BUTYRATE AS A PRECURSOR OF MILK CONSTITUENTS IN THE INTACT DAIRY COW*

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(Received for publication, February 1, 1954)

Butyric acid is a major product of feed fermentation in the intestinal tract of various animals, especially the rumen of cattle and sheep. This has been known for 70 years (1).

Elssen, Hitchcock, Marshall, and Phillipson (2) noted that the rumen and reticulum of an ox contained an average of 329 gm. of volatile acids (calculated as acetic acid), and that the mixture of volatile fatty acids in the intestines of cattle, sheep, horses, pigs, and rabbits had a similar composition, namely an average of 67 per cent acetic, 19 per cent propionic, and 14 per cent butyric acid. (These figures presumably represent molar percentages.)

The relative rate of absorption of fatty acids from the rumen depends on the acidity of the rumen content (3).

Kiddle, Marshall, and Phillipson (4) suggest that a greater part of the butyrate than of the other fatty acids is catabolized in the rumen wall, and Pennington (5) demonstrated that tissue slices of rumen epithelium convert butyrate to acetoacetate. Butyrate is thus a most interesting substance in the nutrition of cattle and sheep, its importance in biochemistry, however, not being limited to ruminants. It may play a rôle as a link between fat and carbohydrate metabolism. Its function as such in rats may, at least in part, be independent of acetate (6), and a considerable part of its contribution to the synthesis of animal carbohydrate by-passes the carbonate pool (7).

We injected C14-labeled butyrate as a single dose into the jugular vein of lactating cows. We then measured the C14 concentration in the expired CO2 and in lactose, casein, milk albumin, and butter fat as a function of time after injection of the tracer. These measurements permitted estimates of the relative rates at which butyrate provides carbon for respiratory CO2 and for the synthesis of the organic milk constituents.

* This investigation was supported by the United States Atomic Energy Commission.

† Predoctoral Research Fellow, United States Atomic Energy Commission

‡ Research Fellow of the National Institutes of Health, United States Public Health Service.
Method

Cows—Table I summarizes the characteristics of the three Jersey cows and one Holstein cow used in the experiments described here.

Tracer—The sodium butyrate-1-C\textsuperscript{14} was prepared by carbonation of propylmagnesium bromide with C\textsuperscript{14}O\textsubscript{2} at -20° (8). Yields were approximately 95 per cent, based on the barium carbonate-C\textsuperscript{14} used.

The sodium butyrate-2-C\textsuperscript{14} was synthesized by condensation of potassium cyanide with propyl-1-C\textsuperscript{14} iodide, followed by hydrolysis of the nitrile and steam distillation of the butyric acid. Propyl-1-C\textsuperscript{14} iodide was prepared by high pressure hydrogenation of cadmium-nickel propionate and iodination of the resulting alcohol with phosphorus and iodine (9).

TABLE I
Data on Experiments with Cows

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Cow No.</th>
<th>Body weight</th>
<th>Food intake per day</th>
<th>Milk yield per day</th>
<th>Plasma volume</th>
<th>Relative injected dose of C\textsuperscript{14}</th>
<th>Position of C\textsuperscript{14} in butyrate</th>
<th>Butyrate injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>905</td>
<td>914</td>
<td>6.4</td>
<td>12.1</td>
<td>1-C\textsuperscript{14}</td>
<td>9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>963</td>
<td>547</td>
<td>6.8</td>
<td>20.2</td>
<td>7.3</td>
<td>1-C\textsuperscript{14}</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1100</td>
<td>554</td>
<td>22.4</td>
<td>31.9</td>
<td>9.0</td>
<td>2-C\textsuperscript{14}</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>941</td>
<td>450</td>
<td>16.3</td>
<td>20.3</td>
<td>11.1</td>
<td>2-C\textsuperscript{14}</td>
<td>4.4</td>
<td></td>
</tr>
</tbody>
</table>

Injection and Dose—The use of plastic tubes for the intravenous injections performed in these trials has been described before (10).

The doses in microcuries per kilo of body weight and the amount of butyrate injected in millimoles are reported in Table I. From the data given by McClymont ((11) p. 95, Table I), we may estimate that 1 liter of arterial blood of a cow contains on the average 1.46 mM of acetate, 0.04 mM of propionate, 0.04 mM of butyrate, and 0.03 mM of higher fatty acids.

For an estimated average of 32 liters of blood, a cow thus contains in its blood about 1.3 mM of butyrate. According to this estimate, which neglects the difference between peripheral and intestinal blood, we injected about 3 to 8 times as much butyrate as is normally present in the blood of the cow. Considerable variation in the rate of production of fatty acids in the rumen and their appearance in the blood stream is to be expected. Our injection of butyrate, therefore, presumably did not produce abnormal conditions.

Respiration Trials and C\textsuperscript{14} Measurements—The methods of measuring
CO₂ production and radioactivity in the respiratory CO₂ have been described in two earlier papers (12, 13).

![Graph](image)

**Fig. 1**

**Results**

In Fig. 1 the standardized specific activity of C¹⁴ in the expired CO₂ ((microcuries of C¹⁴ per mole of CO₂)/(microcuries of C¹⁴ injected per kilo of body weight)) is plotted against time after the injection of the labeled butyrate. The curve for the specific activity after injection of carbonate-C¹⁴ is shown for comparison. As observed earlier with labeled acetate (14)
and propionate (15), the butyrate-1-C\textsuperscript{14} leads to a higher specific activity in respiratory CO\textsubscript{2} than does butyrate-2-C\textsuperscript{14}. This result indicates that, from butyrate as well as from the other fatty acids, the carboxyl carbon is transferred to CO\textsubscript{2} faster than the carbon in the 2 position of the molecule.

The maximal specific activity in CO\textsubscript{2} from butyrate-1-C\textsuperscript{14} is reached in 10 minutes after injection, that from butyrate-2-C\textsuperscript{14} in 30 minutes. The curves for specific activity in CO\textsubscript{2} from butyrate follow those from acetate more closely than those from propionate.

**Table II**

*Influence of Position of Label in Butyrate on Mean C\textsuperscript{14} Level in Respiratory CO\textsubscript{2} As Function of Time*

<table>
<thead>
<tr>
<th>Time after Injection (hrs)</th>
<th>Mean C\textsuperscript{14} level in respiratory CO\textsubscript{2}</th>
<th>Ratio of mean levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial I</td>
<td>Trial II</td>
</tr>
<tr>
<td>1</td>
<td>27.6</td>
<td>28.1</td>
</tr>
<tr>
<td>2</td>
<td>22.0</td>
<td>22.5</td>
</tr>
<tr>
<td>3</td>
<td>18.1</td>
<td>18.6</td>
</tr>
<tr>
<td>6</td>
<td>12.0</td>
<td>11.4</td>
</tr>
<tr>
<td>12</td>
<td>6.7</td>
<td>6.2</td>
</tr>
<tr>
<td>24</td>
<td>3.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* C\textsuperscript{14} levels are calculated as $\frac{1}{t} \int_{0}^{t} \rho dt$, where $\rho$ is expressed as $\mu$c. per mole CO\textsubscript{2} $\mu$c. injected per kilo body weight.

Table II shows that the mean level of C\textsuperscript{14} in the respiratory CO\textsubscript{2} for the 1st hour is over twice as high after injection of butyrate-1-C\textsuperscript{14} as it is after injection of butyrate-2-C\textsuperscript{14}. With increasing time after injection, the two levels tend to become equal. The mean C\textsuperscript{14} levels in CO\textsubscript{2} after injection of butyrate-1-C\textsuperscript{14} are between the levels after acetate-1-C\textsuperscript{14} and those after propionate-1-C\textsuperscript{14}.

The mean levels of C\textsuperscript{14} in CO\textsubscript{2} after injection of butyrate-2-C\textsuperscript{14} are strikingly similar to those after acetate-2-C\textsuperscript{14} (14) and propionate-2-C\textsuperscript{14} (15).

The relative rate of loss of the carboxyl carbon of butyrate appears to be between that of acetate and of propionate, while the relative rate of oxidation at the C\textsubscript{2} position, however, seems to be nearly the same for acetate, propionate, and butyrate. The injected acetate amounted to only 16 per cent of the normal acetate in the blood; the injected propionate and butyr-
ate, however, were from 3 to 8 times as great as the amount normally present in the blood. We do not have enough information to decide

**TABLE III**

*Relative C\textsubscript{14} Retention in Cow’s Body after Intravenous Injection of C\textsubscript{14}-Labeled Butyrate and Carbonate*

<table>
<thead>
<tr>
<th>Time after injection (hrs.)</th>
<th>Butyrate-1-C\textsubscript{14}</th>
<th>Butyrate-2-C\textsubscript{14}</th>
<th>Carbonate-C\textsubscript{14}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial I per cent</td>
<td>Trial II per cent</td>
<td>Trial III per cent</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
<td>68</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>52</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>40</td>
<td>66</td>
</tr>
</tbody>
</table>

**TABLE IV**

*Specific C\textsubscript{14} Activity in Milk Constituents*

The results refer to microcuries per gm. atom of C in milk constituents per microcuries injected per kilo of body weight.

<table>
<thead>
<tr>
<th>Milk constituents</th>
<th>Period No.</th>
<th>Time after injection (hrs.)</th>
<th>Butyrate-1-C\textsubscript{14}</th>
<th>Butyrate-2-C\textsubscript{14}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C\textsubscript{14} in milk constituents from</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trial I per cent</td>
<td>Trial II per cent</td>
</tr>
<tr>
<td>Lactose</td>
<td>1</td>
<td>3</td>
<td>1.77</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>3.07</td>
<td>3.33</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22</td>
<td>0.32</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>34</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>46</td>
<td>0.22</td>
<td>0.23</td>
</tr>
<tr>
<td>Casein</td>
<td>1</td>
<td>3</td>
<td>2.02</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>0.65</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22</td>
<td>0.45</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>34</td>
<td>0.24</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>46</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>1</td>
<td>3</td>
<td>0.32</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>0.60</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22</td>
<td>0.23</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>34</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>46</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>

whether or not this difference in the ratio between the injected dose and the average normal pool content affects the biokinetic behavior of the fatty acids.
Our cows produced 102, 137, 157, and 108 liters of CO₂ (0°C, 1 atmosphere, dry) per hour in Trials I to IV respectively. On the basis of these metabolic rates, the C¹⁴ retention during the 3 hours after injection has been calculated. The results are given in Table III. The relative retention of carbon atom 2 of the butyrate is similar to that of carbon atom 2 of acetate (14) and propionate (15). The carboxyl of butyrate is retained for a longer time than that of propionate but not for as long a time as that of acetate.

### Table V

**Transfer of C¹⁴ from Injected Acetate (A) and Butyrate (B) to Organic Milk Constituents**

<table>
<thead>
<tr>
<th>Position of C¹⁴ in injected acetate or butyrate</th>
<th>Per cent injected C¹⁴ in organic milk constituents in 46 hrs.</th>
<th>Per cent distribution of total C¹⁴ in organic milk constituents among Total Per kilo milk</th>
<th>Lactose</th>
<th>Casein</th>
<th>Milk fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>1-C¹⁴</td>
<td></td>
<td></td>
<td>16</td>
<td>6</td>
<td>0.7</td>
</tr>
<tr>
<td>2-C¹⁴</td>
<td></td>
<td></td>
<td>19</td>
<td>22</td>
<td>1.1</td>
</tr>
</tbody>
</table>

### Table VI

**Partition in Transfer of C¹⁴ from Butyrate to Milk Constituents**

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Position</th>
<th>Comparison after injection hrs.</th>
<th>Transfer via CO₂ in per cent of total transfer from butyrate to</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1-C¹⁴</td>
<td>46</td>
<td>Lactose</td>
</tr>
<tr>
<td>II</td>
<td>1-C¹⁴</td>
<td>46</td>
<td>Casein</td>
</tr>
<tr>
<td>III</td>
<td>2-C¹⁴</td>
<td>48</td>
<td>Fat</td>
</tr>
<tr>
<td>IV</td>
<td>2-C¹⁴</td>
<td>48</td>
<td></td>
</tr>
</tbody>
</table>

* Lactose sample lost.

Table IV shows the specific C¹⁴ activities in lactose, casein, and milk fat obtained during the 2 days following the injection of the labeled butyrate. As in previous trials (13–15), there is a definite delay in the labeling of milk fat in comparison to the labeling of lactose and casein. The high specific activity in lactose and casein in comparison to that of milk fat is surprising.

Table V summarizes the distribution of the C¹⁴ from butyrate in lactose, casein, and milk fat for a period of 46 hours following the injection. Table VI, calculated as described in our paper on acetate ((14) p. 377), indicates that only a fraction of the transfer of carbon from butyrate to organic milk constituents involves the carbonate pool.
DISCUSSION

Our data indicate that, in the lactating cow, butyrate in contrast to acetate is more closely related to the synthesis of lactose than to that of milk fat.

This result is in line with that of Buchanan, Hastings, and Nesbett (6), who fed 1-C\textsuperscript{14}-labeled acetate, propionate, and butyrate mixed with glucose to fasted rats, and concluded that propionic and butyric acids are converted to liver glycogen but that acetate is not. The latter statement is derived from the observation that the labeling of glycogen after acetate feeding could be entirely accounted for by transfer of the carboxyl carbon to sugar via carbonate.

Lifson et al. (7) fed C\textsuperscript{132}-labeled acetate and butyrate to rats, and then determined the C\textsuperscript{13} excess in the liver glycogen and its degradation products. In contrast to Buchanan et al. (6), Lifson and his coworkers concluded that, at least, the \(\alpha\)-carbon of acetate can enter glycogen by a pathway other than CO\textsubscript{2} fixation. The labeling pattern of glycogen after feeding butyrate-C\textsuperscript{13} was consistent with \(\beta\) oxidation of butyrate and transfer of carbon from butyrate to glycogen via acetate.

Our trials indicate that a considerable part of the transfer of C from butyrate as well as from acetate (14) in either position 1 or 2 by-passes the carbonate pool.

The comparison of the C\textsuperscript{14} distribution among organic milk constituents after injection of labeled acetate and butyrate (Table V) reveals a radical difference between the metabolic behavior of butyrate and that of acetate, which supports the idea that a considerable part of the carbon transfer from butyrate to lactose by-passes the acetate pool.

These observations, combined with earlier results of other workers, favor the hypothesis that the transfer of carbon from butyrate to lactose is part of a net synthesis of lactose rather than the result of a redistribution process of C atoms in a closed Krebs cycle. Blixenkrone-Moller (16) observed an increase of glycogen in cat liver perfused with butyrate. That was undoubtedly a net synthesis. Since he did not use a tracer, he had no direct evidence that the carbon in the glycogen formed came from the butyrate. But the combination of Blixenkrone-Moller’s results with those of Buchanan et al. (6) and Lifson et al. (7) on rats, of those of Schambye, Wood, and Popjak (17) on lactating rabbits, and of our own on lactating cows with labeled carbon forms a rather strong case for the hypothesis that butyrate participates in a net carbohydrate synthesis.

Blixenkrone-Moller’s scheme of a transfer of butyrate to carbohydrate via oxidation to succinate does not fit the labeling pattern obtained by Lifson et al. (7); namely, an excess labeling of the 3 and 4 carbons of glucose after feeding of butyrate-3-C\textsuperscript{14}. Also, the more recent scheme of
acetone formation from butyrate by oxidation at the 3 position and de-
carboxylation (Plaut and Lardy (18)) and the subsequent direct oxidation
of the acetone to pyruvate (Sakami and Lafaye (19)) would lead to the
prediction that injected or fed butyrate-3-C\textsuperscript{14} labels glucose in the 2 and 5
rather than in the 3 and 4 positions.

Our observation, that the labeling pattern of milk constituents after the
injection of C-1- and C-2-labeled butyrate differs markedly from that
observed after the injection of labeled acetate, does not preclude the \( \beta \)
oxidation of butyrate if interpreted on the basis of the "two species theory"
of C\textsubscript{2} intermediate metabolites of Crandall \textit{et al.} (20). Such an explana-
tion, however, would indicate that the carboxyl type C\textsubscript{2} unit is more di-
rectly involved in carbohydrate than in fat synthesis.

A trial is under way in our laboratory to measure directly the involve-
ment of the acetate and the blood glucose pools in the transfer of carbon
from butyrate to organic milk constituents.

**SUMMARY**

1. Intact normal dairy cows were injected with sodium butyrate-1-C\textsuperscript{14}
and -2-C\textsuperscript{14} in single doses ranging from 7 to 12 \( \mu \)c. of C\textsuperscript{14} per kilo of body
weight.

2. The specific activity of C\textsuperscript{14} in the expired CO\textsubscript{2} reached a maximum 10
minutes after injection of butyrate-1-C\textsuperscript{14} and 30 minutes after injection of
butyrate-2-C\textsuperscript{14}.

3. The relative rate of oxidation of the carboxyl carbon of butyrate ap-
ppears to be between that of acetate and that of propionate, and the rela-
tive oxidation rate of the carbon in the 2 position of the molecule nearly the
same for butyrate as for acetate and propionate.

4. About one-third of the transfer of carboxyl carbon of butyrate to
lactose apparently passes through the carbonate pool, and about one-fifth
of the corresponding transfer to casein and less than one-tenth of that to
milk fat pass via carbonate. The carbonate pool is by-passed by over 80
per cent of the transfer of carbon atom 2 in butyrate to lactose and by over
90 per cent of that to casein and milk fat.

5. About 6 per cent of the 1-C\textsuperscript{14} and 22 per cent of the 2-C\textsuperscript{14} of the in-
jected butyrate appeared in organic milk constituents.

6. More of the 1-C\textsuperscript{14}, as well as of the 2-C\textsuperscript{14}, of the injected butyrate
appeared in lactose and in casein than appeared in milk fat.

7. This pattern of labeling milk constituents differs from the correspond-
ing one after injection of C\textsuperscript{14}-labeled acetate.

8. In contrast to acetate, butyrate behaved in the lactating cow in a
more glyconeogenic than lipogenic manner.

9. Our results are consistent with the participation of butyrate in net
synthesis of carbohydrate.
We are pleased to acknowledge the valuable help of Patricia Adams of the Radiation Laboratory at Berkeley, who synthesized the C¹⁴-labeled butyrate for us, and the faithful assistance of Mr. T. Chernikoff of the Department of Animal Husbandry, who analyzed all the gas samples for our C¹⁴ trials.

BIBLIOGRAPHY

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