Note

The Fate of Urea-N by the Anaerobic Culture of Cecal Microbes of Rats

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Not only ruminants but also monogastric animals have been known to use non-protein nitrogen for their body protein synthesis.¹⁾ Urea is thought to be the metabolic end product of mammals, but is reused by the host through the action of intestinal microbes under low protein conditions.²⁾ Urease activity of many intestinal strains was examined by Suzuki³⁾ and one of us found ammonia assimilating bacteria such as *Bacteroides rumenicola* strains.⁴⁾ Our objective is to find the effects of rat cecal

microbes on the fate of urea in anaerobic cultures.

The composition of the medium was: 0.2% trypticase BBL; 0.5% yeast extract; 0.5% L-cysteine HCl; 0.05% saccharose; 0.05% cellobiose; 0.05% soluble starch; 3.75% mineral solution (0.78% K_2HPO_4 ; 0.47% KH_2PO_4 ; 1.18% NaCl; 1.20% $(NH_4)_2SO_4$; 0.12% $CaCl_2$; 0.25% $MgSO_4$); 0.0001% hemin; 0.0001% resazurin; 0.02% tween 80; 0.05% ascorbic acid; 0.4% Na_2CO_3 ; 0.31% volatile fatty acid mixture (36 ml of acetic acid, 1.8 ml of isobutyric acid, and 2.0 ml each of *n*-valeric, DL-2-methyl butyric, and isovaleric acids); 0.1% urea; and CO_2 gas phase. Urea labeled with 30.0 atom% ^{15}N was used.

Some cecal contents of 3 rats were anaerobically added to the above medium bottled in 100-ml amounts. The final concentration of cecal contents in the medium was 0.02%. The medium was anaerobically incubated at 37°C for 48 hr. The animals used were male rats of the Wistar strain, 6 weeks old, their body weights ranging from 262 to 272 g and fed on a commercial stock diet (Type MF, Oriental Yeast Co., Ltd., Tokyo) with 2% urea added for 2 weeks.

Figure 1 shows the changes in total bacterial counts by direct microscopic clump counts stained by methyl violet 6B. Table I shows the bacterial composition after 48 hr of incubation. Bacterial cultivation and counting methods were described in our previous paper.⁵⁾ The total bacterial

FIG. 1. Changes in Total Bacterial Counts in Anaerobic Culture of Cecal Contents of Rats.

FIG. 2. Changes in Nitrogen Compounds during Anaerobic Culture of Cecal Contents of Rats. ○, urea; ×, NH_3 ; ●, protein nitrogen; ▲, non-protein nitrogen—(urea-N + NH_3 -N).

TABLE I. BACTERIAL COMPOSITION AFTER 48-HR INCUBATION OF CECAL CONTENTS OF RATS (Log number/ml)

Bacterial group	0 hr	48 hr
<i>Enterobacteriaceae</i>	4.4	7.6
<i>Streptococcus</i>	8.3	8.6
Yeast	3.3	—
<i>Lactobacillus</i>	7.1	8.6
<i>Bacteroidaceae</i>	7.3	9.2
<i>Clostridium perfringens</i>	—	—
<i>Clostridium</i> others	8.3	—
<i>Bifidobacterium</i>	—	7.6
<i>Eubacterium</i>	7.3	9.0
<i>Peptococcaceae</i>	8.8	8.8
Curved rods	8.7	7.3
<i>Veillonella</i>	—	7.3

—, under 1.3.

TABLE II. FREE AMINO ACID PATTERN CAUSED BY ANAEROBIC INCUBATION OF CECAL CONTENTS OF RATS IN MEDIUM WITH ADDED UREA ($\mu\text{g/ml}$)

Amino acid	0 hr	48 hr
Lys	88.6	1.4
His	9.7	—
Arg	43.4	—
Asp	19.3	1.5
Thr	16.0	—
Ser	24.7	—
Glu	37.4	2.3
Pro	9.9	—
Gly	8.3	0.6
Ala	23.4	1.4
Cys	94.1	6.2
Val	32.6	77.9
Met	26.1	5.8
Ile	26.2	46.7
Leu	81.0	91.0
Tyr	13.0	—
Phe	50.5	—

counts, *Enterobacteriaceae*, *Bacteroidaceae*, and *Eubacterium* were increased during the 48 hr of this anaerobic incubation. Figure 2 shows the changes in nitrogen compounds during 48 hr of incubation. The methods for chemical analysis of ammonia nitrogen ($\text{NH}_3\text{-N}$), non-protein nitrogen (NPN), and protein nitrogen (PN) were the same as those described previously.⁵⁾ Urea was measured by Conway's method using urease.⁶⁾ Urea decreased promptly and $\text{NH}_3\text{-N}$ and PN increased. NPN exclusive of urea-N and $\text{NH}_3\text{-N}$ reached a peak at 12 hr, and then decreased gradually. From this result, 22% of the urea was calculated to be decomposed to $\text{NH}_3\text{-N}$ and 40%

TABLE III. INCORPORATION OF ^{15}N FROM ^{15}N -UREA INTO NITROGEN COMPOUNDS BY CECAL BACTERIA OF RATS

	Nitrogen compound	0 hr	48 hr
N (mg/100 ml)	Protein nitrogen	3.50	7.52
	Nonprotein nitrogen	79.64	80.51
	NH_3	1.69	55.20
	Lys	1.67	0.03
	Val	0.37	0.92
	Leu + Ile	1.10	1.52
^{15}N (atom% excess)	Protein nitrogen	1.20	6.59
	Nonprotein nitrogen	14.03	11.88
	NH_3	5.67	17.27
	Lys	1.19	ND
	Val	1.14	1.67
	Leu + Ile	1.14	3.89
^{15}N (mg $^{15}\text{N/ml}$)	Protein nitrogen	0.05	0.53
	Nonprotein nitrogen	11.96	10.25
	NH_3	0.10	10.23
	Lys	0.02	ND
	Val	0.00	0.02
	Leu + Ile	0.01	0.06

Lys- ^{15}N after 48 hr was not detected because its amount was too small.

of the urea to be assimilated to this NPN exclusive of urea-N and $\text{NH}_3\text{-N}$ after 12 hr.

Table II shows the free amino acid pattern after 48 hr of incubation. Amino acids were measured using a Hitachi 835 amino acid analyzer. The concentrations of branched-chain amino acids were increased and those of the other amino acids were decreased after 48 hr.

Table III summarizes the incorporation of ^{15}N from urea- ^{15}N to some nitrogen compounds by rat cecal bacteria. ^{15}N was analyzed by the optical spectroscopic analysis as reported previously.⁷⁾ A large amount of ^{15}N incorporation was found in $\text{NH}_3\text{-N}$. The urease activity of anaerobes is thought to be high, because the medium used was poor in nitrogen concentration as compared with the nitrogen concentration in the cecum of rats fed on the low or normal protein diet.⁸⁾ Incorporations of ^{15}N to PN, isoleucine + leucine, and valine after 48 hr were scarcely found.

From these results, it was suggested that the reuse of urea by rats is mostly due to NH_3 formation by intestinal microbes and its absorption.

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