THE METABOLISM OF VOLATILE FATTY ACIDS IN THE LIVER SLICES OF RUMINANTS

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By

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Introduction

Carbohydrate taken in ruminants is decomposed in the rumen into volatile fatty acids such as acetate, propionate and butyrate by the function of rumen microorganisms and then they are absorbed directly through the rumen wall (1–9). On this occasion, part of such volatile fatty acids is metabolized to form the ketone body in the rumen epithelium. However, a considerable amount of volatile fatty acids reaches the liver through the ruminous vein and portal vein, where it is metabolized in various ways (10–15). This is evident from that comparatively high concentration of volatile fatty acids in the portal vein is lowered in the hepatic vein (10). Even in case of non-ruminants, an important role of the liver to the fatty acids metabolism has been confirmed with various experiments in which the method of hepatectomy (16), and liver perfusion (17) were adopted, and liver slice, liver homogenate (18–22) and liver mitochondria (25–28) were used as the experimental materials. Moreover, the metabolic pathway and the enzyme system concerned have been for the most part clarified (29). The writer and his associates have traced many problems on the metabolism of volatile fatty acids in the rumen epithelium (11–14) and in mammary glands of ruminants (30, 31). As the result, we were led to the conclusion that it is inappropriate to apply the present metabolic pattern which is found in the tissues of non-ruminants directly to the corresponding tissues of ruminants.

The results of our investigation about the aerobic metabolism of volatile fatty acids in the liver slices of ruminants will be reported in this paper.

Methods and Materials

The method used in all the experiments was the shaking culture method in which Krebs-Ringer phosphate buffer (pH 7.2) containing liver slices of cattle obtained from the slaughter-house and substrates, were incubated with oxygen

gas for three hours at 38°C. The amounts of the used liver slices and substrates are described in each paragraph of the experiment. The measurement of oxygen uptake was made with the Warburg manometer (14) and the determination of volatile fatty acids was made with the Conway's diffusion method (32), while the ketone body and glucose were measured with the Thin-Robertson's method (33) and the Hagedorn-Jensen's method (34) respectively.

Results and Discussion

1. *The metabolism of volatile fatty acids and glucose in the liver slice of ruminants.*

The first investigation was made on the metabolic pathways of acetate, propionate and butyrate which occupied the greater part of the volatile fatty acids in the rumen contents.

Table 1. The volatile fatty acids and glucose consumptions and ketone body production in the liver slice of ruminants.

Additions	Fatty acid used(A)	Ketone body formed(B)	(B)/(A)×100
—	0.7μM	0.8μM	— %
Acetate	28.9	3.7	12.8
Propionate	26.9	0.3	1.1
Butyrate	29.6	15.2	51.4
Glucose	32.3	0.7	2.2

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100 μM of each substrate.

As shown in Table 1, the results showed that the liver slices consumed a considerable amount of volatile fatty acids though smaller than that of glucose. In this case a large amount of the ketone body was formed from butyrate. The formation of the ketone body was also seen in the case of acetate although its amount was smaller than that of butyrate. When propionate was used as the substrate, the amount of formed ketone body became smaller than in the case of no substrate. Similar result was obtained in the case of glucose. These tendencies were entirely the same as in the case of the rumen epithelium. However, the consumption of propionate and acetate was extremely abundant in the liver slices as compared with the rumen epithelium. It may be said that liver is an important organ for ketone body production, the same as rumen epithelium, even *in situ*. Amount of ketone body formed in the liver, therefore, has great influences on the intact animal.

In the next experiment, the measurement of oxygen uptake by liver slices when incubated with volatile fatty acids and glucose was made for the purpose of determining the degree of the oxidation of volatile fatty acids consumed.

Table 2. The oxidations of volatile fatty acids and glucose in liver slices of ruminants.

Additions	Oxygen uptake
—	153 μl
Acetate	183
Propionate	198
Butyrate	169
Glucose	175

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10 μM of each substrate.

As shown in Table 2, the results demonstrated that each substrate did not considerably increase the oxygen uptake of the liver slices. The propionate increased only 29 per cent of the oxygen uptake in maximum as compared with that of no substrate. The fact that the addition of the substrate was not so influential on respiration may be due to the great influences of the substances which are naturally contained abundantly in the liver slice on respiration. However, the fact that only small amounts of the oxygen uptake occured in spite of the greater consumption of each volatile fatty acid, may lead to an assumption that in the liver slice volatile fatty acids are metabolized greatly in amount in another way than that of the oxidation.

2. *The relationship between the metabolism of volatile fatty acids and T. C. A. Cycle in liver slice of ruminants.*

The formation of C_2 compound from fatty acid by β-oxidation has been clarified since the classical work of Knoop and, in addition, the formation of the C_2 compound in the liver tissues has been known. At present, it is known that fatty acids metabolism is closely related with T. C. A. Cycle at the initiation and termination of their metabolism. Many workers have so far confirmed that the addittion of a small amount of the materials belonging to T. C. A. Cycle to fatty acid accelerate the activation of fatty acids by placing them on the belt of metabolism and induce complete oxidation by T. C. A. Cycle at the terminal stage so as to smooth the metabolism of them (35, 36).

Table 3. The effect of succinate on the oxidation of acetate.

Additions	Oxygen uptake
—	173 μl
Acetate	235
Succinate	302
Acetate + Succinate	395

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10 μM of each substrate.

An additional investigation was made on succinate belonging to the members of T. C. A. Cycle to examine the relationship between T. C. A. Cycle and the metabolism of acetate, propionate and butyrate in the liver slice of ruminants. As shown in Table 3, the results showed that the coexistence of succinate and acetate increased the oxygen uptake considerably, although succinate itself had a comparatively high oxygen uptake.

Then, the consumption of acetate and formation of the ketone body were investigated. As shown in Table 4, the results indicated that succinate accelerated the consumption of acetate and inhibited completely the formation of the ketone body from acetate.

In addition, as shown in Tables 5 and 6, the consumption and oxidation of propionate were accelerated by succinate.

Table 4. The effects of succinate on the acetate consumption and ketone body formation.

Additions	Fatty acid used (A)	Ketone body formed (B)	(B)/(A)×100
—	(2.2)μM	0.9μM	— %
Acetate	32.5	4.8	14.8
Succinate	(2.6)	0.2	—
Acetate+Succinate	38.7	0.7	1.8

One g of liver slices were incubated in 10*ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

Table 5. The effects of succinate on the propionate consumption and ketone body formation.

Additions	Propionate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.8)μM	1.2μM	— %
Propionate	27.3	0.2	0.7
Succinate	(2.1)	0.4	—
Propionate+Succinate	31.1	0.2	0.6

One g of liver slices were incubated in 10*ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

Table 6. The effect of succinate on the oxidation of propionate.

Additions	Oxygen uptake
—	131μl
Propionate	180
Succinate	224
Propionate+Succinate	272

One hundred mg of liver slices were incubated in 2*ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

As shown in Table 7, the investigation on the effects of succinate on butyrate metabolism showed that the coexistence of butyrate and succinate

increased the oxygen uptake more than that of each substrate alone.

Table 7. The effect of succinate on the oxidation of butyrate.

Additions	Oxygen uptake
—	186μl
Butyrate	202
Succinate	264
Butyrate + Succinate	307

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

Table 8. The effects of succinate on the butyrate consumption and ketone body formation.

Additions	Butyrate used(A)	Ketone body formed (B)	(B)/(A)×100
—	(1.2)μM	2.4μM	— %
Butyrate	26.7	13.5	50.6
Succinate	(0.9)	2.1	—
Butyrate + Succinate	29.4	7.3	24.8

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

As shown in Table 8, the consumption of butyrate increased in the presence of succinate and the formation of the ketone body from butyrate was inhibited considerably with the addition of succinate.

It is noteworthy that succinate accelerated the oxidation of acetate and butyrate and inhibited the formation of the ketone body. As for succinate which is being used as the curative agent for ketosis in ruminants, the writers recognized that ketosis caused by the intravenous injection of butyrate was avoidable by the simultaneous injection of succinate (37). However, in the rumen epithelium, succinate works to the direction of ketone body formation and the coexistence of succinate with butyrate accelerates the formation of the ketone body from butyrate (38). As the ability to form the ketone body from the volatile fatty acid in excised rumen epithelium was greater than that of the liver slice, the rumen epithelium has been considered as the influential organ for the development of ketosis. The same conclusion has been reached from the arteriovenous difference experiment. It was confirmed that succinate caused a strong antiketogenic function in the liver slice and simultaneously accelerated the aerobic metabolism of acetate, propionate and butyrate. It is assumed that even in the case of the living body of ruminants, succinate would exert an antiketogenic function to butyrate and acetate metabolism in the liver by inhibiting the formation of ketone body. From the observations that succinate has an antiketogenic action not only on the liver but also on the whole body except rumen epithelium in which succinate works as a ketogenic agent,

it is assumed that the liver and rumen epithelium have different metabolic systems for volatile fatty acids.

In view of the fact that succinate smooths the aerobic metabolism of acetate, propionate and butyrate, metabolism of these fatty acids and T. C. A. Cycle should be related closely with each other in the liver slice. Therefore, further investigation was made on the relationship between the aerobic metabolism of acetate, propionate and butyrate and T. C. A. Cycle which was inhibited with malonate.

Table 9. The effect of malonate inhibition on the oxidation of acetate.

Additions	Oxygen uptake
—	124 μl
Malonate	38
Acetate	165
Acetate + Malonate	29

One hundred mg of liver slices were incubated in 2 ml of Krebs-Ringer phosphate buffer containing 10 μM of acetate. Malonate concentration was 0.005M/l.

As shown in Table 9, the results showed that the endogeneous respiration were affected with malonate inhibition markedly and the oxidation of acetate was inhibited completely.

Table 10. The effects of malonate inhibition on the consumption of acetate and the ketone body formation.

Additions	Acetate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(2.5)μM	1.4μM	— %
Acetate	32.9	4.8	14.7
Malonate	(1.9)	1.6	—
Acetate + Malonate	6.3	2.1	33.3

One g of liver slices were incubated in 10 ml of Krebs-Ringer phosphate buffer containing 100μM of acetate. Malonate concentration was 0.005M/l.

As shown in Table 10, malonate inhibited the consumption of acetate to a considerable extent. Accordingly, the absolute amount of the formed ketone body came to decrease, but the rate of formed ketone body to the consumption of acetate increased by the effect of malonate inhibition.

As shown in Tables 11 and 12, the consumption and oxidation of propionate were inhibited with malonate.

Subsequently, investigation was made on the effect of malonate inhibition on the metabolism of butyrate.

As shown in Table 13, the oxidation of butyrate was considerably inhibited with malonate.

Table 11. The effect of malonate inhibition on the propionate oxidation.

Additions	Oxygen uptake
—	162μl
Propionate	228
Malonate	51
Propionate + Malonate	80

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10μM of propionate. Malonate concentration was 0.005M/l.

Table 12. The effect of malonate inhibition on the consumption of propionate.

Additions	Propionate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.3)μM	1.1μM	— %
Propionate	24.7	0.4	1.6
Malonate	(1.2)	1.8	—
Propionate + Malonate	5.3	0.9	17.0

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100 μM of propionate. Malonate concentration was 0.005 M/l.

Table 13. The effect of malonate inhibition on the butyrate oxidation.

Additions	Oxygen uptake
—	160 μl
Butyrate	173
Malonate	47
Butyrate + Malonate	56

One hundred mg of liver slices were incubated in 2*ml* of Krebs-Ringer phosphate buffer containing 10μM of butyrate. Malonate concentration was 0.005M/l.

Table 14. The effects of malonate inhibition on the consumption of butyrate and ketone body formation.

Additions	Butyrate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.7)μM	1.9μM	— %
Butyrate	36.4	14.5	39.8
Malonate	(0.9)	2.4	—
Butyrate + Malonate	4.1	3.3	80.5

One g of liver slices were incubated in 10*ml* of Krebs-Ringer phosphate buffer containing 100 μM of butyrate. Malonate concentration was 0.005 M/l.

Moreover, as shown in Table 14, the consumption of butyrate decreased with the addition of malonate. Accordingly, although the absolute amount of the formed ketone body was a decrease, the rate of the amount of formed

ketone body to the consumpted amount of butyrate showed a considerable increase by the effect of malonate inhibition.

In the rumen epithelium the oxidation of butyrate was not influenced by malonate inhibition, and the consumption of acetate and butyrate and the formation of ketone body from these acids were both scarcely affected with malonate inhibition. In the case of the liver slice, the aerobic metabolism of acetate, propionate and butyrate was considerably inhibited by the action of malonate. Unlike the rumen epithelium, these facts signify that the metabolic systems of these volatile fatty acids and T. C. A. Cycle are closely related with each other in the liver slice.

3. *The influences of glucose and propionate on the metabolisms of acetate and butyrate.*

It has been known that in the liver slice succinate which belonged to the members of T. C. A. Cycle accelerated the aerobic metabolism of the volatile fatty acids, and that malonate which is an inhibitory agent to T. C. A. Cycle inhibited the metabolism of those acids.

It has been well known that glucose is oxidized into CO_2 and H_2O by the function of T. C. A. Cycle under the aerobic condition. In addition, it has been reported that glucose shows generally the antiketogenic actions, for instance, the ketosis caused by high fat diet is suppressed by the simultaneous supply of glucose (39). On the contrary the accumulation of ketone body in blood was brought about by blocking glucose metabolism with alloxan (40). In consideration of these facts, it is not difficult to imagine that the glucose metabolism and the metabolic system of volatile fatty acids mutually influenced each other in the liver slice.

Therefore, investigations were made on the effects of glucose upon the metabolisms of the ketogenic volatile fatty acids, acetate and butyrate.

Table 15. The effect of glucose on the oxidation of acetate.

Additions	Oxygen uptake
—	98μl
Acetate	135
Glucose	131
Acetate + Glucose	148

One hundred mg of liver slices were incubated in 2ml of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

As shown in Table 15, the result demonstrated that the coexistence of acetate and glucose resulted in somewhat higher oxygen uptake than the single use of glucose, although the difference was not so significant.

Table 16. The effects of glucose on the consumption of acetate and ketone body production.

Additions	Acetate used (A)	Glucose used	Ketone body formed (B)	(B)/(A)×100
—	(2.5)μM	(4.3)μM	0.9μM	— %
Acetate	31.7	(4.1)	5.7	18.0
Glucose	(1.9)	26.9	0.7	—
Acetate+Glucose	37.4	19.2	3.1	8.3

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

As shown in Table 16, the consumption of acetate increased and the amount of ketone body formed decreased by the addition of glucose. The consumption of glucose was decreased under the coexistence of acetate as compared with that of glucose alone. Next, an investigation was made on the effect of glucose upon butyrate metabolism.

Table 17. The effect of glucose on the oxidation of butyrate.

Additions	Oxygen uptake
—	170 μl
Butyrate	248
Glucose	266
Butyrate+Glucose	262

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

As a result, the addition of glucose to butyrate showed a similar oxygen uptake same as by the single use of glucose. The consumption of butyrate and glucose showed almost no difference under the conditions either of their coexistence and single use, as shown in Table 17. The formation of ketone body from butyrate was not affected with glucose. These trends were similar to the cases of rumen epithelium. Consequently, the metabolisms of butyrate and glucose in both the liver slice and the rumen epithelium are considered to take place independently.

Table 18. The effects of glucose on the consumption of butyrate and ketone body formation.

Additions	Butyrate used (A)	Glucose used	Ketone body formed (B)	(B)/(A)×100
—	(1.5)μM	(3.7)μM	2.2μM	— %
Butyrate	39.5	(3.2)	13.3	33.7
Glucose	(0.9)	32.4	1.8	—
Butyrate+Glucose	41.7	32.9	13.1	31.4

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

Investigation was made on the effect of propionate, which is a precursor of T. C. A. Cycle, like glucose, and has a strong antiketogenic action, upon the metabolisms of acetate and butyrate.

Table 19. The effect of propionate on the oxidation of acetate.

Additions	Oxygen uptake
—	134μl
Acetate	189
Propionate	228
Acetate + Propionate	126

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

As shown in Table 19, the result indicated that coexistence of acetate and propionate demonstrated a comparatively low value oxygen uptake as compared with that of their single use. However, as shown in Table 20, the addition of propionate to acetate showed 42.3μM in volatile fatty acids consumption, not being particularly lower than the single case of them. The formation of ketone body from acetate decreased considerably by the addition of propionate.

Table 20. The effect of propionate on the ketone body formation from acetate.

Additions	Fatty acids used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.2)μM	0.6μM	— %
Acetate	35.2	5.3	15.1
Propionate	31.8	0.2	0.6
Acetate + Propionate	42.3	0.9	2.1

One g of liver slices were incubated in 10*ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

A considerable consumption of volatile fatty acids with the decrease of ketone body formation and the decrease of oxygen uptake should be regarded as the results of anabolic and migratory natures of acetate and propionate metabolisms.

When the formations of glucose and lactate were measured in another experiment under the same conditions, lactate tended to increase a little under the presence of propionate, though not so great. Meanwhile, the synthesized glucose did not indicate any increase (30).

In the rumen epithelium the addition of propionate to acetate showed a marked increase of oxygen uptake and its increase was inhibited with the use of malonate. This was considered due to the increase of the amounts of acetate and propionate entered to T. C. A. Cycle. On the contrary, in the mammary

gland slice of ruminants, the coexistence of propionate with acetate or butyrate indicate a decrease of oxygen uptake (31). The mechanism of such an oxidation inhibition action of propionate and the process of its antiketogenic action are both causing a great problem.

If volatile fatty acids are assumed to proceed toward the synthetic system, the participation of propionate in the process of formation of lipids, other than the course to glucose, may be taken into account.

Next an investigation was made on the effect of propionate on the metabolism of butyrate.

Table 21. The effect of propionate on the oxidation of butyrate.

Additions	Oxygen uptake
—	174 μl
Butyrate	180
propionate	217
Butyrate + Propionate	206

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10 μM of each substrate.

As shown in Table 21, the result showed that the coexistence of butyrate and propionate did not decrease the oxygen uptake, different from the case of acetate. However, as shown in Table 22, it may be said that the substrates consumption as total volatile fatty acids decreased to a certain extent. The formation of ketone body from butyrate was exceedingly decreased with the addition of propionate.

Table 22. The effect of propionate on the ketone body formation from butyrate.

Additions	Fatty acids used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.7)μM	1.9μM	— %
Butyrate	36.4	12.7	34.9
Propionate	22.0	0.6	2.7
Butyrate + Propionate	32.0	1.2	3.8

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

Glucose inhibited only the formation of ketone body from acetate and did not indicate any influence on the formation of ketone body from butyrate, while propionate inhibited the formation of ketone body both from acetate and butyrate.

Accordingly, the mechanism of antiketogenic actions of glucose and propionate should be regarded as different from each other. In the rumen epithelium, the formation of ketone body from butyrate was not markedly inhibited

by propionate. In view of the fact that in the liver slice the formation of ketone body was completely inhibited by propionate, it may be said that either the mechanism of ketone body formation from butyrate or the process of antiketogenic action of propionate should be different in the liver slice and the rumen epithelium.

4. *The effect of pyruvate on the metabolisms of acetate and butyrate.*

Pyruvate has been placed in an important position in the metabolic pathways of glucose and propionate. As various systems are linked with pyruvate regarded as a center of them, the deployment of different metabolic pictures depends upon how pyruvate acts. Therefore, the effect of pyruvate was investigated on the aerobic metabolism of acetate and butyrate.

Table 23. The effect of pyruvate on the oxidation of acetate.

Additions	Oxygen uptake
—	93 μl
Acetate	128
Pyruvate	147
Pyruvate + Acetate	152

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

As shown in Table 23, the results indicated that the addition of pyruvate to acetate did not show any great difference in oxygen uptake as compared with the single use of pyruvate.

Table 24. The effects of pyruvate on the acetate consumption and ketone body formation.

Additions	Acetate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(2.3)μM	1.5μM	— %
Acetate	24.9	3.1	12.4
Pyruvate	(4.7)	1.9	—
Acetate + Pyruvate	22.5	2.6	11.6

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

As shown in Table 24, pyruvate did not influence greatly on the consumption of acetate. Although the formation of ketone body from acetate tended to decrease somewhat with the use of pyruvate, the metabolism of acetate, on the whole, was not influenced by pyruvate.

As shown in Table 25, the effects of pyruvate on the consumption of butyrate and on the formation of ketone body were not observed.

Table 25. The effects of pyruvate on the consumption of butyrate and ketone body formation.

Additions	Butyrate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.3)μM	1.7μM	— %
Butyrate	38.1	12.6	33.1
Pyruvate	(2.7)	2.4	—
Butyrate+Pyruvate	41.3	12.6	30.5

One g of liver slices were incubated in 100 *ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

Moreover, as shown in Table 26, the coexistence of butyrate and pyruvate showed similar values of oxygen uptake to the single use of pyruvate.

Table 26. The effect of pyruvate on the oxidation of butyrate.

Additions	Oxygen uptake
—	98 μl
Butyrate	135
Pyruvate	161
Butyrate+Pyruvate	158

One hundred mg of liver slices were incubated in 2 *ml* of Krebs-Ringer phosphate buffer containing 10μM of each substrate.

Although pyruvate, like glucose, acted only on the metablism of acetate and had no effect on the metabolism of butyrate, the antiketogenic action of pyruvate was extremely weak as compared with that of glucose. Accordingly, it is conceivable that there was a very small portion of glucose consumed in the liver slice passed through pyruvate. It might be worthwhile to test the antiketogenic function of some substance considered as an intermediate between the sequence from glucose to pyruvate. To clarify this, the effect of phosphoenolpyruvate was investigated on the formations of ketone body from acetate and butyrate.

Table 27. The effects of phosphoenolpyruvate (P. E. P.) on the consumption of acetate and ketone body formation.

Additions	Acetate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.5)μM	1.6μM	— %
Acetate	25.6	4.9	19.1
P. E. P.	(0.9)	0.8	—
Acetate+P. E. P.	32.9	0.8	2.4

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100 μM of each substrate.

As shown in Table 27, the result showed that phosphoenolpyruvate increas-

ed the consumption of acetate and decreased greatly the formation of ketone body.

Table 28. The effects of phosphoenolpyruvate (P. E. P.) on the consumption of butyrate and ketone body formation.

Additions	Butyrate used (A)	Ketone body formed (B)	(B)/(A)×100
—	(1.2)μM	0.8μM	— %
Butyrate	21.9	7.8	35.6
P. E. P.	(0.7)	0.2	—
Butyrate+P. E. P.	22.5	7.7	34.2

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

As shown in Table 28, phosphoenolpyruvate had no effect on the metabolism of butyrate. Since this trend is similar to the case of glucose, glucose is considered not to proceed into pyruvate but to enter T. C. A. Cycle through phosphoenolpyruvate.

5. *The antiketogenic action of propionate and succinate in the liver slice.*

As described above, it has been observed that propionate and succinate showed considerable inhibition for the ketone body formation from acetate and butyrate.

With the use of liver slices, several investigations were made on the antiketogenic actions of propionate and succinate. First, observation was made to determine on which fractions of the ketone body, i. e., acetone fraction and fraction including acetoacetate and β-hydroxybutyrate, were mostly influenced under the condition that the ketone body formation from butyrate inhibited with propionate or succinate.

Table 29. The effect of propionate on the each fraction of ketone body formed from butyrate.

Additions	Fatty acids used	Acetone formed	Acetoacetate+β-Hydroxybutyrate
—	(0.9)μM	0.30μM	2.51μM
Butyrate	25.3	4.35	8.97
Propionate	24.9	0.01	1.74
Butyrate+Propionate	22.5	0.02	4.71

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

As shown in Table 29, the results showed that propionate did not only decrease the amount of acetoacetate and β-hydroxybutyrate but also had a marked effect on acetone formation, and the amount of acetone formed was negligible.

Table 30. The effect of succinate on the each fraction of ketone body formed from butyrate.

Additions	Fatty acid used	Acetone formed	Acetoacetate + β-Hydroxybutyrate
—	(1.8)μM	0.32μM	1.57μM
Butyrate	20.0	3.12	6.98
Succinate	(2.1)	0.13	2.04
Butyrate + Succinate	26.3	0.19	4.35

One g of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 100μM of each substrate.

This result is almost the same as in the cases of inhibition of the ketone body formation from acetate by propionate in the rumen epithelium (30).

As shown in Table 30, succinate also considerably decreased the amount of acetone formed from butyrate.

Although the causes for the antiketogenic action of propionate and succinate are attributable to various reasons, the first consideration can be given to the effect of these materials on the metabolism of acetoacetate and acetone formed from butyrate.

Table 31. The effect of propionate and succinate on the metabolism of acetoacetate.

Additions	Tissue additioned	Acetone formed	Acetoacetate recovered
—	Fresh liver slices	0.07μM	1.59μM
Acetoacetate	None	0.71	28.12
Acetoacetate	Boiled liver slices	0.84	28.01
Acetoacetate	Fresh liver slices	1.01	25.20
Propionate	"	0.03	1.05
Succinate	"	0.01	1.01
Acetoacetate + Propionate	"	0.95	24.02
Acetoacetate + Succinate	"	0.73	24.10

Five hundred mg of liver slices were incubated in 10*ml* of Krebs-Ringer phosphate buffer containing 30μM of acetoacetate or 50 μM of each propionate and succinate.

Table 32. The effects of propionate and succinate on the consumption of acetone.

Additions	Acetone used
—	(0.08)μM
Succinate	(0.02)
Succinate	(0.01)
Acetone	1.65
Acetone + Propionate	1.63
Acetone + Succinate	1.71

Five hundred mg of liver slices were incubated in 10 *ml* of Krebs-Ringer phosphate buffer containing 10μM of acetone or 50μM of each propionate and succinate.

As shown in Tables 31 and 32, propionate and succinate increased somewhat the consumption of acetoacetate. However, the participation of liver slices was not seen in the acetone formation from acetoacetate and, propionate and succinate had no effect on the consumption of acetone.

Therefore, it is still unknown to localize the causes for that propionate and succinate reduce the amount of acetone in the ketone body formed from butyrate markedly.

Summary

It is assumed that in ruminants a large amount of volatile fatty acids produced in the rumen is absorbed into ruminous vein and then proceeds into the portal vein followed by their metabolization in the liver. The following results were obtained from the investigations on the metabolisms of volatile fatty acids with the use of the liver slices of cattle.

1). The liver slice consumed acetate, propionate and butyrate and formed the ketone body from butyrate and acetate. None of the acids showed any great increase of oxygen uptake compared with that of the liver slice alone. Accordingly, a very small portion of volatile fatty acids was considered to proceed into the oxidation system in the liver slice.

2). Succinate accelerated the consumption and oxidation of acetate, propionate and butyrate and inhibited greatly the ketone body formation from acetate and butyrate.

3). Malonate inhibited considerably the aerobic metabolisms of acetate, propionate and butyrate. Consequently, in view of the result of 2), the metabolisms of volatile fatty acids and T. C. A. Cycle in liver slice are considered to have a close relationship.

4). Glucose increased the consumption of acetate and decreased ketone body formation from acetate. Glucose had no effect on the metabolism of butyrate. The same pattern was applicable to phosphoenolpyruvate.

5). Propionate inhibited markedly the ketone body formations from acetate and butyrate. In this case, the coexistence of acetate and propionate indicated lower value of oxygen uptake than the single use of these materials. Since the consumptions of volatile fatty acids also showed no particular decrease, it was assumed that volatile fatty acids proceeded in some other way than the oxidation system by the addition of propionate.

6). Propionate and succinate increased the consumption of acetoacetate a little, but had no effect on the acetone formation from acetoacetate and the consumption of acetone.

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