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Transfer of Carbon from Short-Chain Fatty Acids to Glucose and Lipids in Rumen Epithelium of Cow

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Summary

Ruminants produce large amounts of short-chain fatty acids in their rumen which, during absorption through rumen epithelium, are partly converted into other substances. The present study was designed to elucidate how is transformation is influenced by the presence of glucose or some glucose metabolites. Statistics were obtained on the transfer of ^{14}C to glucose, lipids and CO_2 from ^{14}C-acetate, -propionate, and -butyrate with the use of the rumen epithelium of cow, *in vitro*.

1. Carbon from the propionate was incorporated into the glucose to a greater extent than carbon from other substrates; carbon from butyrate was converted into lipids and CO_2 to a greater extent than carbon from other substrates.

2. Glucose labeling from acetate and propionate was decreased by the addition of glucose and phosphoenolpyruvate; lipid synthesis and CO_2 production from acetate and propionate was increased by the addition of glucose and phosphoenolpyruvate.

3. There was no apparent affect on the transfer of carbon from butyrate to glucose, lipid, or CO_2 when glucose, pyruvate, phosphoenolpyruvate or lactate were added to the system.

From these results and those previously obtained, it may be deduced that the glucose-phosphoenolpyruvate system affects acetate and propionate metabolism, but there appears to be no relationship between butyrate metabolism and glycolysis in rumen epithelium.

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It is generally accepted that large quantities of short-chain fatty acids in the rumen are absorbed through the rumen epithelium and many experiments have shown that some parts of the short-chain fatty acids are converted into ketone bodies (1, 2, 3), succinate (4, 5), malate (4), lactate (4, 5), fumarate (4), citrate (6), pyruvate (5), lipids (5, 7) and CO_2 (7, 8) in rumen epithelium of ruminants.

It has also been recognized that enzymes affecting carbohydrate and lipid metabolism are present in rumen epithelium of bovine (9). But there is not sufficient evidence to evaluate their role in glucose formation from short-chain fatty acids in rumen epithelium. This paper reports our investigation on the glucose formation from ^{14}C-short chain fatty acids in rumen epithelium of cow. It also presents data on lipid synthesis and CO_2 production from short-chain fatty acids in the presence of glucose and its metabolites.

Methods and Material

Fresh cow rumen epithelium was obtained from the slaughter house. It was separated from the muscle layer and cut into pieces of about $1 \times 1 \times 0.3$ cm. Incubation was carried out in 50 ml Erlenmyer flasks fitted with 2 ml center wells. To each flask was added 2 g of rumen epithelium, 10 ml of Krebs-Ringer bicarbonate buffer pH 7.2, and 100 μmoles (1 μC) of ^{14}C labelled sodium acetate, propionate, or butyrate. The flasks were closed with serum caps after gassing with O_2+CO_2 (95:5). Three flasks were incubated for each animal to allow for separate assays of glucose, lipid, and CO_2. Incubations were carried out for 3 hrs. at 38°C with a shaking rate of 100 strokes/minute.

Glucose was isolated as the glucose pentaacetate from the incubation medium by Jone's method (10). Total lipid was extracted from the tissue and medium with a mixture of chloroform and methanol (2:1), followed by aqueous washing of the extract as described by Folch, Lees and Sloane-Stanley (11). Separation of lipid fraction was effected on silicic acid columns. Successive fractions were eluted with 2 per cent ethyl ether in n-hexane (fraction A), 10 per cent ethyl ether in n-hexane (fraction B), 50 per cent ethyl ether in n-hexane (fraction C), 25 per cent methanol in ethyl ether (discarded), and pure methanol (fraction D). Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol and phospholipid, respectively. To trap the CO_2, 1 ml of Hyamine was injected into the center well of the Erlenmyer flask and 0.5 ml of 10 N H_2SO_4 into the medium. Then the flasks were shaken 30 minutes in ice prior to quantitative removal of the Hyamine for estimation of total radioactivity in the trapped CO_2.

The total radioactivity in each of these fractions was measured by using 15 ml of a scintillation system in toluene composed of 0.01 per cent POPOP and 0.5 per cent PPO by the liquid scintillation counter. Nitrogen determinations in rumen

pithelium were carried out by the micro-Kjeldahl method. Results are expressed s mean μmC/3hr/g of tissue nitrogen±standard deviation (S.D.).

Results and Discussion

1. The formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-short-chain ıtty acids

The first investigation was made on the utilization of ^{14}C-1-acetate, ^{14}C-2-cetate, ^{14}C-1-propionate, ^{14}C-2-propionate, ^{14}C-1-butyrate, ^{14}C-2-butyrate, and ^{14}C-3-butyrate, in order to observe what parts of the short-chain fatty acids were ɔnverted into each fraction.

As shown in Table 1, ^{14}C from all positions of acetate, propionate, and buty-ıte was converted into glucose, lipids and CO_2. ^{14}C-Propionate was incorporated ıto glucose to a greater extent than acetate or butyrate. Acetate, propionate and utyrate labeled in the C–2 position produced more ^{14}C-glucose than those labelled ı other positions. These results were almost the same as in the case of sheep-liver ices (12).

TABLE 1. *Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Short-Chain Fatty Acids in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total Lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction C[a]	CO_2
^{14}C-1-Acetate	9.4 ±0.8[b]	68.8 ±3.5	2.8 ±0.3	11.4 ±0.6	4.8 ±0.5	38.2 ±1.1	290.3 ±6.3
^{14}C-2-Acetate	18.7 ±1.1	92.5 ±3.1	5.2 ±0.2	12.8 ±0.9	6.5 ±0.8	56.1 ±3.4	98.6 ±3.5
^{14}C-1-Propionate	32.5 ±2.1	17.1 ±0.8	T[c]	4.8 ±0.5	T	9.4 ±0.6	153.6 ±7.4
^{14}C-2-Propionate	61.2 ±2.4	29.3 ±1.9	T	5.1 ±0.8	T	22.5 ±0.9	28.7 ±1.4
^{14}C-1-Butyrate	15.7 ±0.9	118.3 ±2.4	6.1 ±0.5	14.3 ±1.0	8.4 ±0.6	68.6 ±4.9	961.0 ±23.2
^{14}C-2-Butyrate	25.4 ±1.6	170.9 ±4.9	7.2 ±0.7	32.0 ±1.7	9.1 ±0.5	104.1 ±4.3	214.2 ±9.7
^{14}C-3-Butyrate	16.1 ±0.5	115.1 ±3.5	5.2 ±0.3	15.5 ±1.0	8.1 ±0.7	69.0 ±5.1	414.4 ±16.8

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) Trace.

^{14}C-Butyrate gave rise to more highly labelled lipids than ^{14}C-acetate, and the lipid labelling from ^{14}C-propionate was very low. ^{14}C labelling of phospholipids from all ^{14}C-acetate, propionate and butyrate was considerably higher than any other lipid fractions. These relationships were also seen with sheep rumen epithelium (6, 7, 13). ^{14}C-2-labelled acetate and butyrate produced more ^{14}C-lipids than those labelled in other positions.

^{14}C-butyrate produced the greatest level of $^{14}CO_2$, exceeding acetate, while $^{14}CO_2$ production from ^{14}C-propionate was the lowest. These results were essentially similar to sheep rumen epithelium (6, 7, 13). As expected, ^{14}C-1-labelled acetate, propionate and butyrate produced more $^{14}CO_2$ than those labelled in other positions. These tendencies were the same as in the case of sheep liver slices (12).

2. The influences of glucose and its metabolite upon the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-acetate

It has been shown that the metabolism of short-chain fatty acids and glucose have interacting effects in rumen epithelium (7), liver slices (14) and mammary gland slices (15) of ruminants. Therefore, an investigation was made on the effect of glucose, and some of its metabolites, pyruvate, phosphoenolpyruvate and lactate, upon the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-short-chain fatty acids.

As shown in Table 2, the ^{14}C-glucose formation from ^{14}C-1-acetate and ^{14}C-2-acetate was decreased by the addition of glucose. The incorporation of ^{14}C-1-

TABLE 2. *Effect of Glucose on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Acetate	10.1 ±1.1[b]	69.6 ±4.4	3.4 ±0.7	12.4 ±0.7	5.1 ±0.6	36.9 ±1.5	298.1 ±8.2
^{14}C-1-Acetate +glucose[c]	5.8 ±0.7	86.1 ±3.6	4.1 ±0.8	13.7 ±1.0	5.8 ±0.4	44.8 ±1.2	374.5 ±15.8
^{14}C-2-Acetate	19.3 ±1.2	95.3 ±3.9	5.9 ±0.9	14.1 ±0.8	6.3 ±0.6	53.9 ±2.8	97.5 ±3.7
^{14}C-2-Acetate +glucose[c]	12.6 ±0.9	117.3 ±5.2	6.3 ±0.5	17.9 ±1.2	9.0 ±0.7	69.4 ±4.2	118.0 ±4.5

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) 100 μmoles of cold glucose added into incubation flask.

TABLE 3. *Effect of Pyruvate on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Acetate	10.3 ±0.7[b]	72.5 ±4.0	3.7 ±0.9	13.4 ±0.5	3.8 ±0.7	37.5 ±1.9	296.8 ±9.3
^{14}C-1-Acetate +pyruvate[c]	9.6 ±0.4	56.1 ±3.9	2.8 ±0.7	8.2 ±0.4	3.1 ±0.5	21.1 ±1.3	231.9 ±7.1
^{14}C-2-Acetate	21.4 ±1.2	101.6 ±4.3	6.3 ±0.6	13.9 ±0.9	6.9 ±0.3	49.2 ±3.0	103.3 ±5.1
^{14}C-2-Acetate +pyruvate[c]	21.1 ±0.9	84.4 ±5.1	4.1 ±0.3	11.2 ±0.8	4.8 ±0.5	31.8 ±2.2	64.1 ±3.7

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) 100 μmoles of cold pyruvate added into incubation flask.

TABLE 4. *Effect of Phosphoenolpyruvate (PEP) on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorprated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Acetate	9.6 ±0.7[b]	71.9 ±4.7	3.9 ±0.4	13.6 ±0.9	4.9 ±0.2	40.2 ±1.7	301.6 ±10.4
^{14}C-1-Acetate +PEP[c]	6.2 ±0.5	89.7 ±2.9	4.3 ±0.7	14.8 ±1.1	6.1 ±0.5	48.3 ±2.1	366.4 ±11.6
^{14}C-2-Acetate	20.8 ±1.4	94.1 ±3.5	6.1 ±0.7	14.8 ±1.0	7 3 ±0.6	56.8 ±3.4	106.8 ±4.3
^{14}C-2-Acetate +PEP[c]	15.2 ±0.9	121.1 ±3.7	6.9 ±0.9	18.1 ±1.1	8.7 ±0.7	71.3 ±3.9	121.6 ±3.6

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) 100 μmoles of cold phosphoenolpyruvate (PEP) added into incubation flask.

acetate and ^{14}C-2-acetate into lipids was slightly incresed by the addition of glucose. Unexpectedly, $^{14}CO_2$ production from ^{14}C-1-acetate was increased by the glucose addition and the $^{14}CO_2$ production from ^{14}C-2-acetate was slightly increased by the addition of glucose.

As shown in Table 3, the addition of pyruvate had no effect on the formation of ^{14}C-glucose from ^{14}C-1-acetate and ^{14}C-2-acetate. The incorporation of ^{14}C-1-

acetate and ^{14}C-2-acetate into lipids and $^{14}CO_2$ production from ^{14}C-1-acetate and ^{14}C-2-acetate were slightly decreased by the addition of pyruvate. Thus, the pyruvate effects on the metabolism of acetate differed from those of glucose.

As shown in Table 4, the appearance of ^{14}C in glucose from ^{14}C-1-acetate and ^{14}C-2-acetate was slightly decreased by the addition of phosphoenolpyruvate. The incorporation of ^{14}C-1-acetate and ^{14}C-2-acetate into lipids was slightly increased by the addition of phosphoenolpyruvate. The $^{14}CO_2$ production from ^{14}C-1-acetate was increased by the addition of phosphoenolpyruvate and also the $^{14}CO_2$ production from ^{14}C-2-acetate was slightly increased by the addition of phosphoenolpyruvate. These effects of phosphoenolpyruvate on acetate metabolism were the same as the glucose effects.

As shown in Table 5, there was no affect of lactate addition upon the transfer of ^{14}C into glucose, lipids or CO_2 from ^{14}C-1-acetate and ^{14}C-2-acetate.

TABLE 5. *Effect of Lactate on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Acetate	9.8 ±1.3[b]	67.6 ±5.2	3.2 ±0.7	13.1 ±1.1	4.3 ±0.7	37.1 ±2.2	286.3 ±15.3
^{14}C-1-Acetate +lactate[c]	9.3 ±1.1	65.1 ±4.9	3.1 ±0.6	13.6 ±0.7	4.1 ±0.8	38.3 ±1.6	290.1 ±14.1
^{14}C-2-Acetate	22.9 ±1.7	92.4 ±5.2	4.8 ±0.9	15.4 ±1.3	6.1 ±0.7	47.1 ±4.5	98.1 ±6.1
^{14}C-2-Acetate +lactate[c]	23.4 ±1.2	96.6 ±6.1	4.1 ±0.8	14.3 ±1.2	6.7 ±0.5	48.7 ±4.1	104.6 ±6.5

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) 100 μmoles of cold lactate added into incubation flask.

The affect of glucose, pyruvate, phosphoenolpyruvate or lactate on the ^{14}C-lipids synthesis and $^{14}CO_2$ production from ^{14}C-1-acetate were almost the same as with sheep rumen epithelium (7). As reported previously, ketone body formation from acetate was suppressed by the addition of glucose and phosphoenolpyruvate, but pyruvate and lactate had no effect on the ketone body formation from acetate in the rumen epithelium of sheep (7) or cow (16). Glucose accelerated acetate oxidation but there was no effect of pyruvate and lactate on the acetate oxidation in rumen epithelium of cow (17).

Therefore, it may be assumed that the glucose-phosphoenolpyruvate system has some role in acetate metabolism of rumen epithelium, but there is no apparent

relationship between acetate metabolism and the metabolism of pyruvate and lactate.

3. The influences of glucose and its metabolites upon the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-propionate

As shown in Table 6, the ^{14}C-glucose formation from ^{14}C-1-propionate and ^{14}C-2-propionate was decreased by the glucose addition, while the incorporation of ^{14}C-1-propionate and ^{14}C-2-propionate into lipids was increased by the addition

TABLE 6. *Effect of Glucose on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Propionate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction C[a]	CO_2
^{14}C-1-Propionate	34.6 ±1.8[b]	15.9 ±0.7	T[c]	3.9 ±0.4	T	9.2 ±0.7	148.2 ±9.1
^{14}C-1-Propionate +glucose[d]	25.0 ±1.7	22.1 ±0.8	T	4.2 ±0.3	T	14.4 ±1.2	251.7 ±11.7
^{14}C-2-Propionate	64.2 ±2.7	28.4 ±1.2	T	4.7 ±0.6	T	21.7 ±1.1	31.7 ±1.5
^{14}C-2-Propionate +glucose[d]	51.2 ±2.3	35.3 ±1.6	T	6.1 ±0.8	T	28.1 ±1.0	42.1 ±1.0

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) Trace.
d) 100 μmoles of cold glucose added into incubation flask.

of glucose. Unexpectedly, the $^{14}CO_2$ production from ^{14}C-1-propionate and ^{14}C-2-propionate also increased when glucose was added to the medium.

As shown in Table 7, there was no effect of pyruvate addition on the transfer of ^{14}C to glucose, lipids or CO_2 from ^{14}C-1-propionate or ^{14}C-2-propionate. These results show that pyruvate and glucose have different effect.

As shown in Table 8, the transfer of ^{14}C into glucose from ^{14}C-1-propionate or ^{14}C-2-propionate was decreased by the addition of phosphoenolpyruvate. The incorporation of ^{14}C-1-propionate and ^{14}C-2-propionate into lipids was slightly increased by the addition of phosphoenolpyruvate. The production of $^{14}CO_2$ from ^{14}C-1-propionate and ^{14}C-2-propionate was also increased by the addition of phosphoenolpyruvate. These results were the same as in the case of the glucose addition.

As shown in Table 9, lactate addition had no effect on the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-1-propionate and ^{14}C-2-propionate.

TABLE 7. *Effect of Pyruvate on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Propionate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction C[a]	CO^2
^{14}C-1-Propionate	33.8 ±2.9[b]	17.4 ±1.1	T[c]	3.7 ±0.8	T	9.1 ±0.8	150.9 ±10.3
^{14}C-1-Propionate +pyruvate[d]	34.7 ±3.2	16.9 ±1.4	T	4.2 ±0.9	T	8.9 ±1.0	148.7 ±8.4
^{14}C-2-Propionate	61.6 ±3.1	31.7 ±2.4	T	5.3 ±1.0	T	23.5 ±1.3	30.4 ±1.6
^{14}C-2-Propionate +pyruvate[d]	60.4 ±3.7	29.1 ±2.1	T	4.9 ±0.7	T	22.6 ±1.5	29.4 ±1.8

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) Trace.
d) 100 μmoles of cold pyruvate added into incubation flask.

TABLE 8. *Effect of Phosphoenolpyruvate (PEP) on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Propionate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Propionate	31.1 ±1.5[b]	16.9 ±0.9	T[c]	3.2 ±0.5	T	9.3 ±0.5	149.4 ±8.1
^{14}C-1-Propionate +PEP[d]	19.7 ±1.1	23.5 ±0.8	T	4.9 ±0.3	T	16.2 ±0.9	236.4 ±10.8
^{14}C-2-Propionate	62.2 ±2.3	31.4 ±1.1	T	5.2 ±0.6	T	22.1 ±0.8	29.2 ±1.4
^{14}C-2-Propionate +PEP[d]	46.9 ±2.6	36.2 ±1.5	T	6.8 ±0.5	T	28.5 ±1.2	43.8 ±1.6

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) Trace.
d) 100 μmoles of cold phosphoenolpyruvate (PEP) added into incubation flask.

As presented previously, the production of $^{14}CO_2$ from ^{14}C-1-acetate was markedly increased by the addition of glucose and phosphoenolpyruvate, but pyruvate and lactate addition had no effect on the $^{14}CO_2$ production from ^{14}C-1-propionate in rumen epithelium of sheep (7). This was also true for the glucose accelerated propionate oxidation in rumen epithelium of cow (17).

TABLE 9. *Effect of Lactate on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Propionate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Propionate	32.5 ±2.8[b]	18.0 ±1.4	T[c]	3.6 ±0.5	T	9.7 ±0.8	147.3 ±9.9
^{14}C-1-Propionate +lactate[d]	31.8 ±3.0	18.4 ±1.3	T	3.7 ±0.4	T	9.5 ±0.4	141.1 ±10.2
^{14}C-2-Propionate	62.5 ±3.5	30.3 ±2.1	T	5.4 ±0.6	T	21.4 ±1.4	29.6 ±1.7
^{14}C-2-Propionate +lactate[d]	63.4 ±3.6	31.4 ±2.3	T	5.1 ±0.4	T	22.6 ±1.7	30.1 ±1.4

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) Trace.
d) 100 μmoles of cold lactate added into incubation flask.

Therefore, it may be assumed that there was a very close relationship between propionate metabolism and the glucose-phosphoenolpyruvate system, but that pyruvate and lactate have no role in the propionate metabolism of rumen epithelium.

4. The influences of glucose and its metabolites upon the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-butyrate

As shown in Table 10, the glucose addition had no effect on the transfer of ^{14}C to glucose, lipids or CO_2 from ^{14}C-1-butyrate, ^{14}C-2-butyrate, or ^{14}C-3-butyrate. These results differ from those obtained with ^{14}C-acetate and ^{14}C-propionate.

As shown in Table 11, pyruvate addition had no effect on the transfer of ^{14}C to glucose, lipids or CO_2 from ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate; the same result was obtained with ^{14}C-acetate and ^{14}C-propionate.

As shown in Table 12, the transfer of ^{14}C to glucose, lipids or CO_2 from ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate was not influenced by the addition of phosphoenolpyruvate. These results are different from the results with ^{14}C-acetate and ^{14}C-propionate.

As shown in Table 13, lactate addition had no detectable affect on the transfer of ^{14}C to glucose, lipids or CO_2 from ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate. This result was also obtained with ^{14}C-acetate and ^{14}C-propionate.

On the whole, the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-butyrate was not influenced by the addition of glucose or its metabolites. As presented previously, the addition of glucose, pyruvate, phosphoenolpyruvate and

TABLE 10. *Effect of Glucose on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Butyrate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Butyrate	14.9 ±1.2[b]	120.4 ±4.1	6.4 ±0.3	14.0 ±0.8	8.6 ±0.5	69.2 ±4.3	953.6 ±18.7
^{14}C-1-Butyrate+ glucose[c]	15.3 ±0.9	119.3 ±3.9	5.8 ±0.4	13.8 ±1.3	7.9 ±0.6	67.8 ±5.1	963.1 ±19.0
^{14}C-2-Butyrate	23.9 ±2.0	179.6 ±3.9	7.8 ±0.6	30.8 ±2.3	10.2 ±0.8	108.3 ±6.0	203.5 ±11.2
^{14}C-2-Butyrate +glucose[c]	23.3 ±1.9	182.4 ±4.6	8.2 ±0.5	31.6 ±2.5	9.8 ±0.5	114.2 ±6.7	212.6 ±12.5
^{14}C-3-Butyrate	15.8 ±0.6	118.3 ±4.2	5.6 ±0.3	15.8 ±1.3	8.7 ±0.7	71.5 ±5.2	422.7 ±12.9
^{14}C-3-Butyrate +glucose[c]	16.2 ±0.8	116.5 ±4.8	6.1 ±0.8	16.4 ±1.7	9.2 ±0.8	68.2 ±4.9	419.3 ±13.2

a) Fraction A, B, C and D were cmposed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) 100 μmoles of cold glucose added into incubation flask.

TABLE 11. *Effect of Pyruvate on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Butyrate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Butyrate	16.1 ±1.4[b]	121.0 ±4.5	5.9 ±0.5	15.4 ±1.1	8.7 ±0.4	70.2 ±5.0	965.5 ±21.4
^{14}C-1-Butyrate +pyruvate[c]	15.4 ±1.8	118.9 ±5.1	6.2 ±0.8	16.1 ±0.9	9.2 ±0.5	65.8 ±4.6	959.3 ±18.3
^{14}C-2-Butyrate	24.1 ±1.2	176.1 ±4.1	9.1 ±1.2	32.6 ±2.6	10.6 ±0.8	111.6 ±6.3	208.2 ±11.4
^{14}C-2-Butyrate +pyruvate[c]	25.5 ±2.4	181.0 ±5.6	9.3 ±0.9	33.8 ±2.3	10.2 ±0.6	105.8 ±4.9	214.9 ±13.4
^{14}C-Butyrate	14.9 ±0.7	121.6 ±4.1	6.0 ±0.8	14.9 ±1.0	8.9 ±0.5	71.2 ±4.3	411.8 ±13.5
^{14}C-3-Butyrate +pyruvate[c]	15.4 ±0.6	117.3 ±3.9	5.4 ±0.5	15.4 ±1.6	9.3 ±0.7	68.2 ±4.7	426.7 ±14.1

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g tissue N±S.D. in 10 animals.
c) 100 μmoes of cold pyruvate added into incubation flask.

TABLE 12. *Effect of Phosphoenolpyruvate (PEP) on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Butyrate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction D[a]	CO_2
^{14}C-1-Butyrate	15.7 ±0.9[b]	122.0 ±3.7	6.2 ±0.7	14.8 ±0.3	9.1 ±0.5	69.5 ±4.3	962.2 ±23.7
^{14}C-1-Butyrate +PEP[c]	16.2 ±1.2	117.3 ±4.8	5.7 ±1.0	15.2 ±0.8	8.7 ±0.7	74.2 ±5.1	954.1 ±25.3
^{14}C-2-Butyrate	26.0 ±1.8	175.9 ±6.1	8.2 ±0.5	29.7 ±1.9	9.8 ±0.6	112.5 ±5.4	208.5 ±15.0
^{14}C-2-Butyrate +PEP[c]	25.5 ±1.3	182.5 ±6.6	7.6 ±0.7	28.2 ±1.8	10.5 ±0.7	107.1 ±5.1	202.6 ±18.1
^{14}C-3-Butyrate	17.1 ±0.9	113.9 ±4.9	6.4 ±0.4	13.9 ±1.1	9.0 ±0.8	69.5 ±4.3	430.3 ±16.3
^{14}C-3-Butyrate +PEP[c]	16.4 ±0.4	108.4 ±5.1	7.1 ±0.8	14.3 ±0.8	8.3 ±0.7	70.9 ±5.1	421.0 ±19.4

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) 100 μmoles of cold phosphoenolpyruvate (PEP) added into incubation flask.

TABLE 13. *Effect of Lactate on Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Butyrate in Rumen Epithelium of Cow*

Substrate	^{14}C Incorporated into						
	Glucose	Total lipid	Fraction A[a]	Fraction B[a]	Fraction C[a]	Fraction C[a]	CO_2
^{14}C-1-Butyrate	15.4 ±0.7[b]	127.7 ±3.7	6.3 ±0.7	15.8 ±0.9	7.7 ±0.3	68.1 ±5.2	967.8 ±27.2
^{14}C-1-Butyrate +lactate[c]	16.1 ±0.9	122.7 ±3.5	6.5 ±0.4	16.2 ±0.6	7.8 ±0.4	66.1 ±5.1	951.8 ±25.2
^{14}C-2-Butyrate	24.7 ±1.8	185.9 ±9.2	8.6 ±0.5	32.5 ±2.4	11.0 ±0.6	106.5 ±5.1	221.3 ±17.6
^{14}C-2-Butyrate +lactate[c]	23.9 ±2.0	177.3 ±5.4	8.4 ±0.6	30.6 ±3.0	12.1 ±1.1	111.2 ±6.3	214.0 ±18.2
^{14}C-3-Butyrate	14.2 ±0.6	121.3 ±4.6	5.8 ±0.8	16.3 ±0.9	8.1 ±0.7	73.0 ±5.9	417.3 ±19.6
^{14}C-3-Butyrate +lactate[c]	15.1 ±0.7	111.4 ±5.4	6.3 ±0.5	14.8 ±0.6	7.6 ±0.5	70.8 ±4.2	430.7 ±20.5

a) Fraction A, B, C and D were composed of cholesterol ester, triglyceride, free cholesterol, and phospholipid, respectively.
b) Mean μmC per g of tissue N±S.D. in 10 animals.
c) 100 μmoles of cold lactate added into incubation flask.

lactate did not influence ^{14}C-lipid synthesis or $^{14}CO_2$ production from ^{14}C-1-butyrate in rumen epithelium of sheep (7), nor did they effect ketone body formation from butyrate in the rumen epithelium of sheep (7) or of cow (16).

Therefore, it may be concluded that there is no relationship between butyrate metabolism and glucolysis in rumen epithelium.

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