6. Ammonia- ^{15}N Absorption and its Transfer to Amino-N by the Small Intestinal Sac of Rats *in situ*

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The use of non-protein nitrogen (NPN) by monogastric animals has been studied,²⁾⁻⁶⁾ and ammonia-N ($\text{NH}_3\text{-N}$) is known to be used for non-essential amino acid synthesis under the condition of feeding a low protein diet. However there is little information concerning the organ in which this occurs and how much $\text{NH}_3\text{-N}$ is transferred to amino-N. We have already studied NH_3 absorption and $\text{NH}_3\text{-N}$ transfer to amino-N at the intestinal wall where ammonium-N ($\text{NH}_4\text{-N}$) in the diet is first absorbed into the body.

From the result of *in vitro* experiments using the everted sac of the rat's intestine,^{7),8)} the following observations were obtained: 1) NH_3 was passively transported; 2) 73% of the serosal nitrogen derived from NH_3 in the mucosal fluid was found as $\text{NH}_3\text{-N}$, 21% as α -amino-N and 6.5% as amide- ^{15}N ; and 3) N from NH_3 was incorporated into non-essential amino acids in the following order; alanine>glutamine>arginine>glutamic acid, and uptake of N from NH_3 to essential amino acids was negligible.

The purpose of this report was to determine the response to NH_3 at the intestinal wall of the rat *in situ*. Incorporation of ^{15}N into amino acids and their amides in the blood of the portal vein after injection of $\text{NH}_4\text{-}^{15}\text{N}$ into the rat's intestinal tract *in situ* was elucidated.

Materials and methods. Nineteen adult male rats of the Wistar strain weighing 220–280 g were fed a diet (DAC diet) which contained 2.7% diammonium citrate (DAC) and 3.0% essential amino acid mixture as a nitrogen source for 6 days. Other dietary ingredients were the same as shown in the previous paper.⁶⁾ After adaptation to the DAC diet, the rats were fasted for one day. Under anesthesia with ether and nembutal, the abdomen of each rat was opened by a midline incision, and the greater omentum followed by the distal end of the ileum was ligated. The proximal point of the

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jejunum was cut, and through this cut the test medium was injected into the intestinal tube; then this point was ligated. Immediately after (zero time) and ten min after this operation, the blood from the portal vein was collected. Test medium used was a Krebs-Ringer biphosphate buffer containing 2 mM DAC (95.8 atom % excess ^{15}N), 4 mN eq. of an essential amino acid mixture (3.8% tryptophan, 3.8% histidine, 7.5% lysine, 13.5% leucine, 23.1% isoleucine, 10.2% phenylalanine, 12.4% methionine, 9.1% threonine, and 16.7% valine) and 28 mM glucose, pH 7.4 at 37°C . The blood collected from the portal vein was centrifuged at $2000\times g$ for 30 min. The supernatant, plasma, had trichloroacetic acid (TCA) added to a final concentration of 5%, and has then centrifuged at $2000\times g$ for 20 min. TCA was removed with ethyl ether and $\text{NH}_3\text{-N}$, amide-N and α -amino-N in the plasma were separated as described previously.⁷⁾ Each amino acid was separated by thin layer chromatography. ^{15}N abundance was measured by the spectroscopic analysis previously reported.⁷⁾

To determine the effect of adaptation to DAC diet on the surface of the intestinal wall, the mucosal surface of this wall was observed by the scanning electron microscope (SEM, JSM U-3, JEOL).

Results and discussion. The time course of $\text{NH}_3\text{-N}$, amide-N and α -amino-N in plasma after injection of $\text{NH}_4\text{-N}$ into the intestinal lumen *in situ* is shown in Fig. 1. The concentration of $\text{NH}_3\text{-N}$, amide-

Fig. 1. Time course of $\text{NH}_3\text{-N}$, amide-N and α -amino-N in plasma of portal blood after injection of $\text{NH}_3\text{-N}$ into intestinal lumen *in situ*. $\circ$ — $\circ$: $\text{NH}_3\text{-N}$. $\bullet$ — $\bullet$: Amide-N. $\times$ — $\times$: α -amino-N.

N and α -amino-N was relatively constant for 10 min. The time course of ^{15}N derived from $\text{NH}_3\text{-}^{15}\text{N}$ injected into the intestinal lumen is shown in Fig. 2. The concentration of $\text{NH}_3\text{-}^{15}\text{N}$ and amide- ^{15}N was almost constant, but α -amino- ^{15}N derived from $\text{NH}_3\text{-}^{15}\text{N}$ increased. This means that NH_3 in the intestinal lumen was utilized as the source of α -amino-N by the rat. Table I shows N concentration, ^{15}N abundance and ^{15}N concentration in plasma at zero time and 10 min after injection of $\text{NH}_4\text{-}^{15}\text{N}$ into the lumen. The values at zero time show only the function of the intestinal tube, while the values after 10 min

Fig. 2. Time course of ^{15}N concentration of $\text{NH}_3\text{-N}$, amide-N and $\alpha\text{-amino-N}$ in plasma of portal blood from $\text{NH}_4\text{-}^{15}\text{N}$ injected into intestinal lumen *in situ*. $\circ\text{---}\circ$: $\text{NH}_3\text{-}^{15}\text{N}$. $\bullet\text{---}\bullet$: Amide- ^{15}N . $\times\text{---}\times$: $\alpha\text{-amino-}^{15}\text{N}$.

Table I. N concentration, ^{15}N abundance and ^{15}N concentration in plasma after $\text{NH}_4\text{-N}$ was injected into intestinal lumen (*in situ*)

	0 min	10 min
N concentration ($\mu\text{g N/ml plasma}$)		
$\text{NH}_3\text{-N}$	4.70	4.20
Amide-N	5.77	5.90
$\alpha\text{-amino-N}$	73.4	81.0
^{15}N abundance (atom % excess)		
$\text{NH}_3\text{-}^{15}\text{N}$	22.5	14.2
Amide- ^{15}N	1.55	2.17
$\alpha\text{-amino-}^{15}\text{N}$	0.24	0.85
^{15}N concentration ($\mu\text{g }^{15}\text{N/ml plasma}$)		
$\text{NH}_3\text{-}^{15}\text{N}$	0.981	0.639
Amide- ^{15}N	0.096	0.137
$\alpha\text{-amino-}^{15}\text{N}$	0.189	0.737

show additionally the effect of other organs such as the liver. ^{15}N abundance and ^{15}N concentration at zero time *in situ* agreed with the result of the absorption experiment using the everted sac of the rat's intestine previously reported.⁷⁾ ^{15}N percentages in plasma of NH_3 , amide and $\alpha\text{-amino}$ calculated from the values in Table I were 77, 8 and 15% at zero time and 42, 9 and 48% after 10 min respectively.

Table II shows ^{15}N incorporation from $\text{NH}_4\text{-}^{15}\text{N}$ into each amino acid- ^{15}N . The largest quantity of ^{15}N was incorporated into glutamine and next into alanine, and the incorporation rate of the other amino acids was negligible. In the *in vitro* experiment,⁸⁾ the ^{15}N incorporation rate was in the following order; alanine>glutamine>glutamic acid, and the incorporation rate of the other amino acids was negligible. From these results, $\text{NH}_4\text{-}^{15}\text{N}$ injected into the intestinal lumen was shown to be passively transported and 23% of transported $\text{NH}_3\text{-N}$ was transferred to non- $\text{NH}_3\text{-N}$, mainly glutamine and alanine. The intestinal wall has the ability of transferring N from NH_3 to glutamic

Table II. ^{15}N incorporation of amino acids
from $\text{NH}_4\text{-}^{15}\text{N}$ injected into intestinal
lumen *in situ* (atom % excess)

Amino acid	0 min	10 min
NEAA		
Glu (NH_2)	1.13	2.86
Ala	0.39	1.15
Glu	0.04	0.98
Asp (NH_2) + Gly	0.06	0.20
Ser	0.02	0.05
Asp	0.02	0.06
Arg	0.00	0.33
Cys	0.04	—
EAA		
Lys	0.02	0.02
Val	0.02	0.04
Met + Leu + Ile	0.01	0.00

0 min: Zero time, immediately after injection.
10 min: 10 minutes after injection. NEAA:
Non-essential amino acid. EAA: Essential
amino acid.

Fig. 3. Scanning electron micrograph (SEM) of jejunal surface of rat fed commercial diet. $\times 300$.

acid from the result of the *in vitro* experiment,⁸⁾ but this ability could not be evaluated because ^{15}N incorporation to glutamic acid was negligible *in situ* at zero time. ^{15}N incorporation into glutamic acid is due to L-glutamic dehydrogenase, while ^{15}N incorporation into alanine is thought to be due to both L-glutamic dehydrogenase and glutamic pyruvic transferase because alanine dehydrogenase was found in bacteria⁹⁾ but was not reported in mammalian tissues. Since ^{15}N

Fig. 4. SEM of a part of the jejunum surface of rat fed the commercial diet. $\times 1000$.

Fig. 5. SEM of jejunum surface of rat fed the NPN diet. $\times 300$.

abundance of glutamic acid was considerably lower than that of alanine, the intestinal tissue was thought to have a compartment which has a higher rate of ^{15}N incorporation into glutamic acid.

To determine the effect of the DAC diet on the intestinal wall, the mucosal surface of this wall was observed by a scanning electron microscope. Figs. 3 and 4 show the mucosal surface of intestinal wall of a rat fed a commercial diet (Oriental NMF), and Figs. 5 and 6 show that of a rat adapted to the DAC diet for 7 days. Leaf shaped mucosa of the rat fed the DAC diet were more regular than those of the rat fed the commercial diet. Many mucous plugs from goblet cell

orifices were observed on the mucosal surface of the intestinal wall of rats fed both diets. The surface of the former rat fed the commercial diet seemed to be rough, while that of the latter rat fed the DAC diet was relatively smooth and rounded dome-shaped epithelial cells were clearly observed. Celiac jejunal mucosa with smaller and more rounded dome-shaped epithelial cells was suggested by Asquith *et al.* to show increased cell loss and turn over.¹⁾ The mucosal surface of the latter rat also had rounded dome-shaped cells; therefore this mucosa might have increased the turn over of the epithelial cells.

Fig. 6. SEM of a part of jejunum surface of rat fed the NPN diet. $\times 1000$.

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