STUDIES ON THE SALIVA AND SALIVATION OF RUMINANTS I. THE METABOLISM OF VOLATILE FATTY ACIDS IN THE SLICES OF RUMINANT SALIVARY GLAND

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I. THE METABOLISM OF VOLATILE FATTY ACIDS IN THE SLICES OF RUMINANT SALIVARY GLAND

By

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(Received July 15, 1960)

Introduction

In ruminants, the diets ingested into the animal are utilized as effective nutrient for many organs and tissues only through the rumen digestion in which extensive fermentation is proceeded. Cellulose, the main energy source of ruminants is attacked by the rumen bacteria with the production of the volatile fatty acids, including acetate as the main constituent from which the animal is supplied with caloric energy. Other nutrients are also absorbed and utilized by the animal body after receiving the microbial synthesis or decomposition in the rumen. With the recent advance of ruminology, the effect of the ruminant saliva on rumen conditions has been regarded considerably important (1~7). Expressing this effect briefly, it is the effect on the maintenance of rumen constancy. From the view point of nutritional physiology of ruminants, it may be not too much to say that whether the ruminant body can be maintained physiological normal conditions depends upon the maintenance of the normal rumen conditions. There are several effects of ruminant saliva on the maintenance of the rumen constancy. The volatile fatty acids produced in the rumen are neutralized with the buffer action of saliva. An adult cow secretes about 50 *l*/day of saliva containing 300~350 g of bicarbonate and this amount of bicarbonate can speculatively neutralize 56~60 *l* of 0.1N HCl (8). Ruminant saliva has also another significance as the aqueous solution to the rumen constancy. It can preserve the volume of rumen fluid as the medium for microorganisms in which the growth and development of rumen bacteria and many sorts of the chemical reaction proceed. Other physicochemical important natures of the ruminant saliva is the nature of the low surface tension for retaining the optimum surface

tension of the medium in rumen to accelerate the development of rumen bacteria (9). Since the salivary salts derived from the blood stream may be reabsorbed through the alimentary tract, the circulation of salivary salts should arrest our attention pertaining to acid-base balance in the animal body. Thus, different from non-ruminant saliva, ruminants secrete more effective saliva than non-ruminants not only for mastication, swallowing and digestion to some extent but also for the particular role mentioned above. We studied the metabolism of the volatile fatty acids, mainly oxygen uptake and substrate consumption in the slices of ruminant salivary gland from the point of supply of energy for activation of the gland.

The classical interest that ruminants could maintain their life by the ingestion of cellulose which is the constituent of most of the vegetable foodstuff, in spite of the lack of the celluloytic enzyme in their alimentary tract, seems to be primitive when the recent advancement of ruminology is considered. In reality there are many problems to be solved in the field of ruminology, especially of the nutritional physiology of ruminants. It is a matter of course that the feeds ingested into the rumen do not contain all "true" nutrients for the ruminant body, but they can become effective nutrients through the extensive rumen fermentation. In other words, there are so many significances in the rumen fermentation that we may introduce a conception for the process of the ruminant digestion and absorption that the rumen fermentation changes the superficial nutrients in the feeds to the effective "true" nutrients for the animal body. Thus, rumen fermentation focuses our attention the study of ruminology even though it is a complicated phenomenon. Above all, a problem to be made clear firstly is the fate of the volatile fatty acids produced in rumen in the animal body. The studies by many investigators were required until the fact that volatile fatty acids effective to the maintenance of the ruminant body was acknowledged widely. Looking at these studies successively in the phase of the process of the utilization of volatile fatty acids in the ruminant body, Phillipson (30) and Barcroft (18) showed that the concentration of volatile fatty acids in the ruminal vein was higher than that of any other vein and Umezu (22) also recognized this fact by arterio-venous differences. Tsuda (21) proved the absorption of these acids through the rumen wall by the use of rumen pouch. As many attentions were given to the evidences that the volatile fatty acids produced in the rumen were absorbed by the ruminant body through rumen wall, the studies concerning the metabolism of these acids in the animal body attracted our interests. The fact about the utilization of volatile fatty acids in the ruminant body was proved by many experiments with the use of arterio-venous difference. Reid (15) measured the arterio-venous difference in the head circulation and McClymont (31) showed the utilization of volatile fatty

acids in ruminant mammary gland. Umezu (22, 23) discussed the volatile fatty acid utilization in several tissues and organs of ruminants by the arterio-venous difference in relation to other intermediates (blood glucose, lactate, pyruvate, ketone bodies and carbon dioxide). From these experimental facts, it may be easy to recognize the utilization of volatile fatty acids in the whole ruminant body. On the other hand, few investigators have studied the metabolism of these acids in individual organs and tissues of ruminants and detailed studies concerning this phase were reported by Penington dealing with rumen epitherium (27), Folley with the mammary gland (28), Seto on the rumen epitherium (23~26). As the volatile fatty acids could be considered the particular tissue nutrient, we may not make a big mistake if we assume that these acids may be utilized more than glucose in the whole tissues and organs in the ruminant body. However, Seto (32) reported that the metabolic pattern in the slices taken from the ruminant liver, kidney and heart muscle showed the utilization of glucose higher than acetate, similarly to that of non-ruminants. To apprehend this evidence, it may convince us to consider that the level of these free acids in the blood stream of ruminants was very low in contrast to that of glucose as already well acknowledged. However, here was an organ which showed so different metabolic pattern that the above consideration was difficult to apply to it. Namely, Folley (28) found that on the contrary of non-ruminant, the mammary gland slices taken from the ruminant oxidized acetate more than glucose in vitro. We have merely understood this evidence conceptually as the speciality of the mammary gland of ruminants used as a milking animal. Some questions have given rise to whether the speciality that acetate is utilized more than glucose exists only in the mammary gland, or whether the high acetate utilization would have been applicable to many other organs and tissues of the ruminant. The studies reported here demonstrated as the first step of the further studies on the saliva and salivation of ruminants and also to get a factor to prove the previously noted question.

Materials and Methods

About 500 mg of the cow parotid gland slices taken from a slaughter-house were shaken with 10 *ml* of Krebs-Ringer phosphate buffer, pH 4, containing 100 μM of each substrate in about 100 *ml* Waruburg manometric flasks under 100 per cent oxygen gas phase at 37.5C for three hours. The carbon dioxide produced was absorbed by 1 *ml* of 4N KOH in the center wall. The substrates used were Na-salts of formate, acetate, propionate, butyrate and glucose. The oxygen uptake was observed at the intervals of 15 minutes and after three hours the substrate consumption was measured. The volatile fatty acids were determined by titration of 0.01N NaOH after

Friedmann's vapor distillation (10) and glucose was done by Hagedron-Jensen's method (11). The rat salivary gland slices were also used with substrates of glucose and acetate on behalf of the determination of the volatile fatty acid consumption in contrast to ruminants.

Results and Discussions

Utilization of acetate and non-utilization of glucose in the ruminant salivary gland slices.

We could clearly show that the cow parotid gland slices utilized remarkably acetate owing to its oxygen uptake and the substrate consumption as shown in Table 1.

Table 1. Oxygen uptake and substrate consumption of the cow partid gland slices in the presence of volatile fatty acids and glucose.

Additions	Oxygen uptake*	Substrate consumption**
Acetate	58	11.0
Butyrate	33	6.5
Propionate	18	4.1
Formate	3	1.8
Glucose	−5	2.3

* μl./100mg. wet tissue/hr.
** μM./100mg. wet tissue/3hrs.

The oxygen uptake of the acetate was 58 μl/100 mg wet tissue/hr and was about 1.7 times as much as butyrate, three times as much as propionate. Of the volatile fatty acids used, acetate most consumed oxygen, setting in the order of acetate, butyrate and propionate. Formate and one which was not added with the substrate showed about the same oxygen uptake. The oxygen up take of glucose was slightly below the non-substrate. As well as in the case of oxygen uptake, acetate was most consumed in the ruminant parotid gland slices. The acetate consumption of 11.0 μM/100 mg wet tissue for three hours was the greatest of all the substrates used. The next was butyrate of 6.5 μM and propionate was 4.1 μM. The acetate consumption was about 1.7 times as much as butyrate, 2.7 times as much as propionate. As for these three volatile fatty acids, both their order of magnitude of utilization and the ratio of utilization of butyrate, propionate to acetate were approximately the same in their oxygen and substrate consumption. Very small formate consumption of 1.8 μM was 16 per cent of acetate. From its oxygen uptake and the substrate consumption, it may be hardly recognizable for the formate to be utilized in the ruminant salivary gland. The glucose consumed was 2.3 μM while its oxygen uptake was below the non-substrate.

It may be considered that glucose enters into the metabolic pathway other than oxidation.

Table 2. Glucose and acetate utilization in the rat salivary gland.

Additions	Oxygen uptake*	Substrate consumption**
Glucose	72	7.7
Acetate	39	6.6

* μl./100mg. wet tissue/hr.
** μM./100mg. wet tissue/3 hrs.

Subsequently, in contrast to the ruminant, the rat salivary gland slices were shaken in the same manner as the cow parotid gland slices with acetate or glucose as substrates. Although the activity of the rat salivary gland slices was generally higher than that of the cow, the slices from the rat utilized both the acetate and the glucose as shown in Table 2. It might be said that since the rat salivary gland slices utilized not only glucose but also acetate, the difference between the metabolism of the acetate and that of the glucose in the cow and the rat should be presented by the difference of glucose utilization. There was little to choose between the two, but glucose, if anything, was more utilizable in the rat.

Many investigators (12–27) demonstrated the physiological effect of the volatile fatty acids produced in a large quantity in the rumen for the maintenance of the body of the ruminant. At present, artificial nutrition test by Shibata and Umezu (29) for the goats and the cows, dripping volatile fatty acids as a main energy source under the removal of the rumen contents, showed almost realistically the expected effect. On the other hand, the metabolism of the volatile fatty acids in the organs and tissues has been proceeded by Seto with the kidney, heart muscle, liver, rumen epitherium and mammary gland (23–26), by Penington with rumen epitherium (27) and by Folley with mammary gland (28). We obtained a result that the salivary gland of ruminants also utilized the acetate than the glucose as well as the ruminant mammary gland. This fact not only confirms our long entertained conception that the volatile fatty acids supply the gland with energy to activate the gland, and the glucose may be used as the substrate for the synthesis of the gland secretes, but also may induce the futher conception. It has not yet been proved whether the universality that the utilization of acetate is higher than that of glucose is applicable to all the organs of the body as well as the salivary and mammary gland. However, judging from the fact, as proved by Seto, that the metabolism of the volatile fatty acids in the liver, kidney and heart muscle of the ruminant is generally similar

to that of the rat, it is interesting to notice that both the mammary gland of ruminants almost used as a milking animal and the ruminant salivary gland secreting a large quantity of saliva which plays an important effect in rumen fermentation, utilize actively acetate because of their speciality of being the "gland".

Fffect of glucose on utilization of acetate and propionate in cow paroitd gland slices.

As shown in Table 3, the oxygen uptake of the coexistence of acetate and glucose was almost equal to that of acetate alone. The fact that the oxygen uptake of glucose was innert and the addition of glucose to acetate did not increase the oxygen uptake of acetate, must be investigated.

Table 3. The effect of glucose on oxidation of acetate.

Additions	Oxygen uptake
—	97
Acetate	159
Glucose	91
Acetate+Glucose	154

μl./100mg. wet tissue/hr.

Table 4. The effect of glucose on the acetate consumption.

Additions	Acetate used	Glucose usec
—	(0.0)	(0.0)
Acetate	11.7	(0.0)
Glucose	(0.0)	2.0
Acetate+Glucose	11.2	3.2

μM./100mg. wet tissue/3 hrs.

As shown in Table 4, the consumption of acetate did not undergo a change by the addition of glucose. The consumption of glucose was slightly increased in the coexistence of acetate as compared with that in glucose alone.

Table 5. The effect of glucose on oxidation of propionate.

Additions	Oxygen uptake
—	81
Propionate	111
Glucose	75
Propionate+Glucose	121

μl./100mg. wet tissue/hr.

Next, an investigation was made on the effect of glucose upon the propionate metabolism. The results are shown in Table 5. The addition of

glucose to propionate increased the oxygen uptake in comparison with propionate alone, and the glucose alone showed somewhat lower oxygen uptake than the non-substrate.

Table. 6 The effect of glucose on the propionate consumption.

Additions	Propionate used	Glucose used
—	(0.0)	(0.0)
Propionate	2.9	(0.0)
Glucose	(0.0)	2.0
Propionate+Glucose	3.2	3.6

μM./100mg. wet tissue/3 hrs.

As shown in Table 6, the propionate consumption was not affected by the addition of glucose. Glucose increased in its consumption under the coexistence of glucose and propionate as compared with glucose alone.

From these results, in the cow paroyid gland, it would be supported that the metabolic pathway of the acetate was different from that of the propionate. Thus, the ruminant salivary gland was one of the constractive organs that could utilize the acetate actively.

Summary

The metabolism of the volatile fatty acids was investigated in the ruminant salivary gland in vitro in contrast to that of the rat, a non-ruminant. Of the formate, acetate, propionate, butyrate and glucose used for the experiments, the acetate was most utilized according to its both oxygen uptake and substrate consumption. Glucose should not be regarded as the active substance for the ruminant salivary gland.

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