Butyrate Metabolism in the Lactating Cow *

ARTHUR L. BLACK, MAX KLEIBER, AND ALICE M. BROWN†

From the School of Veterinary Medicine and the College of Agriculture
University of California, Davis, California

(Received for publication, January 19, 1961)

The possibility that butyrate may be glucogenic in ruminants has been suggested by several experimental studies. Intravenous injection of butyrate in sheep was followed by increased glucose production after butyrate injection. In an effort to account for this variability it has been suggested that a plethora of body glucose inhibited "gluconeogenesis from butyrate" (5) and that a state of acidosis interferes with "the glucogenic effect of butyrate" (6).

Studies with C14-labeled fatty acids have provided additional evidence for the apparently anomalous metabolic behavior of butyrate in ruminants. Intact cows injected with acetate-C14 transferred to milk fat 70% of the total C14 recovered from milk products; this demonstrates the lipogenic behavior of this fatty acid (7). Propionate-C14 was markedly glucogenic in the cow with 60 to 70% of the milk C14 in lactose (8). In contrast to these results, which are consistent with current concepts on the metabolic behavior of acetate and propionate in animals, when butyrate-1-C14 or butyrate-2-C14 was injected into lactating cows, approximately twice as much C14 was recovered from lactose and also from casein as was recovered in milk fat (9). Inasmuch as butyrate is a component of milk triglyceride and makes up about 60 to 70% of the milk C14 in lactose (8), one would expect butyrate to be a better precursor of milk fat.

Studies in vitro have indicated a direct role for butyrate coenzyme A in fatty acid synthesis in animal tissues. Butyryl-CoA was reported to be the most effective of several acyl-CoA compounds tested, including acetyl-CoA, for stimulating the conversion of malonyl-CoA to long chain fatty acids by rat liver enzymes (11). It has also been observed that butyryl-CoA was a required component for the transfer of C14 from pyruvate to fatty acids by normal rat liver mitochondria and that it restored to normal the defective lipogenesis of liver mitochondria from alloxan-diabetic rats (12). The butyryl moiety of butyryl-CoA was incorporated intact into palmitic acid synthesized by pigeon liver (13, 14), and it was shown that the same enzymes and cofactors were required for the incorporation of C14 from either acetate or butyrate into long chain fatty acids (13).

The demonstrated behavior of butyryl-CoA in animal tissues considered together with the fact that β oxidation of butyrate produces acetyl-CoA, a common intermediate in the metabolism of acetate, would lead one to expect the metabolism of butyrate to resemble acetate, which is lipogenic, rather than propionate, which is glucogenic.

Isotope studies with the rat (13) and other animals (16) have supported the idea that the intermediates and pathway for transfer of carbon from fatty acids (including butyrate) to glucose includes β oxidation to acetyl-CoA and metabolism via the tricarboxylic acid cycle and reverse glycolysis according to classical schemes; there was no evidence that special pathways had been involved. This explanation, involving a common pathway for the fatty acids, is insufficient to account for the diverse metabolic behavior of acetate and butyrate in the cow. It is recognized that unlike most of the higher animals which receive large amounts of glucose from their digestive tract, the ruminant depends for energy largely on short chain fatty acids produced by fermentation of dietary carbohydrate in the rumen. Carroll and Hungate (17) have estimated that the acetate, propionate, and butyrate produced in the rumen provide approximately 70% of the total energy requirement in the bovine. The quantitative importance of these fatty acids and the paucity of alimentary glucose create in the ruminant a metabolic environment that is unique among the higher animals, with short chain fatty acids assuming major significance. It seemed possible that reactions which appear to be unimportant or absent in other animals may, because of species variation or metabolic adaptations to fatty acids as major nutrients, become obvious in ruminants.

The present study was undertaken to obtain additional information on the pathway of metabolism of butyrate in the intact cow. We have recovered several amino acids from casein at 3 and 10 hours after intravenous injection of cows with butyrate-1-C14, butyrate-2-C14, or butyrate-3-C14. The specific activities of the amino acids and the intramolecular distribution of C14 in serine, alanine, and glutamic acid provide a basis for evaluating the metabolic pathway of butyrate. Our results indicate that the cow metabolizes butyrate in the same manner as do other animals.

EXPERIMENTAL PROCEDURE

Lactose and casein were isolated from milk collected 3 and 10 hours after intravenous injection of six normal lactating cows with a single dose of 3 to 5 mc of butyrate-1-C14, butyrate-2-C14, or butyrate-3-C14. Data on the experimental animals and compounds injected are listed in Table I.

The methods for separation and crystallization of amino acids (18), degradation of amino acids (19), and C14 assay (18) have been discussed in earlier papers.
The methods for sampling blood from cows and isolating glucose as the glucosazone have also been described (20).

RESULTS

Fig. 1 shows the specific activity of plasma glucose at various times after intravenous injection of lactating cows with 5 mc of butyrate-2-Cl4 or acetate-2-U4. The difference in results is striking. Maximal specific activity was 4 times as great after butyrate as after acetate, and occurred much earlier. The time of the maximal specific activity was 4 times as great after butyrate as after acetate. The explanation for the different metabolic fate of carbon from the two fatty acids must account for this more rapid transfer of carbon from butyrate to glucose.

The Cl4 level among the milk products synthesized by cows injected with butyrate-04 was reported earlier (9); the specific activity was high in lactose and casein and much lower in milk fat. The casein amino acids that contained Cl4 are listed in Table II. In all samples, specific activity was greatest in glu-

![Graph](image_url)

**Fig. 1.** Specific activity of plasma glucose at various times after intravenous injection of lactating cows with 5 mc of butyrate-2-Cl4 or acetate-2-U4. The curve for butyrate was published earlier (14) but is reproduced here to simplify comparison with results after acetate-2-Cl4.

The specific activities of lactose from butyrate trials I to IV were published earlier (9). In trials not previously reported, in which butyrate-3-C14 was administered, the specific activities of lactose were 1.17, and 1.10 at 3 hours and 0.89 and 1.02 at 10 hours for butyrate trials VI and VII, respectively.
September 1961  
A. L. Black, M. Kleiber, and A. M. Brown.

**Table III**

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Time after injection (hrs)</th>
<th>Position of isotope in butyrate</th>
<th>Amino acid degraded</th>
<th>Specific activity</th>
<th>C14 Recovery in degradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>to C-1</td>
<td>C-2</td>
<td>C-3</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>-1</td>
<td>Serine</td>
<td>1.22</td>
<td>3.53</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>-1</td>
<td>Serine</td>
<td>0.84</td>
<td>2.03</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>-1</td>
<td>Serine</td>
<td>0.84</td>
<td>2.47</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>-3</td>
<td>Serine</td>
<td>0.91</td>
<td>2.22</td>
</tr>
<tr>
<td>VI</td>
<td>10</td>
<td>-3</td>
<td>Serine</td>
<td>0.91</td>
<td>2.72</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>-3</td>
<td>Alanine</td>
<td>1.14</td>
<td>2.83</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>-3</td>
<td>Alanine</td>
<td>1.15</td>
<td>3.28</td>
</tr>
<tr>
<td>VII</td>
<td>3</td>
<td>-3</td>
<td>Alanine</td>
<td>1.08</td>
<td>3.19</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>-2</td>
<td>Serine</td>
<td>3.40</td>
<td>1.6</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>-2</td>
<td>Serine</td>
<td>2.96</td>
<td>1.43</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>-2</td>
<td>Serine</td>
<td>1.71</td>
<td>1.11</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>-2</td>
<td>Alanine</td>
<td>1.89</td>
<td>1.44</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>-2</td>
<td>Glutamic acid</td>
<td>8.90</td>
<td>11.34</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>-2</td>
<td>Glutamic acid</td>
<td>6.04</td>
<td>7.75</td>
</tr>
</tbody>
</table>

* C-1 recovered by ninhydrin treatment of serine.
† C-2 and C-3 combusted together.
‡ C-2, C-3, and C-4 combusted together.

Serine or alanine were labeled in the same manner, principally in the C-1 position, after injection of butyrate-1-C14 or butyrate-3-C14. This is the expected position of labeling for transfer of carbon via the cycle as shown in Fig. 2. The small amount of C14 in the C-2 and C-3 positions of the amino acids may be due to one or more of the following causes: (a) some carry-over of C14 from the C-1 to the C-2 or C-3 position during the degradation procedures; (b) the pentose cycle which is active in the cow (21) and leads to some redistribution of carbon in glucose and in glycolytic intermediates; and (c) other minor pathways that would introduce C14 into positions other than C-1 of the glycolytic intermediates from which serine and alanine were derived.

Butyrate-2-C14 labeled serine and alanine principally on the C-2 and C-3 positions with approximately equal specific activity on each carbon. These results, like those after butyrate-1-C14 and butyrate-3-C14, also agree with the distribution expected for the transfer of butyrate carbon via the tricarboxylic acid cycle (Fig. 2). A similar distribution of C14 was found in these amino acids after injection of acetate-2-C14 into lactating cows (18) and also into rats (22).

The C14 distribution in glutamic acid was the same for butyrate-1-C14 and butyrate-3-C14, which agrees with the results expected when acetyl-1-C14-CoA enters the cycle at citrate. If butyrate had undergone β-oxidation to succinate, the C14 from butyrate-3-C14 would have appeared in the C-2 and C-3 positions of glutamate. Inasmuch as these positions contained less than 0.5% of the C14 in glutamic acid it appears that 14 oxidation of butyrate could not have been significant, if it occurred at all. The C14 distribution in glucose from rats injected with specifically labeled butyrate was inconsistent with β-oxidation of butyrate in that species also (15).

The labeling pattern in glutamate after butyrate-2-C14 (see Table III) further supports the pathway outlined in Fig. 2.
ketone bodies, acetone, may be directly converted to a glycolytic intermediate in animal tissues (23-27). This evidence has been derived from the C14 distribution in: (a) the glucose of liver glycogen from rats injected with acetone-2-C14 (23); (b) alanine from liver protein after injecting rats with acetone-2-C14 (24); (c) lactate in rat liver minced incubated with acetone-1-3-C14 (25, 26); and (d) in propanediol phosphate and liver glycogen from rats injected with acetone-2-C14 (28). In each case the C14 distribution indicated that acetone was utilized largely by a pathway in which the molecule was converted intact to some, as yet unidentified, glycolytic intermediate. In addition, it was shown that mice injected with two of the suggested intermediates in the pathway from acetone to glycolytic compounds had significantly higher levels of liver glycogen than did control groups (27).

The glucogenic behavior of butyrate, in contrast to that of acetate, could be accounted for by the following scheme if it were functional in the cow:

butyrate → butyryl-CoA → acetacetate3 → acetone + CO2
acetone → 3-C3 → glucose, alanine, serine

If this pathway had been responsible for the glucogenic behavior of butyrate in the cow, one would expect that lactose would contain more C14 (per unit of C14 injected) after injection of butyrate-3-C14 than after injection of butyrate-1-C14 because the carboxyl carbon (C-1) is lost as CO2 in the reaction sequence. In contrast to this expectation, the specific activity of lactose was no greater after injection of butyrate-3-C14 than it was after injection of butyrate-1-C14.2 Serine and alanine, which are synthesized from glycolytic intermediates, also had specific activities that were approximately equal after injections of butyrate-3-C14 and butyrate-1-C14 (see Table II).

Additional evidence that a pathway involving acetone was not quantitatively important in butyrate metabolism can be obtained from the degradation data. The direct oxidation of acetone would transfer C14 from butyrate-3-C14 to the C-2 position of glycolytic intermediates; but in the cow, C14 was found primarily in the C-1 position of alanine and serine after injection of either butyrate-1-C14 or butyrate-3-C14. The low levels of C14 in the C-2 positions of these compounds, may indicate the presence of a direct pathway, e.g. via acetone, but as discussed earlier, other factors may also contribute to the presence of C14 in the C-2 position. In any case, it seems safe to conclude that a pathway via acetone, if present at all, had only a very minor role in the transfer of carbon from butyrate to amino acids and lactose.

In the absence of convincing evidence that the glucogenic behavior of butyrate can be explained on a chemical basis, one could account for the different metabolic fate of acetyl-CoA from acetate and butyrate on an anatomical basis. The udder of the cow is especially active in lipogenesis (28) whereas the liver is a major source of blood glucose and is active in gluconeogenesis. If acetate were preferentially metabolized by the udder, and butyrate by the liver, that would explain the glucogenic fate of butyrate in contrast to the lipogenic fate of acetate in the lactating cow.

Evidence from studies in vitro supports this anatomical explanation for the diverse metabolic behavior of the two fatty acids in the intact animal:

1. Shaw et al. (29) perfused goat livers with C14-labeled fatty acids. After 1 hour, butyrate and most of the propionate had disappeared from the blood whereas 77% of the isotope recovered from acetate-C14 was still present as acetate. The C14 from propionate and butyrate was recovered mainly as lactate and glycerogen. These results support the idea that the liver does not readily metabolize acetate whereas propionate and butyrate are extensively utilized and contribute to the formation of glucose precursors.

Acetate was metabolized more slowly by liver than by several other tissues of the rat in vitro (30). Either propionate or butyrate inhibited the oxidation of acetate when added to rat liver slices (31). Propionate was shown to have a similar effect on acetate oxidation in sheep liver slices although the inhibition was not as marked as it was with rat liver (32). It would appear that the metabolic environment in liver, especially ruminant liver which may contain much propionate and butyrate, would not be favorable for acetate metabolism. Consequently, little carbon from acetate would be expected to appear in glycolytic intermediates from which glucose is synthesized in liver.

2. On the other hand, Peeters et al. (33) demonstrated that perfused mammary gland of cows metabolized acetate more rapidly than propionate or butyrate. The R.Q. value of the gland was less than 1 when butyrate (0.71) or propionate (0.73) was added to the perfusate but was greater than 1, an indication of fat synthesis, when acetate was added. R.Q. values as high as 1.5 were measured in mammary gland perfused with acetate (34).

Tracer studies reflect a similar metabolic picture for mammary gland. The C14 level was high in milk fatty acids and in CO2 when mammary gland was perfused with acetate-1-C14 (28) but was low when butyrate-1-C14 was perfused (35). It is interesting that butyrate had the greatest specific activity among the milk fatty acids when acetate-1-C14 was perfused (28) but had the least specific activity when butyrate-1-C14 was perfused (35). These results support the hypothesis that mammary gland preferentially uses acetate not only for oxidation but also for fatty acid synthesis whereas butyrate is poorly utilized. It appears that mammary gland does not directly esterify butyrate in the synthesis of milk fat but utilizes butyrate carbon to a limited extent, after conversion to 2 carbon units (36). It has already been suggested that a compound other than butyrate, namely β-hydroxybutyrate, may be a direct precursor of the butyrate moiety of milk fat (37, 38).

The metabolism of butyrate together with propionate, in the liver, provides an explanation for the "glycogenic" behavior of butyrate in the ruminant. According to this explanation carbon from propionate traversing the tricarboxylic acid cycle, at least in part during its conversion to glucose, would mix with carbon from butyrate being metabolized in the cycle and carbon from both compounds would appear in the precursors from which glucose is derived. The propionate carbon has an active role in this process in the sense that it provides the influx of carbon into the cycle that is necessary to replace intermediates withdrawn for the biosynthesis of glucose and amino acids in the cow. The role of butyrate would be a more passive one in the sense that it appears in glucose primarily because of the mixing of dicarboxylic acids which contain butyrate or propionate carbon and the random withdrawal of these in the synthesis of glucose or amino acids. This relationship results in a large amount of butyrate carbon appearing in glucose (and lactose) and gives...
the impression that butyrate-C\textsuperscript{14} could be responsible for net synthesis of glucose in the ruminant.

This explanation does not preclude the possibility that other compounds have a role similar to that discussed for propionate; for example, pyruvate or succinate, itself, derived from rumen fermentations. However, the fact that propionate constitutes a major end product of rumen microbial metabolism (17) would indicate that it is quantitatively important in the reactions required to replenish cycle intermediates.

It is also understandable how the uptake of butyrate carbon by liver cells might spare the oxidation of other metabolites, such as lactate or pyruvate, which could then contribute directly to the synthesis of glucose. This, together with a possible pharmacological effect\textsuperscript{4} resulting from intravenous injection of large amounts of butyrate could account for the hyperglycemic effect that has been reported for this fatty acid (1, 2, 4–6).

The anatomical explanation for the "glucogenic" behavior of butyrate obviates the need to postulate special metabolic pathways but does not exclude the possibility that such pathways may have a minor role in the metabolism of butyrate in the cow. The diverse metabolic behavior of organs in the animal makes it important to recognize that the metabolic fate (i.e. lipogenic, glucogenic, etc.) of a compound in animals depends on the site where each is metabolized and the enzymatic environment at that site. It is apparent that one cannot predict the metabolic fate of a compound in an animal from the knowledge alone of its pathway nor can one deduce its metabolic pathway from a knowledge alone of its metabolic fate.

### SUMMARY

Lactating cows were injected intravenously with butyrate-l-C\textsuperscript{14}, butyrate-2-C\textsuperscript{14}, and butyrate-3-C\textsuperscript{14}. At 3 and 10 hours after injection, milk was collected and used to prepare lactose and amino acids from casein.

On a basis of relative specific activities among these compounds, butyrate is a better precursor of glutamate and aspartate than it is for lactose, serine, or alanine. The specific activities in the cow metabolizes butyrate in the classical fashion by \( \beta \) oxidation and then via the tricarboxylic acid cycle, as do other animals, and that the "glucogenic" behavior of butyrate apparently results from its metabolism at a site of active gluconeogenesis, presumably the liver which absorbs butyrate relatively faster than it absorbs acetate.

### Acknowledgments

The authors wish to acknowledge the valuable technical assistance of J. A. Nakagiri and Edna Yeh.

### REFERENCES


\textsuperscript{4} It is known that intravenous injection of hyperphysiological amounts of butyrate may induce muscular twitching in animals. The increased levels of blood lactate, derived from muscle glycogen, could then stimulate gluconeogenesis in the liver.
Butyrate Metabolism in the Lactating Cow
Arthur L. Black, Max Kleiber and Alice M. Brown