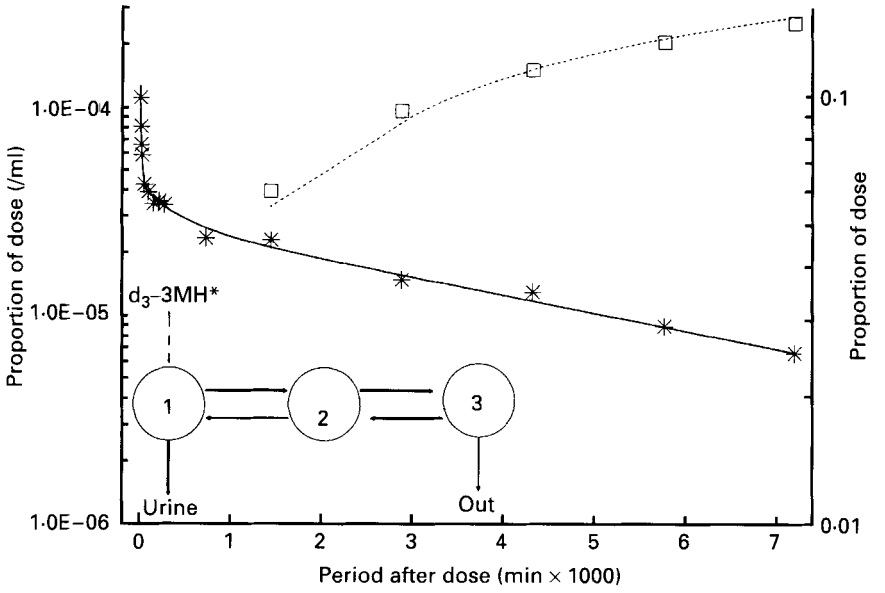

Fig. 3. The simultaneous fit of plasma and urine kinetic data from lamb no. 9183. Plasma is represented as a proportion of dose of 3-[$^2H_3$]methylhistidine (d_3-3MH) per ml (——), while the urine is represented as a proportion of dose of d_3-3MH (----). Also illustrated is the proposed model (model B) compatible with the data, in which urine exit was from pool 1, and another exit occurred from pool 3. For details of procedures, see p. 747.

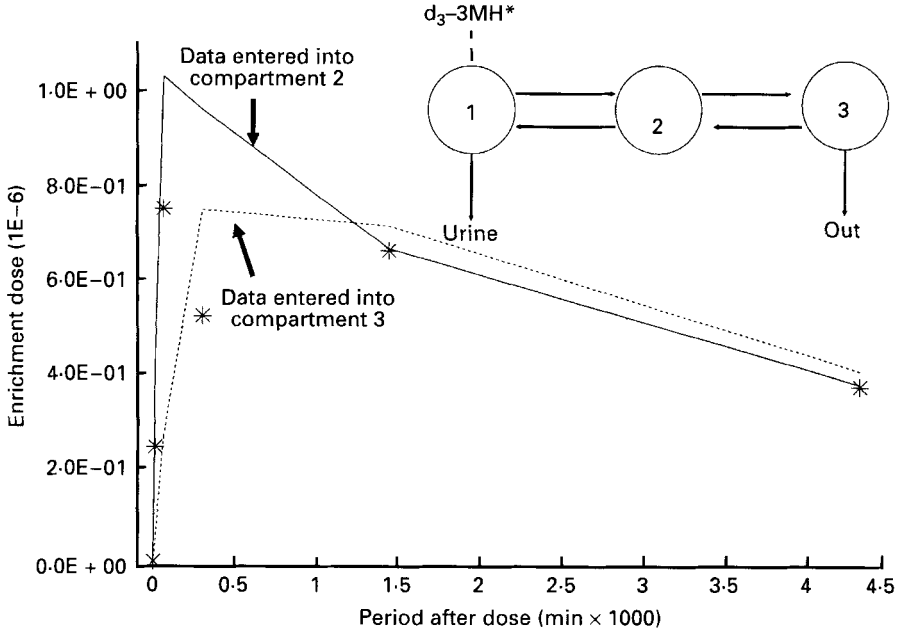

Fig. 4. The predicted enrichment of 3-[$^2H_3$]methylhistidine in muscle when entered either into compartment 2 (——) or 3(····) by using the proposed model B (plasma and urine kinetic model, see Fig. 3; lamb no. 9186). *, Actual observed measurements. For details of procedures, see p. 747.

Table 4. *Lamb status and natural 3-methylhistidine (3MH) measurements*

Lamb no....	9173		9183		9186		9169	
Sex	♂		♂		♂		♀	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Weight (kg)	25·9		27·3		25·0		25·0	
Plasma 3MH (nmol/ml)*	38·5	0·2	30·3	0·2	32·8	0·1	41·2	0·2
Urinary 3MH production (nmol/min)	22·1	1·4	38·6	4·5	26·1	1·9	17·6	1·7
Model calculated urinary 3MH production (nmol/min)*	23·4	1·1	41·4	1·3	34·4	1·3	18·7	1·0
Model calculated 3MH production (nmol/min)‡	191·7	63·8	205·8	5·7	213·2	6·7	205·5	7·1
Muscle 3MH (nmol/g)‡	34·5	4·7	29·0	5·7	20·4	1·3	33·1	4·5

* Urinary 3MH production was calculated from a compartmental model (model B) based on plasma and urinary kinetics of 3-[$^2H_3$]methylhistidine.

† 3MH production calculated from a compartmental model (model A) based on plasma kinetics of 3-[$^2H_3$]methylhistidine.

‡ Non-protein-bound 3MH in muscle.

A summary of 3MH kinetics and measurement of physiological characteristics are shown in Table 4. The mean plasma concentration was 35·7 (SE 2·5) nmol/min, while muscle was 29·2 (SE 3·2) nmol/g (wet) weight of muscle. The urinary production rate estimated from model B was 26·1 (SE 4·5) nmol/min. The actual urinary 3MH production rate was similar to the values calculated from model B, that is 29·4 nmol/min (Table 3). Whereas urinary recovery of 3MH is incomplete, a compartmental model can be used to calculate kinetically a *de novo* production rate based on plasma kinetics (Table 3; 205 nmol/min).

DISCUSSION

The major objectives of the present study were to show that the 3MH kinetics of a stable isotope could be described by a compartmental model and to determine whether a *de novo* production rate of the tracee could be estimated. This method is of value to estimate the fractional breakdown rate of muscle protein because urinary 3MH is invalid for use in species such as sheep and pigs. Other methods for studying muscle protein degradation directly are limited, and methods are available that allow for indirect calculation of muscle degradation if the fractional accretion rate of muscle protein is known (Garlick, 1980).

We have described the *in vivo* kinetics of 3MH in lambs by means of two different models, starting with a very simple three-compartment model requiring only the sampling of plasma and thence to a model requiring the sampling of plasma and urine. The simple plasma model (model A) and the more complex plasma–urine model (model B) gave nearly identical results, demonstrating the usefulness and accuracy of the simple plasma model. In addition, the biopsies from the *longissimus dorsi* were used for representing the total skeletal musculature from which 3MH is primarily produced. These findings used in a simulation of the plasma–urinary model forced skeletal muscle to be the only source of 3MH produced *de novo*. However, it is thought the skin and intestines may also contribute 10–15% of the daily 3MH produced (Nishizawa *et al.* 1977; Young & Munro, 1978; Harris, 1981; Millward & Bates, 1983). Therefore, a lower production rate could be expected for the model when muscle is forced to be the only source of *de novo* production.

The models were constructed based on previous knowledge of 3MH metabolic pools in sheep (Harris & Milne, 1980). It had been demonstrated that only a small percentage of

3MH, which is released from actin and myosin, is actually excreted in the urine, with the remaining majority retained as balenine in muscle. It was assumed that the tracer was initially distributed into a plasma plus extracellular water space of compartment 1, which had an exit into a urine pool (compartment 10). This assumption was based on the space of distribution of compartment 1 (K(1), Table 3) which was 7381 and 7619 for model A and B respectively. This space of distribution for compartment 1 was 30% of the body weight of these four lambs and would be approximately equal to the extracellular water space. Additionally, model B accurately predicted the daily excretion of 3MH in the urine: 26·1 was observed compared with 29·4 nmol/min predicted. Compartments 2 and 3 are most likely to be intracellular pools of free non-protein-bound 3MH, with a second exit from pool 3 being most likely into a balenine 'sink'. The second exit was assumed to be an exit into a balenine pool that was thought to turn over very slowly or not at all during the duration of the study because balenine seems to accumulate in the muscle of sheep. Nevertheless, when the concentration of free 3MH in the *longissimus dorsi* was extrapolated to the total musculature of the lambs the observed pool size of muscle-free 3MH was only half the model calculated compartment size of either 2 or 3. This might be expected if the enrichment of our tracer in muscle differed between muscles or groups of muscles as the *longissimus dorsi* was used to represent the whole musculature, and the contribution of 3MH from smooth muscle actin to the size of compartments 2 and 3 must also be taken into account.

The structural configurations of these models are not unique, and alternative arrangements may also be compatible with the data. It was possible with simple plasma model A to place the exit from the system at any of the three compartments and to obtain identical agreement between observed and model calculated values. Again with model B the second exit could be placed at any of the three compartments. It is also possible to enter the muscle response into either compartment 2 or 3 as a single metabolic pool and to obtain a solution. The present models represent a framework and methodological approach describing steady-state 3MH kinetics in the whole animal and constitute a working theory for testing by further experimentation with designs altering muscle protein breakdown.

In conclusion, the rate of 3MH production is an important tool in understanding the regulation of muscle protein degradation. The advantages of these models are that (1) it does not necessitate quantitative urine collection (plasma model); (2) it reduces error due to the frequency of plasma sampling *v.* the infrequency of urine collection in the other models; (3) it measures the total production rate independent of the determination of free or conjugated forms; (4) it gives information about pool size and transfer rates; (5) it does not require restraint of the animals for long periods. The model does, however, need further validation as a method for estimating 3MH production in other species.

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