ENZYMIC ACTIVITIES IN RUMINAL MUCOSA

I. SURVEYS OF CARBAMYL PHOSPHATE SYNTHETASE AND ORNITHINE TRANSCARBAMYLASE IN GOATS

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It is known that a part of dietary protein taken by the ruminant is decomposed into simpler nitrogenous compounds, such as amino acids and ammonia, by the action of microorganisms dwelling in the rumen, and that an appreciable level of ammonia is maintained in the rumen liquor^{11,12}). Under some dietary conditions, ammonia in the rumen increases in level, and a considerable amount of it is absorbed from the mucosa of the rumen^{8,10}). In the rumen, a large amount of carbon dioxide is produced. The largest part of it is evacuated as eructation, but some part of it may also be absorbed. It is generally understood that the absorbed ammonia is detoxicated by the liver, but the facts noted above raise a hypothesis that ammonia might be metabolized to some extent in the mucosa of the rumen. In the present studies, therefore, to clarify the metabolism of ammonia and its derivative in the mucosa of the rumen, the author surveyed carbamyl phosphate synthetase (CPS) and ornithine transcarbamylase (OTC) in goats. These two enzymes participate in the Krebs-Henseleit cycle of urea synthesis and catalize the following reactions (1) and (2), respectively³).

Legends for abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; and Pi, inorganic orthophosphate.

MATERIALS AND METHODS

The rumen and the liver used were obtained from healthy Saanen hybrid goats immediately after slaughtering. The goats weighed from 10 to 40 kg and were different in age, sex, and dietary conditions. The rumen tissue was rinsed in ice-cold physiological saline, cleaned, and then blotted. The mucosa was detached at its base, cut into small pieces, and homogenized in a Potter-Elvehjem homogenizer with 4 volumes of 0.1% cetyltrimethylammonium bromide (CTB). The homogenate was centrifuged at 4,000 g for 10 minutes at 2°C, and the resultant supernatant was used for enzyme assays.

The mitochondrial fractions used were prepared from the ruminal mucosa by essentially the same procedure as described by HOGEBOM and SCHNEIDER⁵). A mucosal specimen weighing 30 g was blended with 120 ml of 0.25 M sucrose in a Waring blender

for 3 minutes. The homogenate was filtered through one layer of gauze, and the filtrate centrifuged at 700 g for 10 minutes. The resulting supernatant was filtered through two layers of gauze, and centrifuged at 13,000 g for 15 minutes. The resultant precipitate was suspended in 4 ml of 0.1% CTB and stirred. The suspension was centrifuged at 4,000 g for 10 minutes. The resulting supernatant was used for enzyme assays.

As reference, a liver specimen was homogenized with 9 volumes of 0.1% CTB in a Potter-Elvehjem homogenizer. From this, samples were prepared for enzyme assays by the same method as employed for the ruminal mucosa.

Enzyme assays were conducted by the method of BROWN and COHEN¹¹). The assay medium of CPS contained 50 μ M of ammonium bicarbonate, 5 μ M of ATP, 5 μ M of L-ornithine, 3 μ M of N-acetyl-L-glutamate, 10 μ M of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10 μ M of phosphoenol pyruvic acid, 25 μ g of pyruvate kinase, 0.5 mg of OTC, and 0.3 ml of the enzyme solution in a final volume of 1 ml. The medium for OTC contained 20 μ M of L-ornithine, 90 μ M of sodium glycylglycine buffer at pH 8.3, 20 μ M of carbamyl phosphate, and 0.3 ml of the enzyme solution in a final volume of 2 ml. The incubation period was 15 minutes.

Citrulline was determined by the method of RATNER¹³). Protein was determined by the method of LOWRY, ROSEBROUGH, FARR, and RANDALL⁹), with bovine serum albumin as standard.

RESULTS AND DISCUSSION

Table 1 shows the enzyme activities of CPS and OTC determined. In the liver, OTC was higher in activity than CPS. The activities of both enzymes were not so much higher in goats than in rats or man^{2,6,14}). The CTB extract obtained from 1 g of liver showed a CPS activity of 375 to 960. Under the same conditions of assay, no CPS activity was shown by the extract from the ruminal mucosa containing about 4 mg of protein. COHEN and SALLACH³) found the presence of CPS in the mucosa of the small intestine in addition to the liver. They mentioned that the enzyme was labile and that preparations from rat or dog liver were considerably more stable in the presence of Mg^{++} and ATP. Therefore, extracts were prepared from the ruminal mucosa by using 0.1% CTB containing each 0.1 M of ATP and MgSO_4 at pH 7.5. Assays with the extracts also gave negative results.

The presence of OTC was found in the extract from the ruminal mucosa. The activity, however, was extremely low and corresponded to about one-thousandth of that

Table 1. Enzymic Activities of Carbamyl Phosphate Synthetase and Ornithine Transcarbamylase in Liver and Ruminal Mucosa of Goats

Animal No.	Carbamyl phosphate synthetase*		Ornithine transcarbamylase*		
	Liver	Ruminal mucosa	Liver	Ruminal mucosa	Mitochondrial preparation
1	609 (—)	—	17,100 (—)	14.7 (—)	(1.9)
2	469 (6.0)	—	12,500 (160)	12.3 (0.29)	(2.8)
3	960 (6.8)	—	18,700 (133)	13.3 (0.37)	(6.3)
4	480 (4.4)	—	23,500 (216)	14.4 (0.26)	(4.8)
5	375 (3.4)	—	15,500 (141)	12.3 (0.24)	(4.7)
6	450 (3.5)	—	28,200 (218)	17.1 (0.30)	(4.0)
Average	557 (4.8)	—	19,250 (174)	14.0 (0.29)	(4.1)

* The activity is expressed in terms of μ M of product per g of tissue (wet weight) for one hour. The value in parentheses shows specific activity which is expressed in terms of μ M of product per mg of protein for one hour.

in the liver. A higher level of specific activity of OTC was shown by mitochondrial preparations from the ruminal mucosa than by whole-mucosal preparations from the rumen (Table 1).

Requirements for synthesis of citrulline were studied with the mitochondrial preparation from the ruminal mucosa. The results are presented in Table 2. The addition of carbamyl phosphate was essential for synthesis of citrulline. A considerable amount of citrulline, however, was synthesized without addition of ornithine. This might be due to endogenous citrulline contained in the mitochondria.

Table 2. Citrulline Synthesis in Mitochondrial Preparation from Ruminal Mucosa

System	Citrulline synthesized
	$\mu\text{M}/\text{hour}$
Complete system*	0.84
Complete system — Ornithine	0.28
Complete system — Carbamyl phosphate	—
Complete system — Mitochondrial preparation	—

* It contained, in a final volume of 2 ml, 20 μM of L-ornithine, 20 μM of carbamyl phosphate, 90 μM of sodium glycylglycine buffer at pH 8.3, and 0.3 ml of mitochondrial preparation.

In recent years, KRVAVICA et al.⁷⁾ surveyed CPS and OTC on the mucosa of cattle. They mentioned that the activity of OTC in the ruminal mucosa was comparable in level to that in the liver. They failed, however, to detect CPS. The results of the present studies indicate that the ruminal mucosa was much lower in OTC activity than the liver in goats. They are quite different from the results of KRVAVICA et al., who studied the same subject with cattle.

HALL et al.⁴⁾ demonstrated that CPS was present with OTC in the mucosa of the small intestine. In the present studies, no measurable activity of CPS was detected from the ruminal mucosa. This negative finding, however, does not entirely preclude the possibility of the presence of CPS in the mucosa of the rumen. In the liver of goats, CPS is considerably lower in activity than OTC. When the relative activity of CPS to OTC in the liver is adopted in the case of the ruminal mucosa, the activity of CPS in the ruminal mucosa is assumed to be too low to be detected under such conditions of enzyme assay as adopted in the present studies.

The results of the present studies seem to indicate that citrulline synthesized by the ruminal mucosa might be very small in amount, even if ammonia is taken into consideration as its precursor. It is thought that ammonia is possibly metabolized by the ruminal mucosa into glutamic acid or glutamine. The activities of the enzymes catalyzing these reactions will be presented in a paper to come.

SUMMARY

For the purpose of clarifying the metabolism of ammonia and its derivative in the mucosa of the rumen, carbamyl phosphate synthetase (CPS) and ornithine transcarbamylase (OTC) were surveyed in goats. The ruminal mucosa failed to show any measurable CPS activity, but showed the activity of OTC, which was in the order of one-thousandth of that in the liver in level. A higher level of specific activity of OTC was exhibited by mitochondrial preparations from the ruminal mucosa than by whole-mucosal preparations from the rumen.

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反芻胃粘膜の酵素活性

I. Carbamyl phosphate synthetase および Ornithine transcarbamylase の検索

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反芻胃粘膜におけるアンモニアとその関連物質の代謝の可能性を明確にする目的で, Carbamyl phosphate synthetase (CPS) および Ornithine transcarbamylase (OTC) を山羊について検索した。

山羊の第一胃粘膜は, CPS の活性は示さないが, OTC の活性を示し, カルバミル・リン酸お

よびオルニチンの存在下で, チトルリンを生成した。しかし第一胃粘膜の示す OTC の活性は著しく低く, 肝の示す活性のおおよそ 1/1,000 であった。実験結果から, 反芻胃粘膜では, アンモニア→カルバミル・リン酸→チトルリンの代謝がおこなわれる可能性は少ないと考えられた。