THE MECHANISM OF THE KETONE BODIES PRODUCTION WITH RUMEN EPITHELIUM I. THE KETONE BODIES PRODUCTION FROM VOLATILE FATTY ACIDS WITH RUMEN EPITHELIUM AND THE INFLUENCE OF SUBSTANCES CONCERNED WITH THE CITRIC ACID CYCLE ON THEIR PRODUCTION

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I. THE KETONE BODIES PRODUCTION FROM VOLATILE FATTY ACIDS WITH RUMEN EPITHELIUM AND THE INFLUENCE OF SUBSTANCES CONCERNED WITH THE CITRIC ACID CYCLE ON THEIR PRODUCTION

By

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Introduction

In the rumen of ruminants, numerous micro-organisms populate and play an important role in digestion of foodstuffs. Specifically a large quantity of volatile fatty acids would be produced as the result of the decomposition of the carbobydrate, especially cellulose, by the fermentative activity of the microorganisms in the rumen.

The amounts of volatile fatty acids was estimated as 329 g and 64 g in the rumen contents of a cow and a sheep respectively (1). Although the relative amount of those acids would depend on the quality and quantity of the foodstuffs, it was generally said that acetate constituted 67%, propionate 19% and butyrate 14% respectively (1). Volatile fatty acids are also produced and increased in the large intestine.

The studies upon the absorption and the utilization of such large quantities of volatile fatty acids have been begun many physiologists and biochemists.

Reid concluded that the blood content of volatile fatty acids in the carotid artery was lower than that in the jugular vein, recognizing the utilization of volatile fatty acids by the circulation in the head (2). Pennington observed *in vitro* the utilization of volatile fatty acids and the accompanied ketone bodies production in various tissue slices of sheep. He reported that rumen epithelium, liver slice and kidney slice showed utilization of a large quantity of volatile fatty acids and then a large proportion of butyrate converted to ketone bodies (3).

Concerning this point, Sibata measured the oxygen consumption of various tissue slices, using volatile fatty acids as the substrate, and observed the grade of the utilization of volatile fatty acids in liver and kidney slice (4).

The broad experiments on the absorption of volatile fatty acids through the rumen wall were carried out by Phillipson et al. Casting volatile fatty acids into empty rumen, he observed that the amount of volatile fatty acids disappeared from the rumen with the lapse of time.

He recognized that volatile fatty acids were absorbed from the rumen wall directly, and the amount of butyrate in the blood draining rumen was less than the relative amount disappearing from the rumen (5). The latter fact showed the utilization of butyrate in the rumen wall. Then Tsuda confirmed the absorption of volatile fatty acids from the rumen wall by the miniature rumen method (6).

Umezu and his colleagues determined the blood level of several substances in veins and arteries which circulated blood through various organs of a goat and observed the singularity of the ruminants metabolism. According to their results, the volatile fatty acids and ketone bodies of the ruminous vein was the highest and so the volatile fatty acids, produced in the rumen, were absorbed through the rumen wall and some proportion of the absorbed volatile fatty acids converted to ketone bodies in the tissue of the rumen wall. They also confirmed the utilization of volatile fatty acids in systemic circulation too (7).

Volatile fatty acids, such as the above mentioned play an important role as an energy source for ruminants. The utilization of the volatile fatty acids of ruminants becomes higher the more serious the influence of the ketone bodies production on the ruminant. Such circumstances give an account of the fact that ketosis prevails in ruminants (8).

So the writers necessarily inspected *in vitro* the metabolisms of acetate, propionate and butyrate in the rumen epithelium which played an important role on the absorption of volatile fatty acids and produced the largest quantity of ketone bodies.

Methods and materials

Tissues were obtained from neats killed at the slaughter house. They were immediately removed into a cold 1.15% KCl aqueous solution. The experiments were begun within 3 hrs. after slaughtering.

Rumen epithelium could be separated very easily from muscle layer and the thickness was approximately 300 μ. 1 g (wet wt) of epithelium or tissue slice was taken into a 50 ml conical flask, with 10 ml of Krebs-Ringer phosphate buffer, pH 7.2, containing various substrate. The flasks, after oxygen satulation, were shaken in a water bath at 38°C for 3 hours.

After incubation, total ketone bodies, namely β-hydroxybutyrate, acetoacetate and acetone, were determined by Thin and Robertson's method (9).

Volatile fatty acids were determined by Conway's diffusion method (10).

Results and Discussion

(1) Ketone bodies production from butyrate in various tissues of ruminants.

The ketone bodies productions were judged by comparison with various tissues.

Rumen epithelium, liver slice, kidney slice and caecum epithelium were incubated with 50μ-M. of Na-butyrate as the substrate which was the most ketogenic substance among volatile fatty acids.

The experimental results are shown in table 1.

Table 1. Butyrate consumption and ketone bodies production in various tissues. 1g of tissue incubated for 3 hr. at 38°C in Ringer-phosphate containing 50μ-M. of Na-butyrate.

Tissue	Ketone bodies produced	Fatty acid used	Ketone produced / Fatty acid used
	4.3 μ-M.	(2.3) μ-M.	%
Rumen epithelium without substrate			
With butyrate	15.2	30.9	49.0
Liver slice without substrate	0.6	(1.0)	
With butyrate	10.0	36.4	27.5
Kidney slice without substrate	0.9	(2.0)	
With butyrate	2.6	33.9	7.7
Caecum epithelium without substrate	0.1	(2.5)	
With butyrate	0.2	11.0	0.2

The bulk of butyrate was consumed by rumen epithelium, liver slice and kidney slice.

The amount of ketone bodies production was the greatest in rumen epithelium and then in liver slice.

The kidney slice spent the large quantity of butyrate but production power of ketone bodies was weak.

The amounts of the butyrate consumption and ketone bodies production were very little in caecum epithelium.

(II) The ketone bodies production from acetate, propionate and butyrate in rumen epithelium.

The experiments concerning the ketone bodies production from acetate, propionate and butyrate were done with Na-salts of each acids. Since one molecule of ketone bodies is formed from two molecules of acetate or one molecule of butyrate, 100μ-M acetate, propionate or 50 μ-M. butyrate were added to Krebs phosphate buffer respectively.

The experimental results are shown in table 2.

Table 2. Ketone bodies production from acetate, butyrate and propionate in rumen epithelium.
1g of tissue incubated for 3hr. at 38°C in Ringer-phosphate containing 100μ-M. of acetate and propionate, 50μ-M. of Na-butyrate.

Substrate	Ketone bodies produced	Fatty acid used	No. of experiments
No substrate	1.2 μ-M.	(0.7) μ-M.	11
Acetate	2.7	14.0	8
Propionate	0.3	11.9	5
Butyrate	11.2	22.3	7

In the presence of butyrate, a large quantity of ketone bodies accumulated. The amount of ketone bodies production from acetate was one quarter of that from butyrate.

The amount of ketone bodies production of propionate was less than that of control. Butyrate was consumed more vigorously than the other two acids. These results in the rumen epithelium were generally similar to those of Pennington.

(III) The influence of glucose and propionate on the ketone bodies production from acetate and butyrate by rumen epithelium.

Fatty acids, as mentioned, split to 2-carbon units, acetyl-CoA, and in the presence of oxaloacetate C-2-compounds are oxidized by the way of the citric acid cycle.

Glucose and propionate are said to convert to oxaloacetate through pyruvate in most animal tissue.

Therefore glucose and propionate were added to acetate and butyrate medium. The experimental results are shown in tables 3, 4, 5.

Table 3. The influence of propionate on ketone bodies production from acetate in rumen epithelium.
1g of tissue incubated for 3hr. at 38°C in Ringer phosphate containing 100μ-M. of Na-acetate and Na-propionate

Substrate	Ketone bodies produced	Fatty acid used	Ketone produced / Fatty acid used
No substrate	1.76 μ-M.	(0.1) μ-M.	%
Acetate	2.35	8.0	29.3
Propionate	0.01	14.0	0.1
Acetate + Propionate	0.35	18.0	1.9

Propionate suppressed entirely the ketone bodies production from acetate. Glucose also suppressed the ketone bodies production of acetate but not as

markedly as propionate. The consumption of acetate did not increase by addition of glucose.

Table 4. The influence of glucose on ketone bodies production from acetate in rumen epithelium.
1g of tissue incubated for 3hr. at 38°C in Ringer-phosphate containing 100 μ-M. of Na-acetate or 100 μ-M. of glucose.

Ssubstrate	Ketone bodies produced	Fatty acid used	Ketone produced / Fatty acid used
No substrate	2.32 μ-M.	(1.3) μ-M.	%
Glucose	1.17	(0.4)	
Acetate	4.65	26.3	17.8
Acetate+Glucose	2.10	22.0	9.5

These results were considered by us: propionate and glucose converted to oxaloacetate and oxaloacetate accelerated acetate oxidation by way of the citric acid cycle.

Table 5. The influence of propionate or glucose on ketone bodies production from butyrate in rumen epithelium.
1g of tissue incubated for 3hr. at 38°C in Ringer-phosphate containing 50 μ-M. of Na-butyrate and 100 μ-M. of Na-propionate or 100 μ-M. of glucose.

Substrate	Ketone bodies produced	Fatty acid used	Ketone produced / Fatty acid used
No substrate	1.19 μ-M.	(0.6) μ-M.	%
Butyrate	10.80	23.9	45.2
Propionate	0.35	10.1	3.4
Butyrate+Propionate	9.29	41.0	22.6
Glucose	0.8		
Butyrate+Glucose	10.78		

The ketone bodies production of butyrate was also suppressed by propionate but its suppression was very slight, while glucose had no effect upon butyrate metabolism in rumen epithelium. Glucose is said also to enter into citric acid cycle through pyruvate, so it would have to show the same antiketogenic action with propionate. But we observed a differences between the antiketogenic action of propionate and that of glucose. Sibata and Ambo also investigated a differences between the healing effect of propionate and that of glucose in experimental butyric ketosis of a goat (11).

These facts showed that the mechanism of antiketogenic action of propionate differed from that of glucose.

On the other hand, the ketone bodies production from acetate was suppressed

with propionate entirely and with glucose to some degree, while that from butyrate was suppressed only 4% with propionate and was not suppressed with glucose. This would hardly to be explained from the point of view that all of butyrate converts to the intermediate, being formed from acetate and then form acetoacetate with rumen epithelium. Therefore in the case of butyrate metabolism the acetoacetate must be formed through some other intermediate than acetate.

(IV) Influence of malate and succinate on the ketone bodies production from acetate and butyrate in the rumen epithelium.

Since glucose or propionate supplies oxaloacetate to fatty acid metabolism, it is considered they must have the antiketogenic action.

Therefore writers inspected the influence of members in the citric acid cycle, such as malate and succinate, on the ketone bodies production of acetate and butyrate.

Table 6. The influence of malate or succinate on ketone bodies production from acetate and butyrate in rumen epithelium.
1g of tissue incubated for 3hr. at 38°C in Ringer-phosphate containing 50 μ-M. of Na-butyrate and 100 μ-M. Na-acetate and of Na-succinate or of Na-malate.

Substrate	Ketone bodies produced	Fatty acid used	Ketone produced / Fatty acid used
	μ-M.	μ-M.	%
No substrate	1.51	(0.53)	
Acetate	2.12	15.2	13.9
Butyrate	10.28	18.9	67.5
Malate	1.90	(3.8)	
Acetate + Malate	3.96	7.1	55.8
Butyrate + Malate	13.60	17.9	70.8
No substrate	0.46	(1.6)	
Butyrate	4.25	28.8	14.8
Succinate	1.64	(3.3)	
Butyrate + Succinate	10.00	32.0	31.3

The results, as shown in table 6, are that malate and succinate did not only suppress the ketone bodies production from acetate and butyrate, but the ketone bodies were produced from malate and succinate themselves. Volatile fatty acids were formed from malate and succinate, so that probably malate and succinate converted to volatile fatty acids and then produced the ketone bodies.

The amount of produced ketone bodies, when malate was added to acetate or butyrate medium, coincides approximately to the sum of those when malate or acetate or butyrate was incubated individually. But the very large quantity of the ketone bodies were produced from butyrate when succinate was added

to butyrate medium.

(V) Influence of pyruvate and lactate on the production of acetate and butyrate in the rumen epithelium.

An unexpected result was obtained in this experiment: the ketone bodies were produced from succinate and malate, the members of T. C. A. cycle, but not produced from glucose and propionate, the precursors of pyruvate. It was most necessary to inspect the influence of pyruvate and lactate in the ketone-forming reaction from acetate and butyrate. The former two chemical substances were looked upon as the intermediate substances from glucose and propionate toward to the way of the T. C. A. cycle.

Table 7. The influcence of pyruvate or lactate on ketone bodies production from acetate and butyrate in rumen epithelium.
1g of tissue incubated for 3hr. at 38°C in Ringer-phosphate containing 50μ-M. of Na-butyrate and 100μ-M. of Na-acetate and of Na-pyruvate or of Na-lactate.

Substrate	Ketone bodies produced	Fatty acid used	Ketone produced / Fatty acid used
	μ-M.	μ-M.	%
No substrate	1.35	(1.7)	
Acetate	3.52	11.0	32.0
Butyrate	12.00	30.3	39.0
Pyruvate	3.20	(3.1)	
Lactate	2.40	(1.7)	
Acetate + Pyruvate	5.24	6.1	86.0
Butyrate + Pyruvate	14.20	24.7	57.0
Acetate + Lactate	3.30	7.0	47.5
Butyrate + Lactate	12.00	30.8	39.0

The result, as shown in table 7, was that ketone bodies were produced from pyruvate and lactate themselves.

As to this problem, Pennington, having obtained similar evidence, postulated the reaction scheme of succinate formation directly from propionate, instead of indirectly passing through pyruvate to it (12). Again in our experiments, the ketone bodies were produced also from succinate. From the standpiont of ketone bodies production, it would hardly be conceivable for us that the propionate-succinate reaction scheme is the main route of propionate metabolism. It would, however, be necessary to take in to consideration that succinate, in our experiments, was in a non-active form metabolically.

The amount of produced ketone bodies when pyruvate was added to acetate or butyrate medium coincided approximately to the sum of those when pyruvate or butyrate was incubated individually. The consumption in amount of fatty acid as a result of adding pyruvate actually declined. But pyruvate

produced volatile fatty acids so that the amount of used volatile fatty acids did not thus decrease. Lactate was absolutely indifferent either to the ketone bodies formation from acetate and butyrate or to the consumption of butyrate.

Summary

The writers traced the formation of ketone bodies from volatile fatty acids *in vitro* with the various tissues, especially with the rumen epithelium of ruminants, and obtained the following results.

1) The consumption of butyrate was at any rate considerable in the liver, kidney and rumen epithelium. The amount of produced ketone bodies was largest in the rumen epithelium and smallest in the kidney, among the three named organs. The epithelium of caecum consumed butyrate was very little and produced ketone bodies conspicuously less than certain other organs.

2) In the rumen epithelium, butyrate was the highest both in its consumption and in the production of ketone bodies. Acetate was the next in both. Propionate was observed to be consumed but it apparently reduced ketone bodies.

3) In the rumen epithelium, propionate obviously suppressed the formation of ketone bodies from acetate and did more or less those from butyrate.

4) Glucose suppressed the formation of ketone bodies from acetate, but was indifferent to that from butyrate.

5) It would seem probable from the evidence cited above, that the pathways of ketone bodies formation from acetate and from butyrate were different, and that glucose and propionate differed from each other in the mechanism of their antiketogenic function.

6) Malate, succinate, pyruvate, and lactate produced ketone bodies in rumen epithelium. This fact would hardly be able to be explained from the point of view that propionate enters the T. C. A. cycle through pyruvate or succinate.

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