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The Synthesis of Glucose and Lipids from Short-Chain Fatty Acids in Cow Liver Slices: Affect of Glucose and its Metabolites

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Summary

In ruminants, large amounts of short-chain fatty acids are produced in the rumen, where they are absorbed, and then transported via the portal vein to the liver for metabolism. The following results were obtained in a study of the influence of glucose and its metabolites on the formation of ^{14}C-glucose, ^{14}C-lipids, and $^{14}CO_2$ from ^{14}C-short-chain fatty acids by cow liver slices.

1. Propionate formed more glucose than acetate or butyrate. Acetate formed more lipid than butyrate. The lipid formation from propionate was small. ^{14}C-1-labelled propionate produced a large amount of CO_2.

2. Glucose and phosphoenolpyruvate inhibited the glucose formation and CO_2 production from acetate, propionate and butyrate but accelerated the lipid synthesis from those fatty acids.

3. Pyruvate and lactate did not affect the formation of glucose, lipids, nor CO_2 from acetate, propionate, or butyrate.

From these and other results, previously obtained, it may be assumed that there was some relationship between the metabolism of short-chain fatty acids and the glucose-phosphoenolpyruvate system, but pyruvate and lactate did not affect the metabolism of short-chain fatty acids in the liver slices of cow.

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It is generally recognized that short-chain fatty acids, primarily acetate, propionate and butyrate, are produced in the rumen and these short-chain fatty acids are absorbed directly through the rumen wall. During absorption, part of the fatty acid gets metabolized by the rumen epithelium but much reaches the liver to be metabolized in various way. This fact is based on the evidence that the concentration of these short-chain fatty acids is high in the portal vein and is much lower in the hepatic vein of goats (1).

It has been shown that sheep liver converts short-chain fatty acids into glucose, CO_2 (2) and lipids (3). The rate of these conversions is changed by starvation (2, 3). Thus, it appears that the utilization of short-chain fatty acids in the liver of ruminants is affected by factors other than nutrients. Previous studies from our laboratory have shown that glucose and its metabolite affect the oxidation of short-chain fatty acids and their conversion to ketone body in cow liver slices (4, 5).

The present paper extends these earlier studies to measure glucose and lipid synthesis from short-chain fatty acids in cow liver slices and to determine the affect of added glucose or glucose metabolites on these conversions.

Methods and Materials

Fresh cow liver was obtained at the slaughter house and prepared as 0.4 mm thick slices. Incubation was carried out in 50 ml Erlenmyer flasks fitted with 2 ml center wells. Each flask contained 2 g of liver slices, 10 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), and 100 μmoles (1 μc) of ^{14}C-labelled short-chain fatty acid. The flasks were closed with serum caps after gassing with O_2+CO_2 (95:5). Incubation (3 flasks for each animal: ^{14}C-glucose, ^{14}C-lipids, and $^{14}CO_2$ assays were carried out in different flasks) was carried out for 3 hr. at 38°C during shaking at 100 strokes per minutes.

The glucose was isolated from incubation medium as glucose pentaacetate by the method of Jones (6).

Total lipids in the tissue and medium were extracted with a mixture of chloroform and methanol (2:1), followed by aqueous washing of the extract as described by Folch, Lees and Sloane-Stanley (7). Separation of the 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). Fractions A, B, C and D consisted of cholesterol ester, triglyceride, free cholesterol and phospholipid, respectively.

The $^{14}CO_2$ assay was made after incubation by injecting 1 ml of Hyamine into the center well of the Erlenmyer flask and 0.5 ml of 10 N H_2SO_4 into the medium. The flask was shaken for 30 minutes in ice prior to quantitative removal of the

Hyamine for estimation of total radioactivity in the trapped CO_2.

The ^{14}C activity 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 in the liquid scintillation counter. Total nitrogen in the liver slices was determined by the micro-Kjeldahl method, and results were expressed as mean μmC/3 hr/g of tissue nitrogen ± S.D. (standard deviation). Each study involved 10 animals.

Results and Discussion

1. ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ formation from ^{14}C-short-chain fatty acids:

As shown in Table 1, the largest amount of ^{14}C-glucose was formed from ^{14}C-propionate. ^{14}C-Glucose was also formed from ^{14}C-acetate and ^{14}C-butyrate, although the amount was smaller than in the case of propionate. ^{14}C-2-labelled short-chain fatty acids gave rise to more highly labelled glucose than short-chain fatty acids labelled in other positions. Similar results were seen in the case of sheep-liver slices (2).

More ^{14}C-acetate was incorporated into lipids than ^{14}C-butyrate and very

TABLE 1. *The Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Short-Chain Fatty acid in Liver Slices 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	191.3[b] ±9.8	318.6 ±13.9	53.4 ±1.7	21.4 ±0.9	62.2 ±3.1	104.3 ±5.1	2040.5 ±106.6
^{14}C-2-Acetate	598.4 ±24.6	690.2 ±26.5	114.0 ±5.7	52.1 ±1.4	123.0 ±4.6	251.4 ±9.1	835.4 ±34.4
^{14}C-1-Propionate	851.1 ±28.6	113.2 ±5.2	21.4 ±1.3	9.7 ±0.8	15.8 ±0.8	43.8 ±1.3	4227.8 ±138.7
^{14}C-2-Propionate	2852.0 ±96.6	146.1 ±7.1	27.1 ±0.9	11.6 ±0.5	19.2 ±0.7	65.9 ±1.9	638.4 ±27.2
^{14}C-1-Butyrate	228.4 ±11.5	216.8 ±10.6	29.3 ±1.1	31.6 ±0.9	28.6 ±1.0	80.5 ±3.6	3135.8 ±124.8
^{14}C-2-Butyrate	826.8 ±25.9	492.1 ±16.1	79.9 ±3.9	89.1 ±3.6	79.3 ±3.2	181.3 ±7.3	228.1 ±10.6
^{14}C-3-Butyrate	235.2 ±9.3	221.3 ±9.6	31.0 ±1.2	29.2 ±1.1	27.6 ±0.9	83.1 ±3.1	1987.1 ±114.6

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 nitrogen ±S.D. in 10 animals.

little ^{14}C-propionate was utilized. In general, the fatty acids labelled in the C-2-position produced more highly labelled lipids than the fatty acids labelled in other positions. Phospholipids labelling was highest among all lipid fractions from each of the ^{14}C-labelled short-chain fatty acids.

The $^{14}CO_2$ production was greatest from ^{14}C-propionate and decreased with ^{14}C-butyrate followed by ^{14}C-acetate. Fatty acids produced more $^{14}CO_2$ when labelled in the C-1-position. Similar results were obtained in the case of sheep-liver slices (2).

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

As shown in Table 2, ^{14}C-glucose formation from ^{14}C-1-acetate was decreased slightly by glucose addition, although the difference was not significant. Also the ^{14}C-Glucose formation from ^{14}C-2-acetate was decreased by glucose addition.

TABLE 2. *Effect of Glucose upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Liver Slices 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	204.0[b] ±10.5	306.6 ±13.5	50.4 ±1.4	20.2 ±0.7	58.8 ±2.7	98.4 ±4.2	1972.3 ±95.8
^{14}C-1-Acetate +glucose[c]	186.8 ±6.8	364.9 ±12.5	61.7 ±1.7	23.7 ±0.6	72.1 ±3.0	119.6 ±3.6	1766.8 ±92.5
^{14}C-2-Acetate	612.4 ±18.1	687.6 ±23.8	105.1 ±5.1	49.3 ±1.7	119.4 ±4.5	249.9 ±10.2	840.7 ±38.3
^{14}C-2-Acetate +glucose	497.4 ±16.6	736.7 ±24.3	118.5 ±5.2	52.7 ±1.8	125.3 ±4.8	261.8 ±6.5	725.5 ±29.7

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

The incorporation of ^{14}C-1-acetate and ^{14}C-2-acetate into lipids was slightly increased by glucose addition. These tendencies were almost the same as in previous studies with sheep-rumen epithelium (8).

The $^{14}CO_2$ production from ^{14}C-1-acetate and ^{14}C-2-acetate was slightly decreased by glucose addition while in rumen epithelium of sheep (8), $^{14}CO_2$ production from ^{14}C-1-acetate was increased by glucose addition. Thus it appears that liver and rumen epithelium, respond differently to glucose in forms of $^{14}CO_2$-production from ^{14}C-acetate.

As shown in Table 3, pyruvate addition did not affect the formation of ^{14}C-

TABLE 3. *Effect of Pyruvate upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Liver Slices 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	207.5[b] ±10.7	322.7 ±14.5	51.8 ±1.9	22.7 ±0.6	61.9 ±3.7	102.3 ±6.3	1950.9 ±108.5
^{14}C-1-Acetate +pyruvate[c]	198.4 ±11.4	314.8 ±12.7	49.3 ±1.8	21.4 ±0.8	62.3 ±4.1	104.4 ±5.8	2066.9 ±112.7
^{14}C-2-Acetate	605.2 ±28.0	691.5 ±32.2	117.5 ±6.4	41.2 ±2.2	123.8 ±5.9	255.7 ±10.8	836.2 ±42.8
^{14}C-2-Acetate + pyruvate	597.8 ±25.2	687.2 ±25.6	104.8 ±4.8	39.5 ±2.6	118.6 ±4.3	261.3 ±10.3	841.8 ±41.1

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

TABLE 4. *Effect of Phosphoenolpyruvate (PEP) upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Liver Slices 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	193.3[b] ±9.1	309.3 ±11.9	51.1 ±1.3	19.1 ±1.0	58.4 ±4.5	98.1 ±4.7	2042.7 ±99.4
^{14}C-1-Acetate + PEP[c]	171.9 ±9.4	373.6 ±12.4	62.4 ±1.9	23.4 ±0.7	71.8 ±4.1	121.2 ±4.2	1738.6 ±93.2
^{14}C-2-Acetate	618.1 ±21.6	685.0 ±24.4	109.5 ±4.5	46.6 ±1.8	125.6 ±4.7	248.1 ±9.3	833.3 ±39.1
^{14}C-2-Acetate + PEP	506.1 ±18.4	742.2 ±23.8	115.7 ±4.1	57.1 ±2.2	122.8 ±5.9	272.9 ±7.1	719.6 ±32.8

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

glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-1-acetate and ^{14}C-2-acetate. These results were almost the same as those with sheep-rumen epithelium (8).

As shown in Table 4, ^{14}C-glucose formation from ^{14}C-1-acetate was very slightly decreased by the addition of phosphoenolpyruvate, although the difference was not significant. It does appear that ^{14}C-glucose formation from ^{14}C-2-acetate was 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. These results were the same as in the cases of sheep-rumen epithelium (8).

The $^{14}CO_2$ production from ^{14}C-1-acetate and ^{14}C-2-acetate was decreased by the addition of phosphoenolpyruvate. These effects of phosphoenolpyruvate were the same as the glucose effects in the liver slices of cow. But in rumen epithelium of sheep, the $^{14}CO_2$ production from ^{14}C-1-acetate was increased by the addition of phosphoenolpyruvate (8), so that there were some differences between the liver slices of cow and the rumen epithelium of sheep, concerning the effects of phosphoenolpyruvate.

As shown in Table 5, there was no effect from lactate addition on the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-1-acetate and ^{14}C-2-acetate. These results differ from addition of glucose and phosphoenolpyruvate. These results were almost the same as that of sheep-rumen epithelium (8).

TABLE 5. *Effect of Lactate upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Acetate in Liver Slices 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	211.7[b] ±11.2	318.1 ±14.8	52.9 ±1.7	19.7 ±0.8	59.4 ±4.1	93.6 ±5.8	2038.1 ±117.6
^{14}C-1-Acetate + lactate[c]	201.9 ±10.5	324.6 ±13.0	49.7 ±1.9	19.2 ±0.8	59.1 ±4.5	92.5 ±5.7	1905.6 ±106.7
^{14}C-2-Acetate	621.8 ±27.4	706.6 ±29.2	106.1 ±5.8	43.2 ±2.1	128.8 ±5.2	262.0 ±10.6	829.6 ±46.7
^{14}C-2-Acetate + lactate	617.2 ±25.4	698.2 ±28.5	95.4 ±6.1	46.5 ±3.0	131.7 ±5.7	264.8 ±10.3	834.4 ±37.5

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

Thus, the glucose and phosphoenolpyruvate had some effects upon the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-acetate, but the pyruvate and lactate did not influence the utilization of ^{14}C-acetate.

As reported previously, glucose and phosphoenolpyruvate accelerated the acetate utilization and inhibited ketone formation from acetate, but pyruvate and lactate did not influence the acetate utilization nor the ketone formation from the acetate in liver slices of cow (4, 5). Therefore, it may be assumed that there was some relationships between acetate metabolism and the glucose-phosphoenol-

pyruvate system, but that pyruvate and lactate had no detectable effect on the metabolism of acetate in the liver of cow.

3. 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 markedly decreased by the addition of glucose but ^{14}C-lipid synthesis from ^{14}C-1-propionate and ^{14}C-2-propionate was increased by glucose addition. The $^{14}CO_2$ production from ^{14}C-1-propionate and ^{14}C-2-propionate was decreased by the glucose addition. In the rumen epithelium of sheep, $^{14}CO_2$ production from ^{14}C-1-propionate was markedly increased by the glucose addition (8), so that liver slices of cow differs in response to glucose in propionate metabolism compared to the rumen epithelium of sheep.

TABLE 6. *Effect of Glucose upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Propionate in Livers Slices 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	862.3 ±26.2[b]	117.5 ±4.7	21.7 ±1.1	9.2 ±0.5	14.7 ±0.8	41.0 ±1.5	4319.4 ±159.7
^{14}C-1-Propionate + glucose[c]	525.9 ±19.6	182.9 ±6.1	32.1 ±1.7	18.0 ±0.9	23.5 ±1.4	73.9 ±2.3	3280.8 ±140.6
^{14}C-2-Propionate	1910.5 ±105.3	140.8 ±7.3	27.0 ±0.8	12.4 0.6	20.8 ±0.9	64.1 ±1.5	657.2 ±31.5
^{14}C-2-Propionate + glucose	1527.9 ±49.3	276.8 ±11.8	43.2 ± 1.6	21.8 ±1.0	36.3 ±1.5	121.8 ±4.6	463.8 ±15.1

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

As shown in Table 7, there were no effects from pyruvate addition on the formation of ^{14}C-glucose, ^{14}C-lipids, and $^{14}CO_2$ from ^{14}C-1-propionate or ^{14}C-2-propionate. These results were the same as in the case of sheep-rumen epithelium (8), but different from the case of glucose addition with liver slices of cow.

As shown in Table 8, ^{14}C-glucose formation and $^{14}CO_2$ production from ^{14}C-1-propionate and ^{14}C-2-propionate was markedly decreased by the addition of phosphoenolpyruvate while ^{14}C-lipid synthesis from ^{14}C-1-propionate and ^{14}C-2-propionate was increased by the addition of phosphoenolpyruvate. These changes were the same as those obtained after glucose addition. But in the rumen epithelium of sheep, $^{14}CO_2$ production from ^{14}C-1-propionate was increased by the

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

Substrate	^{14}C Inocorporated into						
	Glucose	Total lipid	Fraction A[a)]	Fraction B[a)]	Fraction C[a)]	Fraction D[a)]	CO_2
^{14}C-1-Propionate	848.1[b)] ±32.0	106.3 ±6.6	21.8 ±1.4	10.2 ±0.7	16.2 ±0.9	41.9 ±1.7	4207.7 ±178.1
^{14}C-1-Propionate + pyruvate[c)]	837.7 ±31.4	95.4 ±6.2	19.8 ±1.7	9.3 ±0.8	15.4 ±0.8	38.9 ±1.6	4175.2 ±162.7
^{14}C-2-Propionate	2831.5 ±116.7	154.1 ±8.5	29.0 ±1.0	13.5 ±0.9	21.6 ±1.1	69.8 ±2.3	634.8 ±38.9
^{14}C-2-Propionate + pyruvate	2915.4 ±109.1	149.8 ±7.3	27.1 ±1.2	12.8 ±0.8	21.1 ±0.9	71.1 ±2.0	641.4 ±34.5

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

TABLE 8. *Effect of Phosphoenolpyruvate (PEP) upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Propionate in Liver Slices 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	856.4[b)] ±21.1	113.5 ±4.5	22.4 ±1.2	10.3 ±0.8	14.6 ±0.8	42.4 ±1.1	4124.2 ±148.4
^{14}C-1-Propionate + PEP[c)]	538.7 ±22.5	175.6 ±5.6	32.7 ±1.3	17.5 ±1.0	24.9 ±1.1	75.3 ±1.9	2988.8 ±152.9
^{14}C-2-Propionate	2752.4 ±102.8	148.0 ±7.2	29.8 ±1.2	14.2 ±0.9	19.7 ±0.8	68.0 ±1.2	653.3 ±28.5
^{14}C-2-Propionate + PEP	1429.0 ±119.5	266.2 ±13.6	42.5 ±1.8	21.6 ±0.8	33.9 ±1.6	138.9 ±4.8	458.6 ±16.5

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

addition of phosphoenolpyruvate (8), so this indicates some difference between the liver slices of cow and the rumen epithelium of sheep, concerning the effects of phosphoenolpyruvate on the propionate metabolism.

As shown in Table 9, there was no effect from lactate addition on the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-1-propionate and ^{14}C-2-propionate in contrast to the response to addition of glucose and phosphoenolpyruvate. The

TABLE 9. *Effect of Lactate upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Propionate in Livers Slices 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	845.5[b] ±31.5	118.2 ±5.1	21.3 ±1.1	9.7 ±0.4	15.1 ±0.8	40.9 ±1.4	4238.6 ±159.2
^{14}C-1-Propionate + lactate[c]	858.8 ±29.7	117.6 ±6.2	19.8 ±0.8	10.3 ±0.8	14.3 ±1.1	41.8 ±1.3	4175.6 ±168.3
^{14}C-2-Propionate	2936.4 ±121.7	152.9 ±8.7	26.5 ±1.4	12.5 ±0.9	21.7 ±0.9	65.9 ±1.8	668.1 ±31.2
^{14}C-2-Propionate + lactate	2823.3 ±114.0	148.2 ±6.9	28.3 ±1.1	13.0 ±0.9	20.3 ±1.0	67.5 ±2.3	657.2 ±33.9

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

same results were obtained with sheep-rumen epithelium (8).

Thus, the addition of glucose and phosphoenolpyruvate had great affect on the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-propionate, but that of pyruvate and lactate did not. Therefore, it appears that there may be a close relationships between the propionate metabolism and the glucose-phosphoenolpyruvate system, but pyruvate and lactate have no detectable affect on propionate metabolism of cow-liver slices.

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

As shown in Table 10, the ^{14}C-glucose and $^{14}CO_2$ formation from ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate was decreased by glucose addition while the incorporation of ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate into lipids was increased by glucose addition. Glucose addition did not influence the utilization nor oxidation of butyrate, or ketone bodies production from butyrate in liver slices of cow (4, 5). In contrast, in the rumen epithelium of sheep (8) and cow (9, 10), there was no affect from glucose upon the formation of ketone bodies, lipid, nor CO_2 production from butyrate.

As shown in Table 11, there was no affect from pyruvate addition on the formation of ^{14}C-glucose, ^{14}C-lipids nor $^{14}CO_2$ from ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate, in contrast with the results after glucose addition. These changes in the case of pyruvate were the same as those with ^{14}C-acetate and ^{14}C-propionate, as described above. These results were almost the same as in the case of rumen epithelium of cow (9, 10) and sheep (8). As reported previously

TABLE 10. *Effect of Glucose upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Butyrate in Liver Slices 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	221.7[b] ±11.6	219.3 ±11.3	27.2 ±0.9	31.8 ±1.1	28.8 ±1.4	82.9 ±3.2	3011.7 ±128.3
^{14}C-1-Butyrate + glucose[c]	142.6 ±4.3	283.5 ±13.4	38.5 ±1.5	39.5 ±1.3	38.1 ±1.9	123.8 ±4.6	2590.4 ±107.5
^{14}C-2-Butyrate	829.0 ±26.1	486.6 ±17.6	81.2 ±4.1	86.3 ±3.9	78.7 ±3.6	183.2 ±7.0	232.4 ±12.8
^{14}C-2-Butyrate + glucose	684.1 ±18.7	579.0 ±18.3	97.5 ±4.7	103.8 ±4.4	97.4 ±3.7	228.5 ±11.8	175.9 ±5.1
^{14}C-3-Butyrate	242.5 ±27.5	225.9 ±10.1	30.6 ±1.1	31.4 ±1.2	28.2 ±0.8	77.4 ±2.9	2024.7 ±106.9
^{14}C-3-Butyrate + glucose	147.6 ±5.2	280.3 ±13.2	38.7 ±1.7	37.5 ±1.4	39.7 ±1.2	127.5 ±4.7	1729.3 ±98.2

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

TABLE 11. *Effect of Pyruvate upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from ^{14}C-Butyrate in Liver Slices 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	217.5[b] ±12.4	222.8 ±14.9	27.5 ±1.1	29.8 ±1.3	30.6 ±1.5	78.6 ±4.2	3181.1 ±147.9
^{14}C-1-Butyrate + pyruvate[c]	206.1 ±13.2	218.5 ±15.8	26.3 ±1.2	28.0 ±1.2	29.3 ±1.4	76.3 ±4.8	3068.8 ±150.8
^{14}C-2-Butyrate	836.1 ±31.0	504.5 ±21.2	83.5 ±5.1	89.4 ±5.0	82.1 ±6.2	190.0 ±14.8	224.5 ±13.9
^{14}C-2-Butyrate + pyruvate	834.6 ±29.9	487.5 ±20.3	89.3 ±4.9	77.5 ±4.1	91.8 ±7.1	189.0 ±9.5	233.6 ±15.7
^{14}C-3-Butyrate	238.7 ±22.5	230.2 ±10.7	30.1 ±0.9	31.1 ±1.2	32.8 ±1.2	82.3 ±3.1	2183.7 ±118.2
^{14}C-3-Butyrate + pyruvate	229.3 ±16.6	227.6 ±11.4	29.2 ±1.2	27.5 ±1.1	26.2 ±0.9	86.5 ±2.8	2263.7 ±109.1

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

TABLE 12. *Effect of Phosphoenolpyruvate (PEP) upon Formation of ^{14}C-Glucose, ^{14}C-Lipids and $^{14}CO_2$ from ^{14}C-Butyrate in Liver Slices 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	231.0[b] ±11.9	213.5 ±9.7	28.2 ±1.4	26.4 ±1.0	30.4 ±1.3	81.4 ±3.1	3214.4 ±131.2
^{14}C-1-Butyrate + PEP[c]	139.8 ±5.1	291.4 ±8.5	38.9 ±0.9	36.8 ±1.2	39.7 ±1.0	130.3 ±4.2	2430.1 ±103.7
^{14}C-2-Butyrate	818.8 ±24.3	499.7 ±18.3	83.1 ±4.8	84.5 ±4.0	82.2 ±2.9	192.2 ±7.7	228.3 ±9.2
^{14}C-2-Butyrate + PEP	672.2 ±13.8	586.7 ±15.9	104.2 ±4.5	91.0 ±5.2	104.5 ±4.6	213.9 ±12.5	163.5 ±5.3
^{14}C-3-Butyrate	251.0 ±26.9	218.6 ±9.8	29.2 ±0.9	30.8 ±1.2	29.9 ±1.1	76.0 ±3.1	2017.1 ±102.3
^{14}C-3-Butyrate + PEP	131.5 ±5.4	292.7 ±12.0	41.2 ±1.5	40.1 ±1.3	39.1 ±1.0	131.2 ±5.1	1645.3 ±99.8

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

(4, 5), pyruvate did not influence the utilization nor oxidation of butyrate, or ketone bodies formation from butyrate in cow liver slices. Therefore, the metabolism of butyrate and pyruvate in both the liver slices and the rumen epithelium appear to take place independently.

As shown in Table 12, the ^{14}C-glucose and $^{14}CO_2$ formation from ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate was decreased by the addition of phosphoenolpyruvate, while the incorporation of ^{14}C-1-butyrate, ^{14}C-2-butyrate and ^{14}C-3-butyrate into lipids was increased. Phosphoenolpyruvate addition did not affect butyrate utilization or ketone body production from butyrate in cow liver slices (4, 5). These results were almost the same as the glucose affects on butyrate metabolism, as described above. In rumen epithelium of sheep (8) and cow (9. 10), there was no effect from phosphoenolpyruvate on the formation of ketone bodies, lipids, nor CO_2 from butyrate, or oxidation of butyrate, so liver slices and rumen epithelium showed similar effects of phosphoenolpyruvate on the metabolism of butyrate.

As shown in Table 13, there was no effect from lactate addition on the formation of ^{14}C-glucose, ^{14}C-lipids or $^{14}CO_2$ from ^{14}C-1-butyrate, ^{14}C-2-butyrate, and ^{14}C-3-butyrate, in contrast to results after the addition of glucose and phosphoenolpyruvate. Thus the results were consistantly negative concerning the affects of lactate on the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from all the ^{14}C-fatty

TABLE 13. *Effect of Lactate upon Formation of ^{14}C-Glucose, ^{14}C-Lipid and $^{14}CO_2$ from Butyrate in Liver Slices 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	214.6[b] ±13.6	214.2 ±14.6	26.4 ±1.2	28.3 ±1.4	31.4 ±1.1	82.6 ±4.3	3027.8 ±148.2
^{14}C-1-Butyrate + lactate[c]	220.3 ±12.9	216.9 ±16.1	28.5 ±1.0	29.8 ±1.3	27.8 ±1.3	77.3 ±3.9	3148.4 ±157.3
^{14}C-2-Butyrate	822.7 ±36.9	489.0 ±23.7	82.4 ±4.8	87.5 ±4.1	89.6 ±5.1	181.7 ±11.6	235.6 ±14.7
^{14}C-2-Butyrate + lactate	824.3 ±33.7	492.5 ±25.4	81.5 ±3.9	88.2 ±5.0	86.9 ±6.0	192.5 ±14.1	238.0 ±11.6
^{14}C-3-Butyrate	241.9 ±17.2	231.1 ±9.3	30.7 ±0.8	28.5 ±1.1	33.4 ±1.3	81.4 ±3.3	2149.7 ±119.7
^{14}C-3-Butyrate + lactate	231.4 ±17.9	224.2 ±14.7	27.9 ±1.0	31.2 ±1.3	29.1 ±0.8	86.3 ±3.0	2210.4 ±104.3

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

acids. A similar response to lactate was observed in the rumen epithelium of sheep (8) and cow (9, 10). As reported previously, lactate did not influence the utilization nor oxidation of butyrate, or ketone body formation from butyrate in cow liver slices (4, 5). Therefore, the metabolism of butyrate and lactate in both liver slices and rumen epithelium appear to take place independently.

Since glucose and phosphoenolpyruvate had some affect on the formation of ^{14}C-glucose, ^{14}C-lipids and $^{14}CO_2$ from ^{14}C-butyrate, but none on pyruvate and lactate in cow liver slices, it may be assumed that there some relationship exists between butyrate metabolism and the glucose-phosphoenolpyruvate system, but not with pyruvate and lactate.

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