

Essentiality of Histidine in Ruminant and Other Animals Including Human Beings

Ryoji Onodera

Laboratory of Animal Nutrition and Biochemistry, Miyazaki University, Miyazaki-Shi 889-2192, Japan

ABSTRACT : Concept and establishment of essential amino acids in animals and human beings rendered immeasurable contributions to animal production and human health. In ruminant animals, however, essential amino acids have never been completely established. The present review proposes a hypothesis that histidine may not be an essential amino acid for normal growing cattle (Japanese black) at least at the growing stage after about 450 kg of body weight on the basis of the experimental results of histidinol dehydrogenase activities in some tissues of the cattle together with hints from which the hypothesis was derived. At the same time, histidinol dehydrogenase activities in liver, kidney and muscle of swine, mouse, fowl and wild duck will be shown and the essentiality of histidine in these animals will be discussed. Finally, the essentiality of histidine for adult human will briefly be discussed. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 3 : 445-454)

Key Words : Histidinol Dehydrogenase, Histidine Biosynthesis, Rumen Microbes, Cattle Organs, Swine Organs, Mouse Organs, Fowl Organs, Wild Duck Organs

INTRODUCTION

The time when William C. Rose and his colleagues of the University of Illinois in the US made the discovery of α -amino- β -hydroxy-*n*-butyric acid (McCoy et al., 1935) named as 'threonine' later (Meyer and Rose, 1936) from hydrolyzate of fibrin as an essential growth factor for rats marked a really great moment in the history of the research field of nutritional science. Since the time, especially protein nutrition has grown up to the level of science and the concept of essential amino acids (EAAs) has been established on the basis of a convincing theory combined with sufficient experimental proofs. Then EAAs have been established for many sorts of animals such as rats, human beings, domestic animals and so on. It is needless to say that the concept of amino acids has rendered immeasurable contributions to improvement and maintenance of the health of mankind and enhancement of animal production.

In ruminant animals, however, EAAs have never been fully established (Buttery and Foulds, 1985) due to the technical uneasiness in the cause of the presence of rumen microorganisms. In this regard, we have recently achieved a strong suggestion that especially 'histidine' among ten EAAs for immature rats could be synthesized on a large scale by cattle organs (Wadud et al., 2001b; Wadud et al., 2002), which had been hinted in the course of the experiments on amino acid metabolism by (Wadud et al.,

2001a) and on evaluation of nutritive values of rumen microorganisms (Onodera and Koga, 1987). Incidentally, histidine is the only amino acid on which disputes have continued for as long a period as nearly 25 years especially with regard to its essentiality in adult human (Fuller and Garlick, 1994; Metges et al., 1999).

In the present paper, it will be discussed from the nutritional viewpoint whether or not histidine is essential for ruminant animals on the basis of our recent observations of histidinol dehydrogenase (HLDase) activity of the organs of cattle such as liver, kidney and so on. Then the data on HLDase activity of other animals such as swine, mouse, domestic fowl and wild duck will be shown for comparison. Finally arguments on essentiality of histidine for adult human will be shortly discussed.

ESSENTIALITY OF HISTIDINE IN RUMINANT ANIMALS

Historical background

Since Wilhelm Voeltz (Voeltz, 1920) in Germany firstly demonstrated that urea could be used as a nitrogen source for rumen microorganisms of sheep just after the First World War, a great number of studies on urea utilization have been continued in many countries of the world. Striking experiments of Virtanen (1966) finally indicated that urea given as a sole source of nitrogen could supply the entire amino acid requirements of milking cows by way of rumen microorganisms. Since then, rumen microorganisms have been basically regarded as almighty at least in providing all essential amino acids, although essential amino acids had not been fully determined in ruminant animals at that time and even at present as described above. With regard to the essential amino acids

** This paper was presented at an 2002 International Symposium on "Recent Advances in Animal Nutrition" held in New Delhi, India (September 22, 2002).

* Reprint request to: Ryoji Onodera. Laboratory of Animal Nutrition and Biochemistry, Miyazaki University, Miyazaki-Shi 889-2192, Japan. Tel: +81-985-58-2811, Fax: +81-985-58-2884, E-mail : onoderar@cc.miyazaki-u.ac.jp

for ruminants, it has generally been presumed that all ten amino acids essential for immature rats might also be essential for ruminants. Among those, methionine, lysine and threonine have come to actually be taken as essential for sheep and cattle in the course of experiments for examining supplemental effects of amino acids to rumen microbial protein through infusion of these amino acids into abomasum in order to know the limiting amino acids of rumen microbial protein for the host animals (Nimrick et al. 1970; Richardson and Hatfield, 1978). In addition, some researchers asserted that histidine and arginine were also limiting in rumen microbial protein in sheep (Storm and Orskov, 1984; Buttery and Foulds, 1985) implicating that these amino acids were also essential for ruminants.

In relation to the issue of histidine of rumen microbial protein, Bergen et al. (1968) reported that histidine was the first limiting amino acid in rumen protozoal protein for rats on the basis of plasma amino acid score (PAA-S) method as shown in Table 1, where the score was calculated according to the following equation proposed by McLaughlan (1964): $PAA-S = (PAA \text{ level for fed rat}) / (PAA \text{ level for fasted rat}) \times 100$. The second and third limiting amino acids for rats were cystine and arginine, respectively, although cystine and arginine are regarded as non-essential amino acids in mature rats. On the other hand, in rumen bacterial protein,

cystine, arginine and histidine were the first, second and third limiting amino acids, respectively. If we regard cystine and arginine as non-essential amino acids for adult rats, histidine can be thought as the first limiting amino acid even in rumen bacterial protein for the rats. Thus histidine can be regarded as the first limiting amino acid in both rumen protozoal and bacterial proteins for rats.

In this regard, we also reported (Onodera and Koga, 1987) about the nutritional values of proteins, estimated by a modified chemical score method, in the rumen contents of faunated and defaunated goats given the two kinds of rations and pointed out that histidine tended to be the first limiting amino acid of the protein in the rumen contents when an evaluation was made on the basis of amino acid pattern of beef protein (FAO, 1970) as a reference protein. Typical data of the limiting amino acids of rumen contents were shown in Table 2 together with those of rations and rumen microbes. In spite of the fact that histidine was the third limiting amino acid in the ration (haycube and concentrate, 3:1) fed to the experimental animals as well as those in grasses and legumes, it was shifted to the first limiting amino acid in the protein of the rumen contents after the ration was consumed by the animals. As described previously (Onodera and Koga, 1987), this is due to the fact that histidine was the first limiting amino acid of the

Table 1. The first limiting amino acid of rumen protozoal and bacterial proteins for rats determined by plasma amino acid score method

Amino acids	Rumen protozoal protein			Rumen bacterial protein		
	PAA fed ^a	PAA fasted ^b	PAA score ^c	PAA fed ^a	PAA fasted ^b	PAA score ^c
Methionine	6.3	4.8	131.0	4.1	4.5	89.4
Lysine	95.5	89.6	106.6	43.4	65.7	66.1
Leucine	14.0	15.9	88.3	8.4	13.1	64.3
Arginine	26.5	32.3	82.0	17.4	31.7	55.2
Cystine	1.0	1.6	66.1	0.7	2.1	33.8
Histidine	4.4	7.6	58.5	5.5	8.8	63.0

^a Plasma amino acid (PAA) level of fed rats ($\mu\text{g/ml}$).

(Bergen et al., 1968)

^b PAA level of fasted rats ($\mu\text{g/ml}$).

^c Plasma amino acid score = PAA of fed rats/PAA of fasted rats \times 100.

Table 2. Typical data of the limiting amino acids (LAA) of the proteins in rations, rumen microorganisms and rumen contents taken from a goat obtained by chemical score method

LAA ^a	Grasses ^b	Legumes ^b	Ration fed ^c	Rumen bacteria ^d	Rumen protozoa ^d	Rumen contents ^e	
						6 h	12 h
Methionine	55	55	69	125	103	73	70
Lysine	56	60	62	91	91	80	83
Histidine	63	84	83	46	44	67	64

^a Only the first, second and third limiting amino acids are shown in this table. Chemical score was calculated using beef muscle protein as a reference protein.

^b Calculated using values described by Morimoto (1974)

^c Haycube and concentrate mixture (3:1) fed to goats (Cited from Onodera and Koga, 1987).

^d Calculated using values reported by Czerkawski (1976)

^e Rumen contents collected from a goat (H) 6 and 12 h after feeding haycube and concentrate mixture (3:1) (Onodera and Koga, 1987).

proteins of rumen bacteria and protozoa (chemical scores: 46 for bacteria and 44 for protozoa as shown in Table 2), when the limiting amino acid was calculated using the data of amino acid contents in rumen bacteria and protozoa reported by Czerkawski (1976) and in beef muscle protein (FAO, 1970) as a reference protein.

Incidentally, our recent results demonstrated that not only rumen protozoa but also rumen bacteria collected from goats fed haycube and concentrate could not synthesize histidine from histidinol which had been known as a precursor at the final step of the pathway for *de novo* biosynthesis of histidine in general microorganisms (Andorn and Aronovitch, 1982) and plants (Nagai and Scheidegger, 1991). As shown in Table 3, histidinol, imidazolepyruvate, imidazoleacetate and imidazolelactate were used as substrates in this study, and histidinol and imidazolelactate remained unchanged even after 18 h incubation, although the values shown in Table 3 were those only after 12 h incubation. Imidazoleacetate also remained unchanged for 12 h, but it decreased slightly (by 6.3% in bacteria, 3.4% in protozoa and 4.5% in bacteria + protozoa) during a next 6-h incubation period, although no substances were detected by the HPLC method.

Among the four precursors used in this experiment, imidazolepyruvate was the only substrate utilized for producing histidine in the suspensions of rumen bacteria, rumen protozoa and their mixture. Therefore, metabolic features of imidazolepyruvate by the rumen microorganisms were analyzed in more detail and the results were shown in Table 4. Relatively a large amount of histidine was produced from imidazolepyruvate by rumen bacteria and it is also verified that even rumen protozoa could produce histidine from this precursor, though the quantity was less than one third of that produced by the rumen bacteria. Rumen bacteria tended to produce much imidazolelactate (38.6% of disappeared imidazolepyruvate) as a main product, followed by histidine (25.2% of disappeared imidazolepyruvate). Protozoa, however, converted much imidazolepyruvate to histidine (33.5% of disappeared imidazolepyruvate) as a main product, followed by imidazolelactate (18.4% of disappeared imidazolepyruvate). Thus in the case of the mixed suspension of rumen bacteria

and protozoa, production of histidine increased up to 35.5% of disappeared imidazolepyruvate when compared with that by single bacterial suspension, although imidazolelactate was still a main product (37.7% of disappeared imidazolepyruvate). Values of disappeared imidazolepyruvate are shown in parentheses in Table 3. As mentioned above, histidine was not synthesized from imidazolelactate. Considering these data totally, biosynthesis of histidine from imidazolepyruvate by rumen microorganisms, though not a *de novo* synthesis, have a great importance in protecting histidine from wasteful exhaustion and it can be regarded as a saving effect of histidine in the rumen, and in this respect the role of protozoa is of particular importance. Production of imidazoleacetate was 3.2% of disappeared imidazolepyruvate in the mixed suspension of bacteria and protozoa.

These results led us to the conclusion that rumen microorganisms, especially bacteria and protozoa, collected from goats fed haycube and concentrate did not have any ability to synthesize histidine *de novo* different from the results reported by Sauer et al. (1975), and that rumen microorganisms could synthesize histidine from only imidazolepyruvate as suggested by Allison (1969), although not from imidazoleacetate partially different from his suggestion. Therefore, it is considered that histidine must be an essential amino acid for bacteria and protozoa grown in the rumen of goat given haycube and concentrate. These facts make it easier for us to elucidate the reason why histidine is always the first limiting amino acid in rumen bacterial and protozoal protein for rats (Bergen et al., 1968) and in the chemical score (Onodera and Koga, 1987) described above. Interestingly Land and Virtanen (1959) already pointed out in old times that histidine in milk protein of cows contained especially low ¹⁵N when the cows were fed on a diet containing ammonium salt labeled with ¹⁵N, and they speculated in the paper that this might be attributed to inability of the rumen bacteria to synthesize imidazole ring. It can be said that the present has actually demonstrated their speculation.

By the way, as already described above, in the experiments by Nimrick et al. (1970) using growing lambs and Richardson and Hatfield (1978) using growing cattle

Table 3. Synthesis of histidine from histidinol, imidazolepyruvate, imidazoleacetate and imidazolelactate (2 mM) by rumen bacteria and protozoa and their mixture during a 12-h incubation period at 39°C

Substrates	Bacteria	Protozoa	Bacteria + Protozoa
Histidinol (2 mM)	0 (0)	0 (0)	0 (0)
Imidazolepyruvate (2 mM)	253.8 (1008)	80.4 (240)	301.5 (850)
Imidazoleacetate (2 mM)	0 (126)	0 (68)	0 (90)
Imidazolelactate (2 mM)	0 (0)	0 (0)	0 (0)

Values are shown as net increases in total histidine (μM) (supernate + hydrolyzate of cells) in the media after incubation at 39°C for 12 h. Values in () are decreases in substrates, though only the value of imidazoleacetate is one after 18-h incubation.

(partly cited from Wadud et al., 2001a)

(steers), it was similarly demonstrated that the first, second and third limiting amino acids of rumen microbial proteins for these animals were methionine, lysine and threonine, respectively, based on their experimental results obtained after feeding the animals with pure diets containing urea as a sole source of nitrogen, infusing those amino acids singly or together into the abomasum, and then measuring nitrogen retention and plasma amino acid pattern. They also infused histidine into the abomasum singly and together with methionine, lysine and threonine, but it did not improve nitrogen retention any more. The rumen microorganisms, therefore, seemed to supply sufficient amount of histidine to the host ruminant animals. Bergen et al. (1968) clearly showed that histidine was the limiting amino acid in rumen microbial proteins for rats, but the experiments with ruminant animals by Nimrick et al. (1970) and Richardson and Hatfield (1978) showed completely different results from that with rats, in spite of the fact that histidine was the first limiting amino acid when the amino acid pattern of rumen contents was compared with that of muscle protein of cattle using modified chemical score method (Onodera and Koga, 1987). These two contradictory phenomena have made it possible for us to possess a hypothesis that ruminant animals might be able to synthesize histidine *de novo* by themselves and supply histidine to the extent of almost and/or completely covering its requirement of the animals.

Histidinol dehydrogenase in ruminants

In order to prove the above hypothesis, enzymatic

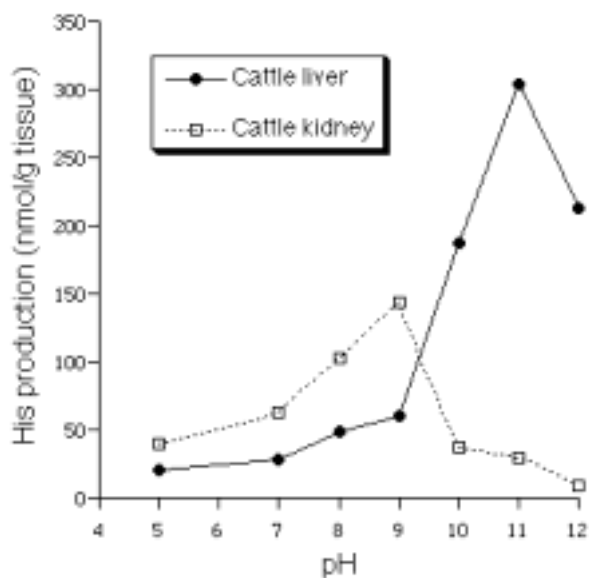


Figure 1. Effect of pH on the histidinol dehydrogenase activities of crude enzyme solutions of cattle liver and kidney at 37°C in 60 min incubation

experiment was conducted in the author's laboratory as reported recently (Wadud et al., 2001b). Namely, histidinol dehydrogenase (HLDase) (EC 1.1.1.23), which catalyzes the reaction from histidinol to histidine at the final step of the histidine *de novo* synthetic pathway as mentioned above, was chosen to be examined with cattle liver and kidney, since in any case it is necessary to know at first the enzyme activity of the final step of the system as has been tested for proving the ability of histidine synthesis in rumen microorganisms (Wadud et al. 2001a). HLDase activity was assayed by determining increase in histidine with crude enzyme solution prepared from liver and kidney collected from Japanese black cattle (delivered cattle) immediately after slaughter. The pH values of buffer solution used for the assay, at which maximum activities of HLDase were observed, were 11.0 in liver with extremely high activity and 9.0 in kidney (figure 1). The actual pH value of the reaction mixture containing the crude enzyme solution of liver and the buffer solution (pH 11.0) was 9.5, when determined later. This optimum pH value was almost similar to those observed in general microorganisms (9.5) (Andorn and Aronovitch, 1982) and plants (9.2) (Nagai and Scheidegger, 1991). However, since pH values usually tended to be expressed with those of buffer solution used, we reported in the first paper (Wadud et al. 2001b) that the optimum pH values of HLDase were 11.0 in cattle liver and 9.0 in cattle kidney. In any case, it was an astonishing new observation which no one had ever imagined before for HLDase activity of animal tissue. In this enzyme reaction, histidinol disappeared and histidine produced was stoichiometrically balanced.

Recently, HLDase activities in some other organs of cattle (Japanese black cattle) were also examined and

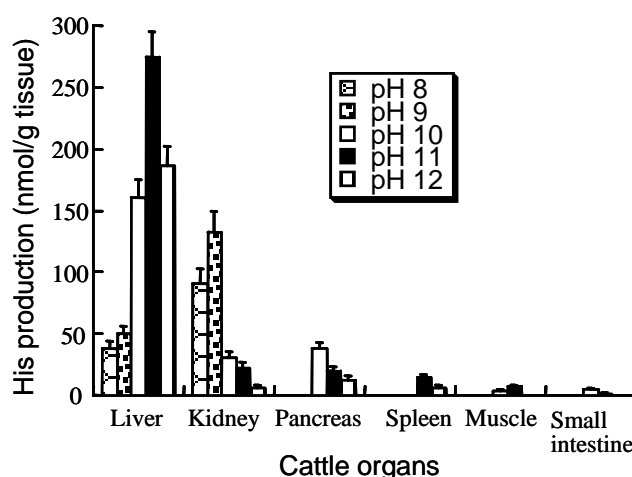


Figure 2. Effect of pH on the histidinol dehydrogenase activities of crude enzyme solutions of liver, kidney, pancreas, spleen, muscle and small intestine collected from cattle at 39°C during a 30-min incubation period

Table 4. Biosynthesis of histidine from imidazolepyruvate (2 mM) by rumen bacteria, protozoa and their mixture during a 12-h incubation period at 39°C

Products	Bacteria	Protozoa	Bacteria + Protozoa
Histidine (µM)	253.8±6.5	80.4±4.5	301.5±8.2
Imidazolelactate (µM)	389.4±18.2	44.2±3.1	320.7±20.1
Imidazoleacetate (µM)	65.8±4.1	39.7±3.4	27.3±2.6
Urocanate (µM)	10.2±1.46	32.5±3.4	15.7±1.6
Histamine (µM)	3.2±0.23	21.1±2.7	7.1±1.6

Values are expressed as the means of nine determinations and standard deviations of the differences between incubation with and without substrate (imidazolepyruvate). (partly cited from Wadud et al. 2001a)

compared with those of liver and kidney (Wadud et al. 2002). The results were shown in figure 2. In this experiment, optimum temperature was determined at first and was shown to be 39°C in liver and kidney, and this time the enzyme activity in each organ was determined at 39°C, as actual body temperature can be thought as 39°C in cattle. On the basis of the results in this experiment, it has been revealed that the crude enzyme of each tissue had its own optimum pH value (expressed with that of buffer solution): liver, spleen and muscle at 11.0; pancreas and small intestine at 10.0; kidney at 9.0 and that the highest activity per unit weight of tissue (g) was observed in liver followed by kidney, pancreas, spleen, muscle and small intestine. Among these tissues, the activities in liver (548.6 nmol His/g tissue/h) and kidney (264.4 nmol His/g tissue/h) appear to be of importance and the activity in muscle cannot also be ignored because of a large quantity of muscle in the body, though the activity per unit weight was very low (13.6 nmol His/g tissue/h).

If daily production of histidine from histidinol by the liver, kidney and muscle in the cattle (body weight, 700 kg) is provisionally calculated using these values and regarding the weight of liver as 7000 g, kidney as 1300 g and whole muscle as 300 kg, it is estimated as follows, where we regarded the molecular weight of histidine as 155.16:

Cattle liver:

$$\begin{aligned} & 548.6 \text{ nmol His/g tissue/h} \times 7000 \text{ g liver} \times 24 \text{ h} \\ & = 92.16 \text{ mmol His/head/day} \\ & = 14.30 \text{ g His/head/day} \end{aligned}$$

Cattle kidney:

$$\begin{aligned} & 264.4 \text{ nmol His/g tissue/h} \times 1300 \text{ g kidney} \times 24 \text{ h} \\ & = 8.25 \text{ mmol His/head/day} \\ & = 1.28 \text{ g His/head/day} \end{aligned}$$

Cattle muscle:

$$\begin{aligned} & 13.6 \text{ nmol His/g tissue/h} \times 300 \text{ kg muscle} \times 24 \text{ h} \\ & = 97.92 \text{ mmol His/head/day} \\ & = 15.19 \text{ g His/head/day} \end{aligned}$$

Thus, the total daily production of histidine from histidinol by the cattle liver, kidney and muscle was estimated to be 30.77 g/head/day.

In the next step, this value should be compared with histidine requirement of cattle, but unfortunately there is no information on amino acid requirements for cattle and other ruminants as described already. One datum on which we can depend is daily requirement of digestible crude protein (DCP) for cattle. Therefore we just mechanically tried to calculate histidine requirement of the cattle using DCP requirement and the histidine content of the muscle protein of cattle, provided that the histidine content of the protein of whole body might be almost similar to that of muscle protein. Japanese Feeding Standard for Beef Cattle (2000) indicated DCP requirement for growing and finishing beef steer (BW 700 kg, DG 0.8 kg) as 429 g/day. On the other hand, the histidine content of the muscle protein of cattle has been indicated as 3.408 g/100 g muscle protein (FAO, 1970). Using these data, speculation has been made about the daily requirement of histidine for the above cattle as follows:

$$\begin{aligned} & 429 \text{ g DCP/head/day} \times 3.408 \text{ g His/100 g muscle protein} \\ & = 14.62 \text{ g His/head/day} \end{aligned}$$

This calculation is based on the concept that requirement of amino acids is determined for the most part by the amount of amino acids necessary for the synthesis of body protein, although some amino acids like histidine are used to some extent for the production of physiologically important substances such as hemoglobin, carnosine and others (Fuller and Garlick, 1994). It is obvious that the estimated values of daily requirement of histidine (14.62 g His/head/day) for the finishing steer (BW, 700 kg) was less than the calculated value of histidine produced from histidinol by the cattle liver, kidney and muscle per day (30.77 g His/head/day). This fact suggests a possibility that histidine may not be an essential amino acid for the growing and finishing cattle, provided that the production of histidinol from its precursor is sufficient in these organs.

However, generally speaking, there is a tendency that requirement of histidine as well as other essential amino acids usually increase in inverse proportion to ages (growing stages) because of higher requirement for growth

Table 5. Histidine requirements and histidine productions in growing and finishing beef steers (Provisionally calculated values)

Body weight ^a (kg)	Daily gain ^a (kg)	DCP requirement ^a (g/day)	Histidine requirement ^b (g/day)	Histidine production ^c (g/day)
250	1.20	569	19.39	10.99
300	1.40	633	21.57	13.19
350	1.40	627	21.37	15.38
400	1.40	619	21.10	17.58
450	1.20	555	18.91	19.78
500	1.20	547	18.64	21.98
550	1.00	490	16.70	24.18
600	1.00	483	16.46	26.37
650	1.00	474	16.15	28.57
700	0.80	429	14.62	30.77

^a These values were indicated in Japanese Feeding Standard for Beef Cattle (2000).

^b Histidine requirement was calculated from DCP requirement and histidine content in beef muscle protein (FAO, 1970)

^c Histidine production in each steer was estimated from the determined value (30.77 g/day) of the steer (BW, 700 kg)(see text), provided that histidine production may increase in proportion to body weight.

in younger animals, while production of histidine may increase in proportion to growing stages, if the HLDase activity per unit weight of tissue does not vary largely with growth. Therefore, it is considered that the relationship between production and requirement (supply and demand) of histidine vary with growing stages. Table 5 shows the histidine requirements in growing and finishing beef steers calculated from DCP requirements and histidine content of beef muscle protein in the same way as described above and the histidine production in these steers calculated on the assumption that histidine production may increase in proportion to growth (body weight). In growing beef steers, histidine requirement increases up to 300 kg body weight and then decreases, while histidine production increases with body weight (as assumed like this). At the body weight of 450 kg, histidine production exceeded histidine requirement. Thus the histidine requirement of the steer more than 450 kg of body weight may be filled with one produced by the animal itself, if the assumptions are correct.

The young steer (BW, 300 kg), for example, self-production of histidine (13.19 g/head/day) is less than the histidine requirement of the animal (21.57 g His/head/day) (Table 5). It seems to be a problem at this stage whether or not rumen contents can supply the difference, 8.38 g His/head/day, to the host animal. On the basis of our own calculation, 10 kg dry matter of rumen contents can supply about 23 g histidine, assuming that average histidine content in the protein of the rumen contents was 2.3 g His/100 g protein (based on our analytical data), crude protein content in dry matter of rumen contents was 10% and moisture of the rumen contents was 80%. If it is true, it can be said that if at least 30 kg of rumen contents of a steer of 300 kg body weight can be supplied daily to the host animal, 13.8 g/day (ignoring digestibility here) of histidine may be absorbed into the host animal. Based on these

assumptions, the growing beef steer may obtain enough histidine sufficient for meeting requirement. Similar discussion is also possible for the other growing beef steers shown in Table 5. With regard to dairy cows, discussion has already been made in the previous paper (Onodera et al., 2001).

In any case and needless to say, it is necessary that HLDase activity of cattle at each growing stage is actually determined, supply of histidine from rumen contents is accurately estimated and a sensitive means for evaluating histidine requirement in ruminants is established.

Although the demonstration of the existence of histidine dehydrogenase activity in the organs does not necessarily mean the demonstration of actual production of histidine from the first precursor, phosphoribosyl pyrophosphate, it can be considered that the possibility of the actual production of histidine in those organs was strongly suggested by the enzyme activity, because this enzyme catalyzes the final step of a series of reactions in the pathway of histidine *de novo* synthesis as mentioned above. Conversely, lack of this enzyme means the denial of the pathway of histidine *de novo* synthesis. However, it may be clever to refrain from making any definite statement on this point considering that the proof is not sufficient.

HISTIDINOL DEHYDROGENASE ACTIVITY IN OTHER ANIMALS

Swine, mouse, fowl and wild duck

A possibility that cattle might synthesize histidine by themselves on a large scale was suggested as described so far. Then HLDase activities in liver, kidney and muscle of some other animals are to be examined in order to compare the activities with those of cattle. Up to now, the activities in these organs of swine, mouse, fowl and wild duck were

Table 6. Histidinol dehydrogenase activities of crude enzyme solutions of liver, kidney and muscle of some animals at optimum pH values^a

Animal	Liver		Kidney		Muscle	
	pH _{op} ^b	Activity ^c	pH _{op} ^b	Activity ^c	pH _{op} ^b	Activity ^c
Swine	11.0	16.56	9.0	9.48	-	0
Mouse	11.0	11.85	10.0	3.12	11.0	0.48
Fowl	9.0	26.45	12.0	1.21	10.0	2.44
Wild duck	9.0	4.25	10.0	9.99	11.0	3.73
Cattle	11.0	42.56	9.0	20.51	11.0	1.06

^a Numbers of the animals used for the assay of histidinol dehydrogenase activity were as follows: 5 swine, 21 mice, 3 fowls, 3 wild ducks and 5 cattle.

^b Optimum pH was expressed with the pH value of the buffer solution used.

^c Histidinol dehydrogenase activity at optimum pH ($\mu\text{g His/g tissue/30 min}$).

assayed as shown in Table 6, in which activities of cattle were also indicated for comparison. All organs of all animals examined except for only swine muscle showed HLDase activities, which were expressed with the unit of $\mu\text{g histidine/g tissue/30 min}$. The optimum pH values of HLDase activity of the liver of mammals were similarly observed at 11.0, though those of birds were at 9.0. In kidney and muscle, however, no trend was observed. An interesting fact was that the activity of the liver per unit weight at optimum pH in cattle was 2.6 times higher than that in swine and similarly 3.6 times higher than that in mouse. In kidney, almost the same facts were observed.

Here, histidine productions from histidinol in the liver, kidney and muscle of these animals were mechanically calculated on the basis of the estimates of the mean wet weights of the organs and were shown in Table 7. Table 7 also shows total production of histidine from histidinol in each organ of each animal. If these values are provisionally regarded as total histidine production in each animal, the sum of the histidine requirement indicated in feeding standard and the total histidine production ('self-production') can be considered as the true requirement of histidine in each animal as shown in Table 8. Histidine requirement of mouse in Table 7 was estimated from the

value of rat and feed consumption of mouse (Ishibashi, 2002) and histidine requirement of wild duck was estimated as the same as that of fowl because of lack of indication. In swine, for example, as histidine requirement of growing and finishing pig (BW, 70-115 kg) is shown as 5.5 g His/head/day in Japanese Feeding Standard and also as self-production of histidine provisionally calculated from the HLDase activities of three organs is 1.59 g His/head/day, total 7.09 g His/head/day can be considered as true requirement for the pig as shown in Table 8. On the other hand, it is possible to speculate histidine requirement of the pig from DCP requirement and histidine content in the muscle protein of the pig as described for the estimation of histidine requirement of cattle. Histidine requirement estimated from DCP requirement of the pig is 8.87 g His/head/day (Table 8). This value is about 25% higher than that of true requirement probably due to the nutritional allowance in the process of the calculation of DCP. When similar calculation was performed for the fowl, histidine requirement estimated from CP requirement of the layer (438.2 mg His/hen/day) was also higher than that of true requirement (305.5 mg His/hen/day) (Table 8). In spite of the fact that estimated requirements of histidine from DCP requirements tended to be higher than true requirements of

Table 7. Mean weights of liver, kidney and muscle and histidine production from histidinol in organs of some animals^a

Animal	Body weight	Liver		Kidney		Muscle		Total production ^d
		Wet weight ^b	Production ^c	Wet weight ^b	Production ^c	Wet weight ^b	Production ^c	
Swine	110 kg	1,750 g	1.39 g	450 g	0.20 g	33 kg ^e	0	1.59 g
Mouse	41.2 g	2.00 g	1.14 mg	0.58 g	0.087 mg	12.40 g	0.29 mg	1.52 mg
Fowl	2,083 g	29.70 g	37.30 mg	10.68 g	0.62 mg	833.2 g ^e	97.58 mg	135.50 mg
Wild duck	1,393 g	25.38 g	5.18 mg	4.77 g	2.29 mg	557.3 g ^f	99.78 mg	107.25 mg
Cattle	700 kg	7,000 g	14.30 g	1,300 g	1.28 g	300 kg	15.19 g	30.77 g

^a Numbers of the animals used for the assay of histidinol dehydrogenase activity were as follows: 5 swine, 21 mice, 3 fowls, 3 wild ducks and 5 cattle.

^b Average wet weight of the whole organ. In the case kidney, the value includes those of the two.

^c Production of histidine from histidinol by the whole organ (His/head/day)

^d Total production of histidine by liver, kidney and muscle (His/head/day)

^e Calculated in accordance with Okitani (1999)

^f Estimated as 40% of body weight

histidine in swine and fowl, this relationship is reverse in growing beef cattle (BW, 700 kg) (Table 8). This may paradoxically suggest that histidine requirement may be sufficiently filled in the steer with its self-production. Considering all these matters mentioned so far, it is hoped that a suggestion, that HLDase activity in animal organ will be an index for judging the essentiality of histidine, may be possible.

Human beings

Immediately after Rose and his colleagues discovered a new amino acid, threonine (McCoy et al., 1935; Meyer and Rose, 1936), and thereby they made it possible to find the importance of the individual amino acids giving "synthetic" rations to rats in which only proteins were replaced by amino acids, ten essential amino acids for immature rats were determined and reported (Rose, 1937). They also employed their concept and method concerning essential amino acids established with rats right away to human nutrition, and in the course of the experiment, they found that 'histidine is not necessary for the maintenance of nitrogen equilibrium in adult human subjects' (Rose et al., 1943). They asserted again this at a later time (Rose et al., 1951a, 1951b). Albanese et al. (1944) reported that human subject on a histidine-deficient diet remained in nitrogen equilibrium similar to the result of Rose et al. (1943), but lost weight. Thus they commented that they felt some hesitation in concluding that histidine was unessential for man (adult) until further studies clarified the picture. Incidentally, Snyderman, et al. (1963) reported that histidine appeared to be an essential amino acid for the young infant.

In the mean time, voices in objection to the view established by Rose and his colleagues that histidine was a non-essential amino acid for adult man have grown gradually in the field of medical science. Fuerst (1972) reported that histidine was an essential amino acid for

severe uremic patients based on the comparison of ^{15}N -distribution in amino acids in muscle and plasma protein between the uremic patients and healthy subjects after dosing ^{15}N -urea to them intravenously. In this experiment, ^{15}N was recovered in histidine of muscle and plasma protein of healthy volunteer, but not in that of uremic patients. Then Kopple and Swendseid (1975) firstly asserted that histidine was an essential amino acid not only for uremic man but also for normal adult man on the basis of the experimental results using four normal and three chronically uremic men. They observed that with ingestion of histidine-deficient diet, nitrogen balance gradually became negative, serum albumin decreased in six subjects, plasma and muscle histidine and hematocrit fell and serum iron rose. They pointed out that the absence of dietary histidine was associated with failure of normal erythropoiesis. Wixom and Anderson (1979) also demonstrated the incorporation of ^{15}N from $^{15}\text{NH}_4\text{Cl}$ into imidazole ring of histidine of plasma proteins and hemoglobin in a normal adult man during the histidine-free intake. At the same time, they observed in the man that the deletion of histidine for 27 days led to a mild positive nitrogen balance and decreases in plasma and urinary histidine to low stable levels. On the basis of these facts, they considered that this indicated limited synthesis of histidine in the adult man. Thus with regard to the essentiality of histidine for adult man, arguments have continued for as long a period as nearly 25 years as described above. It is generally considered that histidine is different from other essential amino acids in that substantial quantities exist in the form of tissue peptides such as carnosine (-alanyl histidine), anserine (-alanyl-1-methyl histidine) and balenine (-alanyl-3-methyl histidine) and hemoglobin (Fuller and Garlick, 1994). The release of histidine mainly from carnosine and hemoglobin allows experimental animals to maintain nitrogen equilibrium even on histidine-deficient diets (Fuller and Garlick, 1994).

Table 8. Histidine requirements of some animals

Animals	Requirement indicated in feeding standard ^a	Self-production ^b	True requirement ^c	Estimated requirement from DCP requirement ^d
Swine	5.5 g	1.59 g	7.09 g	8.87 g
Mouse	3.2 mg	1.52 mg	4.72 mg	-
Fowl	170 mg	135.5 mg	305.5 mg	438.2 mg
Wild duck	170 mg	107.25 mg	277.3 mg	-
Cattle	-	30.77 g	-	14.62 g

^a Swine, Japanese Feeding Standard for Swine (1998); mouse, Ishibashi (2002); fowl and wild duck, Japanese Feeding Standard for poultry (1997)

Unit: His/head/day

^b Production of histidine from histidinol by the animals (His/head/day) shown as 'total production' in table 6

^c The sum of histidine requirement shown in each Feeding Standard and self-production (His/head/day)

^d Histidine requirement estimated from DCP requirement (see footnote 'a') and histidine content in muscle (FAO, 1970) of each animal (His/head/day). DCP or CP requirement: swine, 328 g DCP/head/day (BW 70-115 kg, growing and finishing pigs); fowl, 16.7 g CP/hen/day (layers); cattle, 429 g DCP/head/day (BW 700 kg, DG 0.8 kg, finishing beef steer, from Japanese Feeding Standard for Beef Cattle, 2000). Histidine content: swine, 27.04 mg/g CP; fowl, 26.24 mg/g CP; cattle, 34.08 mg/g CP.

Nowadays histidine has been recognized as an essential amino acid for human adults (Metges et al, 1999).

One mysterious thing is that in spite of the long-term argument with regard to the essentiality of histidine for human adults, there have been no reports on the activity of histidinol dehydrogenase in animal tissues which had already been revealed in microorganisms (Andorn and Aronovitch, 1982) and plants (Nagai and Scheidegger, 1991), although incorporations of ^{15}N from $^{15}\text{NH}_4\text{Cl}$ and ^{15}N -urea in human adults and ^{14}C from H^{14}COOH into histidine in human fetal liver (Levy and Coon, 1952) were examined. The paper by Kotb and Wixom (1971) was the only report in which histidinol was used. They tested L-histidinol for its possible transformation to histidine in young rats. However, the results indicated that the rats could not convert L-histidinol to L-histidine at a sufficiently fast rate to be detected by growth measurements. Determination of HLDase activities in some organs of human adults will give a basic datum for judging essentiality of histidine more confidently.

ACKNOWLEDGEMENT

The author thanks Yoshimoto Meat Industry Co., Ltd., Miyazaki, Japan for giving the organs of cattle and swine to the researchers of the author's laboratory. The researches concerning histidinol dehydrogenase activity in animal tissues cited in this review were carried out mainly by Drs. Shaila Wadud and Mamun M. Or-Rashid and partially by undergraduate students, Nao Fukumitsu, Junko Kudo, Fumio Miyazaki and Masato Yokota in the author's laboratory with the financial supports of the Grant-in-Aid of Scientific Research (given to Ryoji Onodera, 2001-2002) and the Grant-in-Aid for JSPS Fellows relating to JSPS Postdoctoral Fellowship for Foreign Researchers (given to Shaila Wadud, 2001-2002) awarded from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Monbu-kagakusho) and the research grant from Ajinomoto Co., Inc., Japan.

REFERENCES

- Albanese, A. A., L. E. Holt Jr., J. E. Frankston and V. Irby. 1944. Observations on a histidine deficient diet in man. *Bull. Johns Hopkins Hosp.* 74:251-258.
- Allison, M. J. 1969. Biosynthesis of amino acids by ruminal microorganisms. *J. Anim. Sci.* 29:797-807.
- Andorn, N. and J. Aronovitch. 1982. Purification and properties of histidinol dehydrogenase from *Escherichia coli*. *B. J. Gen. Microbiol.* 128: 579-584.
- Bergen, W. G., D. B. Purser, J. H. Cline. 1968. Determination of limiting amino acids of rumen-isolated microbial proteins fed to rat. *J. Dairy Sci.* 51: 1698-1700.
- Buttery, P. J. and A. N. Foulds. 1985. Amino acid requirements of ruminants. In: *Recent Advances in Animal Nutrition-1985.* (Haresign, W. and D. J. A. Cole eds. Butterworths. London.) pp. 257-271.
- Czerkawski, J. W. 1976. Chemical composition of microbial matters in the rumen. *J. Sci. Food Agric.* 27:621-632.
- FAO. 1970. Amino Acid Content of Foods and Biological Data on Proteins in *FAO Nutritional Studies, No. 24.* (ed by Food Policy and Food Science Service, Nutrition Division, FAO, Rome, 1970).
- Fuller, M. F. and P. J. Garlick. 1994. Human amino acid requirements: Can the controversy be resolved? *Ann. Rev. Nutr.* 14: 217-241.
- Fuerst, P. 1972. ^{15}N -Studies in severe renal failure. II. Evidence for the essentiality of histidine. *Scand. J. Clin. Lab. Invest.* 30:307-312.
- Ishibashi, T. 2002. 'Dobutsu Eiyo Shikhenho (Methods for Animal Nutrition Experiments)' ed. by T. Ishibashi. Yokendo Ltd. Tokyo.
- Japanese Feeding Standard for Poultry. 1997. Agriculture, Forestry and Fisheries Research Council Secretariat (ed). MAFF. Central Association of Livestock Industry. Tokyo.
- Japanese Feeding Standard for Swine. 1998. Agriculture, Forestry and Fisheries Research Council Secretariat (ed). MAFF. Central Association of Livestock Industry. Tokyo.
- Japanese Feeding Standard for Beef Cattle. 2000. Agriculture, Forestry and Fisheries Research Council Secretariat (ed). MAFF. Central Association of Livestock Industry. Tokyo.
- Kopple, J. D. and M. E. Swendseid. 1975. Evidence that histidine is an essential amino acid in normal and chronically uremic man. *J. Clin. Invest.* 55:881-891.
- Kotb, A. R. and R. L. Wixom. 1971. Inability of the growing rat to substitute L-histidinol for L-histidine. *J. Nutr.* 101:1493-1498.
- Land, H. and A. I. Virtanen. 1959. Ammonium salts as nitrogen source in the synthesis of protein by the ruminant. *Acta Chem. Scand.* 13:489-496.
- Levy, L., and M. J. Coon. 1952. Histidine synthesis in yeast and human liver. *Fed. Proc.* 11:248(abstract)
- McLaughlan, J. M. 1964. Blood amino acid studies. 5. Determination of the limiting amino acid in diets. *Canadian J. Biochem.* 42:1353-1360.
- McCoy, R. H., C. E. Meyer, W. C. Rose. 1935. Feeding experiments with mixture of highly purified amino acids. VIII. Isolation and identification of a new essential amino acid. *J. Biol. Chem.* 112:283-302.
- Metges, C. C., K. J. Petzke and V. R. Young. 1999. Dietary requirements for indispensable amino acids in adult humans: New concepts, methods of estimation, uncertainties and challenges. *Mannu. Nutr. Metab.* 43:267-276.
- Meyer, C. E., W. C. Rose. 1936. The spatial configuration of α -amino- β -hydroxy-*n*-butyric acid. *J. Biol. Chem.* 115:721-729.
- Morimoto, H. 1974. *Shiryogaku (Feeds and Feeding).* Yokendo. Tokyo.
- Nagai, A. and A. Scheidegger. 1991. Purification and characterization of histidinol dehydrogenase from cabbage. *Arch. Biochem. and Biophys.* 284: 127-132.
- Nimrick, K., E. E. Hatfield, J. Kaminski and F. N. Owens. 1970. Qualitative assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. *J. Nutr.*

- 100:1293-1300.
- Okitani, A. 1999. 'Niku no Kagaku (Meat Science)' ed. by A. Okitani. Asakura. Tokyo.
- Onodera, R. and K. Koga. 1987. Effect of inhabitation by rumen protozoa on the nutritive value of protein in rumen contents. *Agric. Biol. Chem.* 51:1417-1424.
- Onodera, R., S. Wadud and M. M. Or-Rashid. 2001. Is histidine an essential amino acid in ruminant animals? *Asian-Aust. J. Anim. Sci.* 14 (Special Issue):123-130.
- Richardson, C. R. and E. E. Hatfield. 1978. The limiting amino acids in growing cattle. *J. Anim. Sci.* 46:740-745.
- Rose, W. C. 1937. The nutritive significance of the amino acids and certain related compounds. *Science* 86:298-300.
- Rose, W. C., W. J. Haines, J. E. Johnson and D. T. Warner. 1943. Further experiments on the role of the amino acids in human nutrition. *J. Biol. Chem.* 148:457-458.
- Rose, W. C., W. J. Haines, D. T. Warner and J. E. Johnson. 1951a. The amino acid requirements of man. II. The role of threonine and histidine. *J. Biol. Chem.* 188:49-58.
- Rose, W. C., W. J. Haines and D. T. Warner. 1951b. The amino acid requirements of man. III. The role of isoleucine: additional evidence concerning histidine. *J. Biol. Chem.* 193:605-612.
- Sauer, F. D., J. D. Erfle and S. Mahadevan. 1975. Amino acid biosynthesis in mixed rumen cultures. *Biochem. J.* 150:357-372.
- Snyderman, S. E., A. Boyer, E. Roitman, L. E. Holt, JR. and P. H. Prose. 1963. The histidine requirement of the infant. *Pediatrics* 31:786-801.
- Storm, E. and E. R. Orskov. 1984. The nutritive value of rumen Micro organisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. *Br. J. Nutr.*, 52:613-620.
- Virtanen, A. I. 1966. Milk production of cows on protein-free feed. *Science* 153:1603-1614.
- Voeltz, W. 1920. Der Ersatz des Nahrungseiweisses durch Harnstoff beim wachsenden Wiederkaeuer. Der Futter des nach dem Beckmannschen Verfahren aufgeschlossenen Strohs und der Spreu. *Biochem. Z.* 102:151-227.
- Wadud, S., R. Onodera and M. M. Or-Rashid. 2001a. Studies on the possibility of histidine biosynthesis from histidinol, imidazolepyruvic acid, imidazoleacetic acid, and imidazolelactic acid by mixed ruminal bacteria, protozoa and their mixture in vitro. *Appl. Microbiol. Biotechnol.* 55:219-225.
- Wadud, S., R. Onodera, and M. M. Or-Rashid. 2001b. Synthesis of histidine in liver and kidney of cattle and swine. *Anim. Sci.* 72:253-256.
- Wadud, S., M. M. Or-Rashid and R. Onodera. 2002. Method for determination of histidine in tissues by isocratic high-performance liquid chromatography and its application to the measurement of histidinol dehydrogenase activity in six cattle organs. *Journal of Chromatography B*, 767:369-374.
- Wixom, R. L. and H. L. Anderson. 1979. Histidine – Limited synthesis in normal, adult man. *Histidine: Metab., Clin. Aspects, Ther. Use., Internatl. Workshop*, 1st, pp. 19-33.