Jap. J. vet. Res., **26**, 68~73 (1978)

INCORPORATION OF ^{15}N ADMINISTERED TO GERMFREE AND SPF PIGLETS AS ^{15}N-UREA INTO AMINO ACIDS OF HYDROLYZED LIVER AND MUSCLE PROTEINS

Eisaburo DEGUCHI, Masayoshi NIIYAMA, Katsumoto KAGOTA
and Shigeo NAMIOKA

Department of Veterinary Internal Medicine
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

(Received for publication, September 4, 1978)

^{15}N-urea was administered three times with a diet containing urea to two germfree and one specific pathogen free (SPF) piglets. They were killed on the fifth day after the last administration of ^{15}N-urea. The ^{15}N concentration in the amino acids isolated from the hydrolyzed trichloroacetic acid precipitates of the liver and muscle was determined. In SPF piglet, the ^{15}N was incorporated into all of the essential and nonessential amino acids, except threonine in both the liver and muscle and except histidine in the muscle. The ^{15}N concentration of each of the nonessential amino acids was higher than that of most of the essential amino acids in the liver and muscle. On the other hand, an excess of ^{15}N was not detected in the pooled essential and nonessential amino acids nor in the ammonia and glutamic acid in germfree piglets. It was concluded that the ammonia nitrogen converted from urea by the action of intestinal-bacterial urease in the gastrointestinal tract was utilized for the synthesis of all of the essential and nonessential amino acids, except threonine, in the pigs.

INTRODUCTION

It has been suggested that the feeding non-protein nitrogen (NPN) is mainly used as a source of nitrogen for the synthesis of nonessential amino acids, and that urea is a less effective form than other ammonium salts or certain nonessential amino acids, such as the sources of NPN in rats (ROSE et al.) and in chicks (FEATHERSTON et al.). On the other hand, it has been reported that the ^{15}N from ingested ^{15}N-urea was incorporated into the amino acids, including some essential ones, isolated from the hydrolyzed carcass protein in rats (ROSE & DEKKER), and from the plasma and muscle proteins in man (FÜRST and GIORDANO et al.).

In a previous study (DEGUCHI et al.), it was elucidated that intestinal flora is indispensable for utilizing the nitrogen of urea for protein synthesis. If urea nitrogen were used for the synthesis not only of the nonessential amino acids but also of the essential amino acids in pigs, the recycled urea in the entero-hepatic circulation could be regarded as an important nitrogen source for their nutrition.

The present study was carried out to investigate the synthesis of amino acids from urea nitrogen, using the criteria of the incorporation of ^{15}N into amino acids isolated from the hydrolyzed liver and muscle proteins after the administration of ^{15}N-urea to germfree and specific pathogen free (SPF) piglets.

MATERIALS AND METHODS

Animal and diet

Three germfree piglets (GF pig 1, GF pig 3 and GF pig 4) and one SPF piglet from the same litter (Large White) used in a previous study (DEGUCHI et al.), were employed for this experiment. For 8 days following birth, all piglets were fed an optimal volume of autoclaved (at 121°C for 30 minutes) fluid artificial milk (SPF-lac; Borden Chemical, Borden Inc., Norfolk, Virginia) containing 30 % crude protein (dry-matter basis). Next, all piglets, except GF pig 1, were given a basal diet (21.9 % crude protein and 965.4 kcal gross energy/kg) with 3.62 g urea/kg basal diet (equivalent to 5.8 % crude protein) for days 9 though 21 after birth. GF pig 1 was given only the basal diet for days 9 though 20 after birth. The dietary conditions and the methods for rearing the germfree and SPF piglets were described in detail in a previous report (DEGUCHI et al.).

Administration of ^{15}N-urea

Two germfree (GF pig 3 and GF pig 4) and one SPF piglets given the urea-supplemented diet, were administered 0.478 g ^{15}N-urea (51.7 atom % ^{15}N)/dose orally with the diet, every second day for a total of three times (12th, 14th and 16th days following birth). GF pig 1 was not given ^{15}N-urea, but was used for measuring the natural abundance of ^{15}N.

Isolation of amino acids

Five days after the last administration of ^{15}N-urea, the liver and muscle (*M. gluteus*) were collected after bleeding under a fluothane (Takeda Chemical Industries, Ltd., Osaka) anesthetized condition. The trichloroacetic acid (TCA) precipitates of the liver and muscle prepared by the methods described by DEGUCHI et al. and FÜRST & JONSSON, were dried and hydrolyzed in vaccum hydrolyzing tubes with 6.0 N HCl at 110±1°C for 24 hours. The hydrolyzed solutions were evaporated to dryness by a rotary evaporator, and resuspended in a 0.2 N (Na concentration) sodium citrate buffer, pH 2.2. The individual 17 amino acids and ammonia, except tryptophan, were separated by using an amino acid analyzer (Model KLA-5; Hitachi, Ltd., Tokyo) and collected in test tubes using a fraction collector (Model SF-400L; Toyo Chemical Industry, Co., Ltd., Tokyo).

Determination of ^{15}N concentration

The concentration of ^{15}N was determined in the individual amino acids and ammonia or in the pooled essential amino acids (pooled EAA ; Arg, His, Ile, Leu, Lys, Met, Phe, Thr and Val) and in the pooled nonessential amino acids (pooled Non-EAA ; Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr). The determination of ^{15}N concentration was carried out by using a mass spectrometer after the digestion by a modified macro-Kjeldahl method (HOROWITZ). This procedure was described previously (DEGUCHI et al.).

RESULTS AND DISCUSSION

Role of intestinal flora on synthesis of amino acids from urea nitrogen

In a previous study (DEGUCHI et al.), it was demonstrated that intestinal flora is indispensable for utilizing the nitrogen of urea for protein synthesis, since the incorporation of ^{15}N from ingested ^{15}N-urea into various tissue proteins was found in the SPF piglets but not in the germfree piglets.

A similar result on the incorporation of ^{15}N into amino acids was obtained in this experiment: the ^{15}N from ^{15}N-urea administered to SPF piglet was incorporated into

TABLE 1 *Concentration of ^{15}N (atom % excess ^{15}N) in pooled EAA, pooled Non-EAA and ammonia in liver and muscle*[1]

FRACTION	GERMFREE PIGLETS		SPF PIGLET[2]
	PIG 3	PIG 4	PIG 11
Liver			
TCA precipitate[3]	0.001[7]	None	0.190
Pooled EAA[4]	None	None	0.113
Pooled Non-EAA[5]	None	None	0.246
Ammonia	None	None	0.258
Pooled EAA/Pooled Non-EAA[6]	—	—	0.46
Muscle (*M. gluteus*)			
TCA precipitate	None	None	0.100
Pooled EAA	None	None	0.066
Pooled Non-EAA	None	0.001[7]	0.102
Ammonia	None	0.001[7]	0.279
Pooled EAA/Pooled Non-EAA	—	—	0.65

[1]Natural abundance of ^{15}N was 0.367 atom % ^{15}N.
[2]Specific pathogen free piglet
[3]Concentration of ^{15}N in the trichloroacetic acid (TCA) precipitates of the liver and muscle was obtained from data obtained in a previous study (DEGUCHI et al.).
[4]Pooled EAA ; Arg, His, Ile, Leu, Lys, Met, Phe, Thr and Val
[5]Pooled Non-EAA ; Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr
[6]Ratio of pooled EAA to pooled Non-EAA on the basis of ^{15}N concentration
[7]This value did not show a significant increase of ^{15}N excess in a mass spectrometer ($\pm 0.3\% \times 0.367$ natural abundance of $^{15}N = \pm 0.0011$ atom % excess ^{15}N).

the pooled EAA and the pooled Non-EAA and into ammonia, isolated from the hydrolyzed TCA precipitates of the liver and muscle; however, an excess of ^{15}N was not detected in any of these fractions in two germfree piglets (tab. 1). Moreover, glutamic

TABLE 2 *Concentration of ^{15}N (atom % excess ^{15}N) in glutamic acid in liver*[1]

FRACTION	GERMFREE PIGLETS		SPF PIGLET[2]
	PIG 3	PIG 4	PIG 11
Glutamic acid	None	0.001[3]	0.260

[1]See footnote 1 in table 1.
[2]See footnote 2 in table 2.
[3]See footnote 7 in table 1.

TABLE 3 *Concentration of ^{15}N in amino acids isolated from hydrolyzed trichloroacetic acid precipitates of liver and muscle in SPF piglet*

AMINO ACIDS	^{15}N CONCENTRATION (ATOM % EXCESS ^{15}N)[1]	
	LIVER	MUSCLE
Essential		
Arginine	0.248	0.110
Histidine	0.003	None
Isoleucine	0.108	0.098
Leucine	0.120	0.104
Lysine	0.029	0.002
Methionine	0.045	0.027
Phenylalanine	0.030	0.020
Threonine	0.001[2]	0.001[2]
Valine	0.107	0.104
Nonessential		
Alanine	0.283	0.133
Aspartic acid	0.263	0.103
Cystine	0.170	0.091
Glutamic acid (Glu)	0.260	0.122
Glycine	0.218	0.089
Proline+Glu	0.216	0.118
Serine	0.175	0.106
Tyrosine	0.170	0.066

[1]See footnote 1 in table 1.
[2]See footnote 7 in table 1.

acid, which is the first amino acid to be formed from ammonia nitrogen in liver (MEISTER and SALLACH & FAHIEN), never contained an excess of ^{15}N in the germfree piglets (tab. 2).

From these results, it was clear that urea is a possible source of the nitrogen needed for the synthesis of amino acids only when the urea is converted into ammonia nitrogen by the action of intestinal-bacterial urease in the gastrointestinal tract.

Synthesis of amino acids from urea nitrogen in SPF piglet

A finding that the individual nonessential amino acids had higher ^{15}N concentrations than most of the essential amino acids (tab. 3) was in accord with the reports on rats (ROSE & DEKKER) and on man (FÜRST and GIORDANO et al.), and it was in agreement with hypothesis that urea may be mainly used as a source of nitrogen for the synthesis of nonessential amino acids (FEATHERSTON et al. and ROSE et al.). The ratio of pooled EAA to pooled Non-EAA on the basis of the ^{15}N concentration was higher in the muscle (0.65) than in the liver (0.46) (tab. 1). This was due to the finding that the ^{15}N concentrations in valine, leucine and isoleucine were higher than those in some of the nonessential amino acids in muscle, though the ^{15}N concentrations in these amino acids were lower than those in all of the nonessential amino acids in liver (tab. 3).

The high ^{15}N concentrations in alanine, aspartic acid and glutamic acid in liver and in alanine and glutamic acid in muscle (tab. 3) were consistent with the central role of these amino acids for distribution of ammonia nitrogen to other amino acids in each tissue (MEISTER and SALLACH & FAHIEN).

Among the essential amino acids, the highest ^{15}N concentration was found in arginine, which had a higher ^{15}N concentration than some of the nonessential amino acids (tab. 3); arginine is synthesized in the urea cycle in the liver (SCULL & ROSE), but not at a necessary rate for maximum growth in rats (ROSE) and in pigs (MERTZ et al.).

In other essential amino acids, the ^{15}N was incorporated into leucine, isoleucine, valine, methionine, phenylalanine, lysine and histidine (arranged in a descending order of ^{15}N concentration) in the liver, and into leucine, valine, isoleucine, methionine, phenylalanine and lysine in the muscle, respectively (tab. 3). However, an excess of ^{15}N was not detected in threonine in both the liver and muscle, and in histidine in the muscle (tab. 3). These results, in addition to the finding that the ^{15}N was incorporated into all of the nonessential amino acids in the liver and muscle (tab. 3), indicated that the ammonia nitrogen from urea was used for the synthesis of all of the essential and nonessential amino acids, with the exception of threonine.

FÜRST reported that the ^{15}N from ingested ^{15}N-urea to man was not incorporated into lysine and threonine in plasma and muscle proteins. On the other hand, GIORDANO et al. found the incorporation of ^{15}N into these two amino acids isolated from plasma albumin in man after the administration of ^{15}N-urea.

Histidine, lysine and threonine had the lowest ^{15}N concentrations, or did not increase in ^{15}N excess (tab. 3), indicating that the ammonia nitrogen of urea was not used sufficiently for the synthesis of these amino acids as compared to other essential amino acids in pigs.

REFERENCES

1) DEGUCHI, E., NIIYAMA, M., KAGOTA, K. & NAMIOKA, S. (1978): *J. Nutr.*, **108**, 1572
2) FEATHERSTON, W. R., BIRD, H. R. & HARPER, A. E. (1962): *Ibid.*, **78**, 198
3) FÜRST, P. (1972): *Scand. J. clin. Lab. Invest.*, **30**, 307
4) FÜRST, P. & JONSSON, A. (1971): *Acta chem. scand.*, **25**, 930
5) GIORDANO, C., DEPASCALE, C., BALESTRIERI, C., CITTADINI, D. & CRESCENZI, A. (1968): *Am. J. clin. Nutr.*, **21**, 394
6) HOROWITZ, W. (1965): Official methods of analysis, 10 ed., 744, Washington: Association of Official Agricultural Chemists (AOAC)
7) MEISTER, A. (1956): *Physiol. Rev.*, **36**, 103
8) MERTZ, E. T., BEESON, W. M. & JACKSON, H. D. (1952): *Archs Biochem. Biophys.*, **38**, 121
9) ROSE, W. C. (1938): *Physiol. Rev.*, **18**, 109
10) ROSE, W. C., SMITH, L. C., WOMACK, M. & SHANE, M. (1949): *J. biol. Chem.*, **181**, 307
11) ROSE, W. C. & DEKKER, E. E. (1956): *Ibid.*, **223**, 107
12) SALLACH, H. J. & FAHIEN, L. A. (1969): Metabolic pathways, 3 ed., 1, New York: Academic Press
13) SCULL, C. W. & ROSE, W. C. (1930): *J. biol. Chem.*, **89**, 109