STUDIES ON THE METABOLIC CONVERSION OF VOLATILE FATTY ACIDS IN THE RUMEN EPITHELIUM I. RUMINAL ARTERIO-VENEOUS DIFFERENCES OF BLOOD ORGANIC ACIDS AND LIPIDS

STUDIES ON THE METABOLIC CONVERSION OF VOLATILE FATTY ACIDS IN THE RUMEN EPITHELIUM

I. RUMINAL ARTERIO-VENEOUS DIFFERENCES OF BLOOD ORGANIC ACIDS AND LIPIDS

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Introduction

It has been generally recognized that in the rumen of ruminats, volatile fatty acids including acetate as the main constituent, propinate and butyrate, are produced from the ingested diets (mainly cellulose and other organic constituents) by complicated ruminal fementation. These volatile fatty acids are absorbed directly from the rumen eptithelium(1-7), enter into the ruminal vein and reach th the liver via the portal vein, and are utilized by the animal body providing the main caloric energy. In recent years, many studies have been carried out on the metabolism of volatile fatty acids in ruminants and its nutritional significance in ruminants has been clarified in general.

The molar ratio of these volatile fatty acids in the rumen are variable according to the feeding condition. It has been reported that the molar ratio of acetate : propionate : butyrate in the rumen was approximately 6 : 3 : 1 when the animals were fed with hay in the barn, 1 : 1 : 1 at the time of field grass feeding (8).

As metioned, the volatile fatty acids produced in the rumen are actively absorbed through the rumen epithelium. As reported by Shibata(8) and Tsuda (6-7), each acids in the rumen was absorbed at a uniform rate in spite of the molar ratio. On the other hand, the main volatile fatty acids which appears in the portal vein is acetic acid. Butyrate and propionate are exceedingly low (9). Namely, these volatile fatty acids, which are absorbed through the rumen epithelium, do not appear in the portal vein as the former status. On the metabolism of these volatile fatty acids in the rumen epithelium, serial studies

have been reported by Seto (10-13) and Pennington (14-16) in detail. In these studies, they showed that some part of propionate and acetate incubated with rumen epithelium were oxidized to carbon dioxide via T. C. A. cycle and the ketone body was formed from butyrate and acetate. Okabe (17) confirmed that when volatile fatty acids solution and buffered physiological saline were divided with a piece of the rumen epithelium, volatile fatty acids disappeared quickly from the side of the rumen epithelium, i. e. the volatile fatty acids solution, but the minor part of the disappeared acids appeared in the side of the submucosa, i. e. buffered physiological saline.

On the other hand, from the stand point of the liver, to grasp quantitatively the process of these volatile fatty acids entering the liver through the rumen epithelium, Ambo (18) measured the total amounts of volatile fatty acids and other nutrients which entered from the digestive tract into the liver by the method of multiplying the blood flow rate of the portal vein (19) by arterio-veneous differences of the nutrients in the portal blood. The result was that the caloric energy of volatile fatty acids and ketone body flowing into the liver was only 22 per cent to 26 per cent of the digestive energy of the given feeds (18). Washing out the rumen contents through the rumen fistula and isolating the rumen by the method of the reticulo-omasal olifice plugging, Shibata *et al.*, in their artificial nutrition experiments, recognized that the amouts of volatile fatty acids flowed into the liver was only 30 per cent of the amounts of volatile fatty acids absorbed through the rumen epithelium(21). Stewart(22) calculated that the total amounts of volatile fatty acids produced in the rumen per day correspond to 63 per cent of the maintenace energy of ruminants. Moreover, Shibata (9), in artificial nutrition for goats and cow, demonstrated that the animal, peovided 73 per cent to 78 per cent of their maintenance energy with volatile fatty acids, could maintain body weight, secreted milk and recovered from the fasting status.

From these experimental results, as mentioned above, we hold doubt that the volatile fatty acids might be changed to some other substances in the rumen epithelial tissue when they pass into the ruminal vein through the rumen epithelium. We thought the metabolic converson of volatile fatty acids in the rumen epithelium to be an important and indispensable subject for understanding the nutritional physiology of ruminants. To solve this subject, we considered that the analysis of the ruminal arterio-veneous difierences of blood chemical substances might be a clue to understand the metabolic converson of volatile fatty acids in the rumen epithelium. In the experiments, as the first step, we analyzed the ruminal arterio-veneous differences of several blood organic acids, total esterified fatty acids and total cholesterol.

Materials and Methods

Animals and blood sampling

Mature female goats of Saanen breed, weighing 25 to 35 kg in body weight were used for blood sampling. The goats were fed with orchard hay, corn silage and concentrate. At three to five hours after feeding, under local anaesthesis with procaine-hydrochloride, the abdomen and jugular part of the animal were opend. Blood sample of the ruminal vein was obtained from the left ruminal vein and arterial blood was taken from the carotid artery by means of puncture with an injecting syringe respectively at the same time. To prevent blood coagulation, sodium-fluoride was used. Our object in this experiments was to compare the chemical differences of the ruminal arterial and veneous blood, but we substituted the arterial blood for the ruminal arterial blood because previously in our laboratory, it has been recognized that the chemical components of arterial blood were uniform in any part of the body (4).

Analytical method

1. Total esterified fatty acids and total cholesterol.

The determination of total esterified fatty acids was made on the whole blood and serum by the method of Zak (24). Total cholesterol of blood was determined by Stern and Shapiro's method (25). The determinations were made within 12 hours after the blood sampling, because the value of the measurment might get to be low.

2. Separation and determination of blood organic acids.

The principle of the method was as follows: Deprotenization and extraction of the organic acids was proceeded with methanol (final concentration of methanol was 80 %). The methanol extracts obtained were passed through the annion exchanging resin immediately after extraction. The annion captured in the resin was eluted with ammonium carbonate solution. And then the eluate, after condensing under vacuum, was analyzed for organic acids by the method of silicic acid column chromatography. In this method, the excess ammonium carbonate was removed decomposing to ammonia and carbon dioxide in the process of condensation. This removal of the excess salts was convenient to the following procedure.

(A) Reagents

Methanol: Traces of the volatile acids were removed by redistillation adding 2 g of KOH per liter. 1 N-ammonium carbonate. Annion exchanging resin: Amberlite IRA 400 (analytical grade, granule). Silicic acid: Silicic acid (Mallinckrodt, for chromatograph use, 100 meshes) was prepared for use by removing the smaller particles which lessens the flow rate of the solvent through the column. Such removal was attained by suspending 1 lb of silicic acid in two liters of water. Let it settle for 15 min and then discarded the supernatant portion. This procedure was repeated twice. The settled particles were dried and sifted with a sieve of 270 meshes. For column preparation, the particles smaller than 270 meshes was dried in an oven at 105°C.

(B) Preparation of annion exchanging resin column.

The resin column was prepared by triturating 10 g of Amberlite IRA 400 with suitable amounts of ammonium carbonate solution and transfering to a glass chromatographic tube, the inside diameter of 10 mm and 30 cm long. After the particles settled, the resin was activated with the elution of 400 to 500 ml of 1 N-ammonium carbonate solution. Next, the excess alkali in the column was washed out with water. In order to transfer the methanol extracts directly into the column, 100 ml of 80 per cent methanol was poured into the column to displace the water phase with the methanol phase previously. In our previous experiment, the displacement with methanol did not affect the ion exchanging capacity of the resin.

(C) Extraction of blood organic acids.

Fifty milli-litre of blood was poured drop by drop into 200 ml of redistilled methanol to deprotenize and extract the blood organic acids (final concentration of methanol, 80 %). It was centrifuged at 3000 rpm for 10 min and decanted the supernatant solution. Then 50 ml of 80 per cent methanol was added to the precipitated protein, centrifuged and the supernatant decanted. The supernatant was put together with the former. To perfect the extraction, this washing procedure was repeated several times. The total volume of the methanol extract was 300 to 400 ml. The pH of the extracts was adjusted to ca. 8 with 0.5 N-NaOH. The adjusted extracts were transfered into the resin column and the annion (acid radicals) captured. After the run was finished, lipid, blood glucose and other inpurities remained in the column were washed with 100 ml of 80 per cent methanol. Organic acids were eluted as ammonium salts with 400 to 500 ml of 1 N-ammonium carbonate. By evaporation under vacuum at 35°C, the volume of the eluate was reduced to about 10 ml. The condensate was transfered to an evaporating-dish and the final stage evaporation to dryness was conducted in a vacuum-desiccator. This dried sample was used for the following analysis, i. e. separation of organic acids.

(D) Separation of organic acids by silicic acid column chromatography.

Organic acids were separated by Marshall's silicic acid column chromatographic method (26) with exception of the glass chromatographic tube and the sample mounting technique. The glass chromatographic tube was 10 mm for inside diameter and 40 cm long. The column was preapred by triturating 10 g of silicic acid (270 meshes) with 6.0 ml of 0.05 N-H_2SO_4 to a free-flowing powder. The powder was slurried in chloroform saturated with 0.05 N-H_2SO_4 and transfered to the column. The dried salts of organic acids was acidified to pH 2 with 0.5 to 1.0 ml of 10 N-H_2SO_4 using cresol red as an indicator and added 1 to 2 g of silicic acid. The acidified mixture of organic acids and silicic acid were triturated to a free-flowing powder and transfered into the column. The eluate was collected in 2 ml fractions. Cresol red was added as an indicator and titrated under nitrogen against 0.01 N methanolic KOH.

Results and Discussions

(1) Total esterified fatty acids and total cholesterol.

We could not find marked arterio-veneous difference of total esterified fatty acies as shown in Table 1. Namely, in the goat, No. 1, the contentration of total

esterified fatty acids in the blood of ruminal vein was 0.67 mEq/dl serum (0.48 mEq/dl whole blood). In the arterial blood, 0.81 mEq/dl serum (0.53 mEq/dl whole blood) being greater than in the blood of the ruminal vein by 0.14 mEq/dl serum. In the goat, No. 2, the ruminal arterio-veneous difference of total esterified fatty acids was 0.06 mEq/dl serum.

Table 1. Ruminal arterio-veneous differences of total esterified fatty acids.

No. of goat	Sample	Ruminal vein mEq/dl	Artery mEq/dl	Arterio-veneous differences mEq/dl
1	serum	0.67	0.81	−0.14
	whole blood	0.48	0.53	−0.05
2	serum	0.48	0.42	+0.06

Seto and Umezu (10), in their studies on the metabolism of volatile fatty acids in the rumen epithelium, pointed out that in the case of propionate as the substrate, the rumen epithelium consumed the substrate but its oxygen uptake was lower than the case of the non-substrate and the propionate might enter into the metabolic pathway other than oxidation, probably to the synthetic pathway. However, in the studies reported here, we could not define any active synthesis of esterified total fatty acids by the method of arterio-veneous differences.

Table 2. Ruminal arterio-veneous differences of total cholesterol.

No. of goat	Sample	Ruminal vein mg/dl	Artery mg/dl	Arterio-veneous differences mg/dl
1	serum	94.5	112.9	−18.1
2	serum	88.3	89.3	− 1.0

Chaikoff (26) showed that in the slices of the small intestinal epthelium of the rat, cholerterol was produced from acetate. From these evidences, we considered that the rumen epithelium also might have such cholesterol synthesizing ability, so that we measured the ruminal arterio-veneous difference of the total cholesterol. However, as shown in Table 2, we could find no differences. The concentration of total cholesterol in the blood of the ruminal vein was rather lower than in the arterial blood.

(2) Organic acids in the blood of the ruminal vein.

The fractionated pattern of organic acids in the blood of the ruminal vein and the artery are showe in Table 3.

In regard to the ketone body, acetoacetate and β-hydroxybutyrate were found in the blood of the ruminal vein, but not in the arterial blood. The levels of pyruvate, lactate, acetate and propionate in the blood of the ruminal vein was greater than in the arterial blood. These results were in good agreement with

Table 3. Organic acids concentration in the blood of the ruminal vein and artery.

Organic acid	Ruminal vein	Artery	A-V differences
Propionate	45 μM/dl	0 μM/dl	45 μM/dl
Acetate	177	96	81
Acetoacetate	39	0	39
Pyruvate	55	37	18
Fumarate	29	11	18
β-hydroxybutyrate	101	0	101
Lactate	349	255	94
Succinate	20	13	7

many other investigators. As Annison (8) confirmed on the volatile fatty acids in portal vein, we certified that the volatile fatty acids in the blood of the ruminal vein was mainly acetic acid. The level of the propionate was exceedingly low and butyrate could not be detected. Only acetate was found in the arterial blood. The levels of succinate and fumarate in the ruminal vein were greater than in the arterial blood, especially the former.

It was easy to suppose that these organic acids found in the blood of the ruminal vein might be produced from the absorbed volatile fatty acids by the metabolic conversion. But it was possibly due to the metabolic conversion of the constituents in the arterial blood. To make the origin of these organic acids clear, further sudies should be undertaken.

Summary

From the investigation mentioned above, we supposed that in the rumen epithelium, volatile fatty acids might be converted to some other substances when they were absorbed through the rumen epithelium.. To make this clear, we measured the blood chemical constituents of the ruminal vein and the artery. The following results were obtained.

(1) On the total esterified fatty acids and total cholesterol, there were no significant arterio-veneous differences and we considered that the active synthesis of these lipids did not take place in the rumen epithelium.

(2) In regard to organic acids in the blood, we confirmed that the concentration of ketone body, pyruvate, lactate, acetate and propionate in the ruminal vein were greater than in the arterial blood. Succinate and fumarate were detected in the ruminal vein and their concentrations were greater than in the arterial blood.

References

1) Phillipson, A. T. and R. A. McAnally (1942). J. Exptl. Biol. **19**, 199.
2) McAnally, R.A. and A.T. Phillipson (1943). J. Physiol., **101**, 13.

3) Barcroft, J., R.A. McAnally and A.J. Phillipson (1944). J. Exptl. Biol., **20**, 120.
4) Umezu, M., T. Tsuda, A. Shibata, K. Kimura, S. Tsuda, K. Ambo and Y. Tanaka (1955). J. Jap. Biochem. Soc., **27**, 218.
5) Phillipson, T. H. (1951). J. Physiol., **113**, 267.
6) Tsuda, T. (1956). Tohoku J. Agr. Res., **7**, 231.
7) Tsuda, T. (1956). *ibid.*, **7**, 241.
8) Shibata, F. (1963). Memoirs of the Laboratory of Animal Nutrition, Nagoya University, No. 3.
9) Annison, E. F. (1957). Biochem. J., **66**, 592.
10) Seto, K., T. Tsuda and M. Umezu (1955). J. Jap. Biochem. Soc., **27**, 213.
11) Seto, K., T. Tsuda and M. Umezu (1955). Tohoku J. Agr. Res., **6**, 91.
12) Seto, K., T. Tsuda and M. Umezu (1956). J. Jap. Biochem. Soc., **28**, 143.
13) Seto, K., T. Tsuda and M. Umezu (1957). *ibid.*, **29**, 17.
14) Pennington, R. J. (1952). Biochem. J., **51**, 251.
15) Pennington, R. J. (1955). *ibid.*, **60**, xxxvii.
16) Pennington, R. J. (1956). *ibid.*, **63**, 618.
17) Okabe, I., M. Thesis, Tohoku Univ., 1960.
18) Ambo, K., Ph. D. Thesis, Tohoku Univ., 1961.
19) Ambo, K., S. Matsuda and M. Umezu (1963). Tohoku J. Agr. Res., **14**, 25.
20) Watanabe, Y. and M. Umezu (1962). Tohoku J. Agr. Res., **13**, 221.
21) Shibata, F., K. Ambo, Y. Shoji and M. Umezu, unpublished.
22) Stewart, W. E., D. G. Stewart and L. H. Shults (1958). J. Anim. Sci., **17**, 723.
23) Zak, B. (1957). Amr. J. Med. Technol., **23**, 283.
24) Stern, I. and B. Shapiro (1953). J. Clin. Path., **6**, 158.
25) Marshall, L. M. (1952). Anal. Chem., **24**, 185.
26) Chaikoff, I.L. and M.D. Morris (1959). J. Biol. Chem., **234**, 1095.