British Journal of Nutrition (1983), **50**, 691–700

N^{τ}-methyl histidine excretion by poultry: not all species excrete N^{τ}-methyl histidine quantitatively

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(*Received 17 February 1983 – Accepted 20 June 1983*)

1. The rate of elimination of administered N^{τ}-[$^{14}CH_3$]methyl histidine was used to assess the validity of N^{τ}-methyl histidine excretion as an index of muscle protein breakdown in poultry.

2. Broiler chicks (2–3 and 4–5 weeks old), laying hens, adult quail (*Coturnix coturnix japonica*), adult cockerels and turkey poults (2–4 weeks old) were tested.

3. All except the turkey poults showed quantitative recoveries of N^{τ}-[$^{14}CH_3$]methyl histidine within 1 week.

4. Turkeys showed a different pattern of N^{τ}-[$^{14}CH_3$]methyl histidine output; the mean total recovery after 14 d was less than 50% of the injected dose. The majority of the label remaining after this time was found in breast muscle.

5. All birds tested excreted N^{τ}-methyl histidine unchanged, although a small amount sometimes appeared as another metabolite.

6. No significant oxidation of N^{τ}-[$^{14}CH_3$]methyl histidine by broiler chicks, turkey poults or adult quail was found.

7. The results show that excretion of N^{τ}-methyl histidine is a useful measure of muscle protein breakdown in the domestic fowl and quail but not in turkeys.

Skeletal muscle is the major protein deposit of the body in avian species. The major protein components of the tissue are actin and myosin which constitute 60–70% of total protein (Dickerson & McCance, 1960). As in mammals, both these polypeptides contain the unusual amino acid N^{τ}-methyl histidine (N^{τ}-MH), sometimes referred to as 3-methyl histidine (Kuehl & Adelstein, 1970; Krzysik *et al.* 1971). If the excretion of N^{τ}-MH released during degradation of actin and myosin is quantitative, this can be used to estimate muscle protein catabolism. Despite some recent controversy concerning the contribution of N^{τ}-MH from intestinal muscle sources (see Harris, 1981), it is widely accepted that in man and in the rat, rabbit and cow N^{τ}-MH excretion is a valid measure of muscle protein breakdown. Pigs and sheep do not eliminate N^{τ}-MH quantitatively but appear to incorporate it into a muscle dipeptide, balenine (β-alanyl-N^{τ}-methyl histidine), also called ophidine or cetasine (Harris & Milne, 1980*a*, 1981).

Investigations into protein metabolism in poultry would be made considerably easier if excretion of N^{τ}-MH gave a valid measure of muscle protein degradation in these species. Only one study of N^{τ}-MH excretion in birds has been previously reported (Cowgill & Freeburg, 1957). This work tested only very young chicks of unspecified strain but did find quantitative recovery of N^{τ}-MH. Fisher *et al.* (1975), who measured histidine and histidine derivatives in breast muscle of adult cockerels, suggested that the N^{τ}-MH method would be invalid due to high muscle levels of free N^{τ}-MH. Chicken muscle also contains the dipeptide balenine, the existence of which invalidates the N^{τ}-MH method for sheep and pigs (Harris & Milne, 1980*a*, *b*, 1981) and thus also casts doubt on whether the technique could be successfully used with poultry.

The work reported in the present paper was carried out to establish if the excretion of N^{τ}-MH by various types of poultry is quantitative and can therefore be used as a measure of muscle protein degradation.

https://doi.org/10.1079/BJN19830141 Published online by Cambridge University Press

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MATERIALS AND METHODS

Animals

Broiler chicks, 1-d-old, were obtained from D. B. Marshall Ltd, Newbridge, Midlothian. Laying hens of Warren type were obtained at 1-d-old from Robert Thomson Chicks Ltd, Lannhall, Tynron, Thornhill, Dumfries and raised to maturity at the Poultry Research Centre (PRC). Adult cockerels derived from a White Leghorn type (S-line), turkey poults of a large-bodied commercial strain (British United Turkeys Big 5) and adult Japanese quail (*Coturnix coturnix japonica*) were all from stocks maintained at the PRC.

Animals were given diets according to age and species as described in Bolton & Blair (1974). The adult cockerels received the diet formulated for laying hens; all broilers were given the starter ration. The diets were given for at least 4 weeks prior to the experiment or, in the case of the young birds, since hatching.

Birds were maintained in heating and lighting regimens according to age and species as routinely used at the PRC.

MATERIALS

N^{τ}-[$^{14}CH_3$]methyl histidine (N^{τ}-[$^{14}CH_3$]MH) was obtained from Amersham International, Amersham, Bucks; specific activity was 22 mCi/mmol and radiochemical purity was 98%.

N-acetyl-N^{τ}-MH was prepared as described by Young *et al.* (1972).

Fisofluor 1 and Fisofluor 3 liquid scintillation cocktails and Fisosolve tissue solubilizer were obtained from Fisons Ltd, Loughborough, Leicestershire.

All other chemicals were of analytical grade.

METHODS

Determination of N^{τ}-[$^{14}CH_3$]MH excretion

Birds used for the experiments were transferred to individual cages of an appropriate size and type and allowed to adapt to their new surroundings for 3 d.

N^{τ}-[$^{14}C_3$]MH was administered either intravenously (laying hens, adult cockerels, 4-week-old broilers) or intraperitoneally (turkey poults, quail, 2- and 4-week-old broilers). Using 4-week-old broilers no difference was found in N^{τ}-[$^{14}CH_3$]MH excretion between the two routes of administration. The ^{14}C-labelled material was distributed into all body tissues in less than 75 min when injected intraperitoneally. Oral administration of N^{τ}-MH cannot be used in birds because faecal and urinary excreta cannot be separated in normal birds.

Excreta were collected into 0·1 M-hydrochloric acid (200–500 ml) in plastic trays. This limits microbial destruction and drying out of excreta, thus losses in collection were minimized. There was a small, variable and unquantifiable loss in collection due to material sticking to cage floors and sides and to the animals' feet and feathers. It was not always possible to retrieve all such material. The excreta acid mixture was collected into 2-l (or 1-l for small birds) beakers, the volume adjusted with distilled water to 1 l (or 500 ml) and stirred for 30 min at 20°. The mixture was allowed to settle for a few minutes then 50-ml samples of the upper layer were taken and centrifuged for 15 min at 4000 ***g***. This produced a clear yellow supernatant fraction which was used for determination of radioactivity without further treatment. N^{τ}-MH is very soluble in dilute acid and preliminary tests showed that added N^{τ}-[$^{14}CH_3$]MH was completely recovered by this procedure.

Identification of radioactive components of excreta

Radioactive components of excreta were separated by the column chromatography system described by Young *et al.* (1972). Extracts were obtained as described previously from excreta collected 24 h after injection of N^{τ}-[$^{14}CH_3$]MH. These extracts ($\sim$ 1–2 l) were

evaporated to dryness and taken up in a small volume (~ 50 ml) of water. This was applied to an Amberlite CG120 (Type 1, H^+ form; 30 × 280 mm) column and eluted as described by Young *et al.* (1972) except that 10-ml fractions were collected. All the radioactivity appeared as a single peak. The relevant fractions were combined, evaporated, taken up in water (~ 25 ml) and applied to a column (30 × 240 mm) of Dowex 50 × 8 (H^+ form). Components were eluted as described by Young *et al.* (1972). Fractions of 10 ml were collected and assessed for radioactivity.

For comparison, samples of N^{τ}-[$^{14}CH_3$]MH and *N*-acetyl-N^{τ}-[$^{14}CH_3$]MH were passed through the Amberlite and Dowex column procedures. N^{τ}-[$^{14}CH_3$]MH showed three peaks of activity on Dowex separation. A small amount (~ 2%) of the radioactivity appeared in two early fractions while the remaining (98%) activity appeared as a single peak much later (see Fig. 1 (*a*), p. 694). The *N*-acetyl-N^{τ}-[$^{14}CH_3$]MH preparation separated as a major peak of activity in early fractions with some unesterified N^{τ}-[$^{14}CH_3$]MH and its contaminants also present (Fig. 1(*a*)).

Thin-layer chromatography (TLC) was performed using silica gel TLC plates, 0·25 mm thick, developed in tetrahydrofuran–water (1:1, v/v). R_f values for reference compounds were: N^{τ}-MH 0·25, *N*-acetyl-N^{τ}-MH 0·63, N^{π}-MH 0·26, histidine 0·32, anserine 0·08. Where required, compounds were visualized using ninhydrin (in butan-l-ol, 10 g/l).

Paper chromatography using Whatman no. 1 paper was run in descending direction with ethanol–diethyl ether–water (15:12:4, by vol.). R_f values on this system were: N^{τ}-MH 0·32, *N*-acetyl-N^{τ}-MH 0·49, N^{π}-MH 0·25, histidine 0·20, anserine 0·21. Again ninhydrin was used to visualize these materials on chromatograms.

Tissue distribution of N^{τ}-[$^{14}CH_3$]MH

Blood samples were taken from a wing vein into heparinized syringes and centrifuged. Erythrocytes were removed and plasma was deproteinized in sulphosalicylic acid (25 g/l); 0·5 ml samples were used for determination of radioactivity.

Birds were killed by cervical dislocation and tissues rapidly excised and kept on ice. Samples (0·5–1·0 g) of each tissue were homogenized in perchloric acid (100 g/l) using a Polytron homogenizer (Kinematica GmbH, Kriens/Luzern, Switzerland) for 10–45 s depending on the tissue type. After removing protein by centrifugation, 0·5 ml samples of the supernatant fraction were used for determination of radioactivity. Protein precipitates were solubilized in Fisosolve and were used for determination of radioactivity using Fisofluor 3.

Radioactive samples

Liquid-scintillation determination of radioactive samples was carried out using either Beckman LS-230 or LKB 1216 liquid-scintillation counters. Aqueous samples (0·5 ml) were mixed with 4·5 ml Fisofluor 1 scintillation cocktail in plastic mini-vials. Counting efficiency was calculated using an internal standard and varied from 37 to 55% depending on the type of sample and the presence of coloured compounds. Samples of $^{14}CO_2$ contained in ethanolamine-2-methoxyethanol (1:2, v/v; 0·8 ml) were mixed with 2-methoxyethanol–toluene (1:2, v/v; 4·0 ml) and radioactivity determined as described previously.

To determine radioactivity on TLC plates a grid was marked out on the plate and squares of silica scraped off into glass scintillation vials. To this, 1 ml water was added, vigorously shaken and mixed with 9 ml Fisofluor 1 before radioactivity was measured. Areas of activity were assessed quantitatively. Grids were also marked out on the chromatograms, squares cut out and counted for radioactivity as for silica gel samples.

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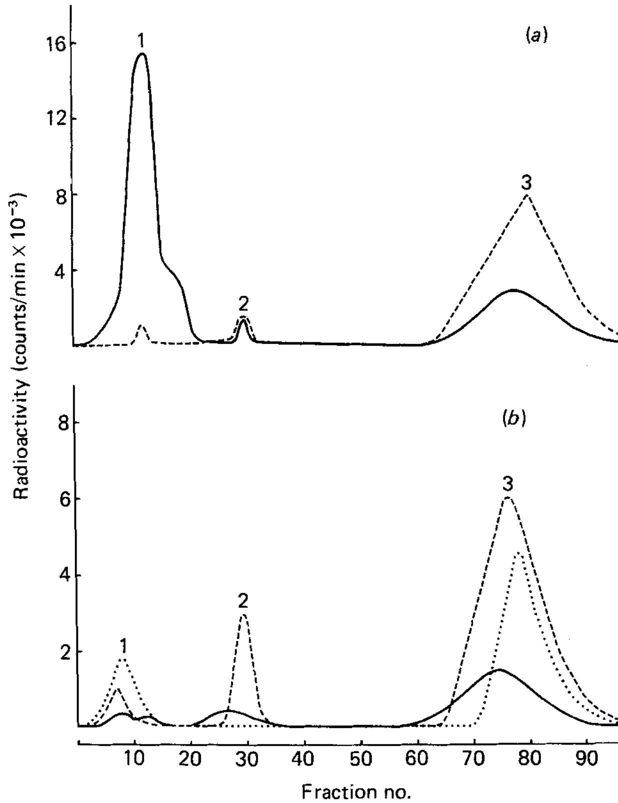

Fig. 1. Separation of radioactive metabolites in excreta extracts by Dowex chromatography (for details of methods, see p. 693). (*a*) (——), Synthesized *N*-acetyl-N^{τ}-[$^{14}CH_3$]methyl histidine; (---), N^{τ}-[$^{14}CH_3$]methyl histidine. (*b*) (——), Excreta extract from broiler chicks; (– – –), excreta extract from turkey poults; (···), excreta extract from adult quail (*Coturnix coturnix japonica*). For a discussion of fractions 1, 2 and 3, see pp. 695 and 696.

In vivo metabolism on N^{τ}-[$^{14}CH_3$]MH

A sealed glass metabolism chamber able to accommodate one small bird (< 500 g body-weight) was constructed. The wire-mesh floor was raised over an aluminium-foil tray in which excreted matter was collected. Air was drawn through the system by a small electric pump at a rate of 2–3 l/min. $^{14}CO_2$ was collected hourly in 10-ml volumes of ethanolamine–2-methoxyethanol (1:2, v/v).

Oxidation of N^{τ}-MH was examined in broilers (3 weeks old), turkey poults (2 weeks old) and adult quail by intraperitoneal injection of 1·45 μCi N^{τ}-[$^{14}CH_3$]MH and following $^{14}CO_2$ output over 6 h. At the end of this time the animals were killed by cervical dislocation and tissue samples rapidly removed for examination of tissue distribution and protein incorporation of ^{14}C.

Amino acid analyses

Samples of pectoral muscle (removed within 1 min of death) were homogenized in ice-cold sulphosalicylic acid (30 g/l) containing norleucine as internal standard. The protein was removed by centrifugation and the supernatant fraction used without further treatment for analysis. Analyses were performed using a Chromaspek J180 analyser (Hilger Analytical

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Table 1. *Thin layer (TLC) and paper chromatography* of radioactive metabolites from poultry excreta extracts and of reference compounds*

Material	Fraction no. of Dowex separation	R_f values TLC	R_f values Paper
Extract from			
Turkey poult	3	0·25	0·35
Quail	3	0·25	0·32
Broiler	3	0·25	0·32
Laying hen	3	0·25	0·32
Cockerel	3	0·25	0·32
N^{τ}-MH	3	0·25	0·32
N-acetyl-N^{τ}-MH	1	0·63	0·49
Histidine	—	0·32	0·20
N^{π}-MH	—	0·26	0·25
Anserine	—	0·08	0·21

N^{τ}-MH, N^{τ}-methyl histidine.
* Solvent systems used were: TLC, tetrahydrofuran–water (1:1 v/v); paper, ethanol–diethyl ether–water (15:12:4, by vol.).

Ltd, Westwood, Margate, Kent). N^{τ}-MH was eluted using a gradient of lithium citrate buffer from pH 1·99, Li^+ 0·15 M to pH 11·55, Li^+ 0·30 M. Elution time was 1 h 52 min. N^{τ}-MH is satisfactorily separated from N^{π}-MH and histidine by this procedure.

RESULTS

Separation of radioactive components of excreta extracts

The results of separation of radioactive components of excreta extracts on Dowex columns are shown in Fig. 1(*b*) for quail, broilers and turkeys. Samples of the radioactive N^{τ}-MH used for injection and *N*-acetyl-N^{τ}-MH synthesized by us are included for comparison (Fig. 1(*a*)). For the sake of clarity, the results from the cockerels and laying hens have been omitted, but these showed essentially the same pattern as quail excreta extracts. It can be concluded from these chromatograms that the major radioactive component (containing > 90% of the activity) corresponded to N^{τ}-MH. (Differences in activity between different species were due to the variation in amount of material injected into birds and to different volumes of extract added to the columns.) Two minor contaminants of the N^{τ}-[$^{14}CH_3$]MH preparation appeared to be excreted by all the species, and also to be present in the *N*-acetyl-N^{τ}-[$^{14}CH_3$]MH. After combining and concentrating fractions comprising each peak (1, 2 and 3 in Fig. 1) these were further examined by TLC and paper chromatography. Results of these procedures on Dowex fraction 3 are shown in Table 1. R_f for reference compounds on each system are included for comparison. In all cases fraction 3 corresponded with the original N^{τ}-[$^{14}CH_3$]MH on both separation systems. No other areas of radioactivity were found on chromatograms. This clearly implied that fraction 3 did represent N^{τ}-[$^{14}CH_3$]MH.

Although we considered that the small early peaks (fractions 1 and 2) represented contamination in the N^{τ}-[$^{14}CH_3$]MH, the fractions containing this activity were examined by the same TLC and paper chromatographic methods. In both these systems radioactivity spread over the chromatogram and no one 'spot' of activity was discernible. The amount of radioactivity in the sample applied was necessarily small, and this also made identification more difficult. However, concentrated material from the N^{τ}-[$^{14}CH_3$]MH preparation

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Table 2. *Excretion of administered* N^{τ}-[$^{14}CH_3$]*methyl histidine* (N^{τ}-[$^{14}CH_3$]*MH*) *by poultry*

(Mean values and standard deviations. Birds were housed and fed as described on p. 692)

Bird	No. of animals tested	N^{τ}-[$^{14}CH_3$]MH excreted (% injected dose) After 2 d Mean	SD	After 7 d Mean	SD
Quail (*Coturnix coturnix japonica*) (♂+♀, 8-month-old)	6	86	9	95	8
Laying hen (9 and 10-month-old)	3	68	4	95	10
Cockerel (16-week-old)	3	62	4	89	4
Broiler (♂, 2-week-old)	6	72	5	85	6
Broiler (♂, 4-week-old)	6	74	5	90	6
Turkey poult (2-week-old)	6	40	6	49	6

Table 3. *Oxidation of* N^{τ}-[$^{14}CH_3$]*methyl histidine* (N^{τ}-[$^{14}CH_3$]*MH*) *by broiler chicks, turkey poults and adult quail* (Coturnix coturnix japonica)

(Mean values and standard deviations for three birds. $^{14}CO_2$ output was measured at hourly intervals. Results expressed as a cumulative total for 6 h following injection of N^{τ}-[$^{14}CH_3$]MH)

Bird	$^{14}CO_2$ output (% of given dose) Mean	SD
Broiler chick	0·01	0·01
Quail	0·23	0·12
Turkey	0·03	0·03

(fractions 1 and 2) after Dowex chromatography also behaved in this way. While the position of peak 1 on Dowex column chromatography might suggest that this activity is present as *N*-acetyl-N^{τ}-[$^{14}CH_3$]MH, the behaviour on TLC and paper chromatograms would imply that this was not the case. However, the results were not totally conclusive as staining showed the presence of much ninhydrin-positive material in this fraction. This may have caused anomalous behaviour of the ^{14}C-labelled material on paper and TLC. Due to the small amounts of these materials isolated, it was impossible to investigate their properties further, either by purification or by hydrolysis. Since the total ^{14}C material in fractions 1 and 2 represented only a small amount of the radioactivity excreted (2–10% depending on species) we consider this to be unimportant compared with the major excretory product and therefore unlikely to interfere with the use of N^{τ}-MH as an indicator of muscle protein degradation.

Excretion of N^{τ}-[$^{14}CH_3$]*MH*

Excretion of administered N^{τ}-[$^{14}CH_3$]MH by the various types of poultry tested is shown in Table 2. The quail, laying hens, cockerels and broilers all eliminated the ^{14}C rapidly, most of the excretion being within 48 h of injection. The failure to show a 100% recovery within 1 week was probably due to the losses incurred on collection (see p. 692). There may also have been some slight variation in the amount of ^{14}C material injected. The N^{τ}-[$^{14}CH_3$]MH

Table 4. *Rate of excretion of administered N^{τ}-[$^{14}CH_3$]methyl histidine by various types of poultry*

(Mean values and standard deviations)

Bird	Collection period (d)	^{14}C excreted (% injected dose)		Average daily output (% injected dose)
		Mean	SD	
Turkey	0–2	40	6	20
	2–4	4·7	0·8	2·3
	4–7	4·0	0·9	2·0
	7–9	4·5	1·6	2·25
	9–11	4·6	2·2	2·3
Broiler (2-week-old)	0–2	72	5·0	36
	2–4	8	1·0	4
	4–7	5	0·2	2·5
Cockerel	0–1	42	8	42
	1–2	20·5	1	20·5
	2–3	11	3	11
	3–4	8·5	2	8·5
	4–7	7	2	2·3

solution used was highly labelled (5·8 μCi/ml) and the volume injected was necessarily small ($\sim$ 0·25 ml) in the smaller birds.

It was unlikely that significant losses due to oxidation occurred in any of the species shown in Table 2. The results of oxidation of N^{τ}-[$^{14}CH_3$]MH to $^{14}CO_2$ by broiler chicks, turkey poults and quail are shown in Table 3. While the broiler chicks and turkey poults showed negligible $^{14}CO_2$ output, the quail apparently oxidized a noticeable amount of N^{τ}-[$^{14}CH_3$]MH (equivalent to $\sim$ 1%/d). We cannot explain this difference. However we do not think the oxidation by quail was significant for two reasons: (1) the $^{14}CO_2$ output by all three birds tested declined rapidly over the 6 h of the experiment to almost zero between 5 and 6 h after injection (results not shown), (2) the very high level of recovery of ^{14}C material in the excreta of quail (see Table 2).

Samples of nine tissues (breast, leg and heart muscles, liver, kidney, intestine, pancreas, brain and skin) were examined for free and protein-bound ^{14}C activity at the end of the 6 h experiment. All tissues showed the presence of ^{14}C in the perchloric acid supernatant but absolutely no activity in acid-precipitable cell components. Thus the N^{τ}-[$^{14}CH_3$]MH reached all tissues but was not incorporated into protein.

Excretion of N^{τ}-[$^{14}CH_3$]MH in the turkey was very different from that of the other birds tested. Only 40% of the injected material was recovered within the first 2 d. Further analysis of the results, shown in Table 4, reveals a more obvious difference. Turkeys, after an initial peak, excreted N^{τ}-[$^{14}CH_3$]MH fairly constantly from day to day, whereas with other birds the ^{14}C eliminated declined with each successive collection.

The distribution of label in the major tissues of turkeys was measured 11 d after the administration of N^{τ}-[$^{14}CH_3$]MH. The results are shown in Table 5. The vast majority of the label remaining was in the supernatant fraction of breast muscle. Analyses of the free amino acids in pectoralis muscle of turkey poults (as well as broiler chicks and adult quail) showed no measurable quantities of N^{τ}-MH. This finding suggested that free N^{τ}-MH in pectoralis muscle of these birds must have been at a low concentration i.e. less than 0·02 μmol/g wet muscle (see Table 6, p. 698).

All results suggest that in turkey breast muscle the N^{τ}-MH present is neither protein

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Table 5. *Distribution of N^{τ}-[$^{14}CH_3$]methyl histidine remaining in supernatant fractions of turkey tissues 11 d after injection*

(Results are expressed as means of duplicate measurements on tissues of three different birds)

Tissue	^{14}C (disintegrations/min per g wet tissue)
Blood plasma	727*
Liver	220
Kidney	320
Heart	260
Leg muscle (gastrocnemius)	763
Breast muscle (pectoralis major)	7330

* Counts/min per ml deproteinized plasma.

Table 6. *Histidine and histidine derivatives (μmol/g wet weight) in breast muscle of various birds*

(Results given are mean values and standard deviations of duplicate analyses on tissues of four birds of each species in the present study, and means of analyses performed on four birds for the adult cockerel)

Bird	Histidine		N^{π}-methyl histidine		N^{τ}-methyl histidine	Carnosine		Anserine		Source of values
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Turkey poult	0·13	0·04	nd		nd	12·71	3·70	6·79	2·08	Present study
Adult quail (*Coturnix coturnix japonica*)	0·04	0·01	0·04	0·01	nd	8·90	2·80	38·27	2·90	Present study
Broiler chick	0·085	0·01	0·05	0·02	nd	26·65	1·44	49·29	7·62	Present study
Adult cockerel	0·30		0·04		63·80	38·80		—		Fisher *et al.* (1975)
New Hampshire chick										
Normal	—		—		$18·9 \times 10^{-3}$	—		—		Hillgartner *et al.* (1981)
Dystrophic	—		—		$27·0 \times 10^{-3}$	—		—		Hillgartner *et al.* (1981)

nd, not detectable (i.e. < 0·02 μmol/g wet tissue).

bound nor in the free form. That the radioactive material is not precipitated by sulphosalicylic acid suggests it is of low molecular weight. It is possible that it is the formation and/or turnover of this compound (or compounds) which invalidates the use of N^{τ}-MH excretion as an index of muscle protein turnover in the turkey.

DISCUSSION

One of the earliest studies of the metabolism of N^{τ}-MH in animals (Cowgill & Freeburg, 1957) provided, until now, the only evidence of quantitative N^{τ}-MH elimination by birds. In agreement with that study, our results demonstrate that in quail and domestic fowl, excretion of administered N^{τ}-MH is quantitative. This means that in these species, excretion of N^{τ}-MH can be used as an index of muscle protein catabolism. Provided the proportion of skeletal muscle in the body and the N^{τ}-MH content of the muscle proteins are known, rates of muscle protein breakdown in vivo can be calculated. Surveys of the N^{τ}-MH content of tissues of the rat and cow have been reported (Nishizawa *et al.* 1977, 1979). Muscle content of N^{τ}-MH in 4-week-old male New Hampshire chicks measured by Hillgartner *et al.* (1981) was 2·9 μmol/g dry muscle. However, no values are available for quail or adult domestic fowl or indeed for tissues other than skeletal muscle in birds.

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Although further information is required for the calculation of rates of muscle protein degradation, N^{τ}-MH excretion can be used to compare treatments (e.g. different dietary regimens) between birds of the same strain. In rats urinary N^{τ}-MH:creatinine is valid in the comparison of dietary treatment (Ward & Buttery, 1980) and, in humans, this ratio is particularly useful in the study of disease and stress (e.g. Seashore *et al.* 1981), since creatinine is proportional to muscle mass (Graystone, 1968). In birds N^{τ}-MH:creatine can be used. We have found (C. L. Saunderson, unpublished results) that this ratio remains relatively constant between birds of the same strain and age and under the same conditions.

That birds do not oxidize a significant amount of N^{τ}-MH is not unexpected. Significant levels of oxidation ($> 5\%$) have never been found in any other species including those which do not excrete N^{τ}-MH quantitatively (Young *et al.* 1972; Long *et al.* 1975; Harris & Milne, 1980*a*, *b*).

The presence of a small ($< 10\%$) amount of radioactivity in the excreta of birds which does not correspond with N^{τ}-MH, is not of great significance. It is possible that this activity represents *N*-acetyl-N^{τ}-[$^{14}CH_3$]MH or balenine or some other conjugate. If this is so, then free N^{τ}-MH would be released by acid-hydrolysis of excreta extracts. Otherwise this would introduce only a small error in the calculation of muscle protein degradation; an error that is of the same order of magnitude or less than that introduced by sources other than skeletal muscle contributing to N^{τ}-MH in excreta. These errors would become negligible where N^{τ}-MH is being used as an indicator of protein degradation (or turnover) in comparative studies.

Our results do not agree with those of Fisher *et al.* (1975) who found a high tissue content of free N^{τ}-MH. The presence of a large pool of free N^{τ}-MH within the body tissues would invalidate the relationship between muscle protein breakdown and N^{τ}-MH excretion. A high level of tissue-free N^{τ}-MH would also, because of the distribution of the small amount of N^{τ}-[$^{14}CH_3$]MH, allow only slow elimination of the radioactive material. Even in adult cockerels (the type of bird used by Fisher *et al.* 1975) we found little or no retention of N^{τ}-[$^{14}CH_3$]MH after 7 d. Also in broiler chicks we did not find a significant level of free N^{τ}-MH. Although the muscle contents of free N^{τ}-MH measured by Fisher *et al.* (1975) are very different from those found in our study, histidine and other histidine derivatives have similar ranges of concentration. This is shown in Table 6. Hillgartner *et al.* (1981) found muscle-free N^{τ}-MH at concentrations of 1000 times less than those of Fisher *et al.* (1975), which would agree with our findings. It is possible that age and sex may contribute to the difference between the values since the adult cockerels used by Fisher *et al.* (1975) were much older than those used by us. We do not consider this probable, but this may warrant further investigation into age–sex related changes in skeletal muscle composition. Another explanation for the difference between conclusions is that the strain of bird used by Fisher *et al.* (1975) had some defect in N^{τ}-MH metabolism.

It is interesting that turkeys do not eliminate administered N^{τ}-MH quantitatively within a short period of time. In this respect, the turkey falls into the same category as pigs and sheep. These animals have an active pool of the N^{τ}-MH-containing dipeptide balenine in muscle. Although chicken muscle contains balenine, the pool is either small or does not interact with free N^{τ}-MH to any extent. In any case, the presence of balenine does not preclude quantitative excretion of N^{τ}-MH since rat and human muscle contain the dipeptide also (Harris & Milne, 1980*b*).

We have not as yet demonstrated the presence of balenine in turkey breast muscle, but we suspect that it forms the other pool of N^{τ}-[$^{14}CH_3$]MH in this tissue. The observations that the radioactive N^{τ}-MH remains almost exclusively in turkey pectoralis muscle after 11 d and that free N^{τ}-MH in this tissue is very low both support this belief. Harris & Milne (1980*a*, 1981) showed that sheep and pig muscle contain low concentrations of free N^{τ}-MH but, after acid-hydrolysis of protein-free muscle extracts, very much greater ($>$ tenfold)

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concentrations were found. We could not show this for turkey muscle due to very large carnosine- and anserine-derived histidine and N^{π}-MH peaks which overlap with N^{τ}-MH on our analytical system. This makes quantitation impossible (results not given).

Little is known of the function of the dipeptide carnosine and other β-alanyl imidazoles in muscle. Recently, Brown (1981) has suggested that the dipeptides are involved with intracellular copper transport. On the other hand, Fisher *et al.* (1978) have suggested that the dipeptides are involved closely in histidine and histamine metabolism. These hypotheses assume that all β-alanyl dipeptides have the same function and they fail to explain why different species contain relatively different proportions of the various dipeptides. While it is possible that the various dipeptides serve similar functions, it is by no means certain.

Two major conclusions can be drawn from the work reported here. First that N^{τ}-MH excretion in quail and domestic fowl can be used as a measure of muscle protein degradation. This is not the case for turkeys, since the method is only valid if N^{τ}-MH released during catabolism of muscle proteins is quantitatively recovered in the urine of the intact animal. Second, we have demonstrated the need for testing quantitative elimination of N^{τ}-MH by any animal before N^{τ}-MH can be used as an index of muscle protein breakdown. It is not possible to predict how any animal will metabolize N^{τ}-MH even by examining a closely-related species. Perhaps in the light of the results of Fisher *et al.* (1975) it may even be unwise to predict metabolism in related strains.

The authors would like to thank Mr D. Greenhill for skilled technical assistance and Mr R. K. Scougall and his staff for the amino acid analyses.

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Printed in Great Britain

https://doi.org/10.1079/BJN19830141 Published online by Cambridge University Press