THE TRANSFER OF CARBON FROM PROPIONATE TO AMINO ACIDS IN INTACT COWS*

BY ARTHUR L. BLACK AND MAX KLEIBER

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

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Propionate, like acetate, is an active metabolite which arises from a variety of sources in animal tissues. It is produced during the metabolism of fatty acids which contain an odd number of carbon atoms (2) and which, as Shorland and Hansen (3) have shown, occur naturally in small amounts in triglycerides from various animal tissues. It arises also during the catabolism of certain amino acids including isoleucine (4), valine (5), methionine (6, 7), threonine (in part) (7, 8), and norleucine (7). In ruminants, large quantities of acetate and propionate arise in the rumen and together constitute an appreciable part of the daily caloric intake for these animals (9, 10).

The metabolic fate of these two fatty acids is different, propionate being largely glucogenic (11, 12) while acetate is largely lipogenic (13) or ketogenic (14). Thus, one might expect a difference in their utilization for amino acid synthesis. In an earlier study with acetate-C14, it was shown that, among the amino acids, those derived directly from tricarboxylic acid cycle intermediates, glutamic and aspartic acids, had the greatest specific activities (15).

The present study was undertaken to evaluate the role of propionate as a precursor of amino acids in casein. Five cows were injected intravenously with propionate-1-C14 or propionate-2-C14, and amino acids were recovered from nineteen samples of casein collected at different times after injection. The results indicated that propionate was equally as important as a precursor for aspartic acid and serine as it was for carbohydrate. Surprisingly, alanine, a glucogenic amino acid, was formed with relatively small specific activity. These results are considered together with those previously obtained from cows injected with acetate-C14 (15) and glucose-C14 (16).

Methods

Casein samples were prepared from milk collected 3, 10, 23, and 34 hours after injecting five normal lactating cows intravenously with a single dose

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of 1 to 5 mc. of propionate-1-C\textsubscript{14} or propionate-2-C\textsubscript{14}. The details concerning the animals used and compounds injected are given in Table I.

Earlier publications have described the methods for casein hydrolysis, ion exchange chromatography, crystallization of amino acids, and C\textsubscript{14} assay (15).

### Table I

**Data on Experimental Animals Used in Propionate Trials**

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Cow No.</th>
<th>Weight</th>
<th>Injected dose</th>
<th>Propionate injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>905</td>
<td>414</td>
<td>5.0</td>
<td>1-C\textsubscript{14}</td>
</tr>
<tr>
<td>II</td>
<td>913</td>
<td>495</td>
<td>4.6</td>
<td>1-C\textsubscript{14}</td>
</tr>
<tr>
<td>III</td>
<td>965</td>
<td>469</td>
<td>5.0</td>
<td>2-C\textsubscript{14}</td>
</tr>
<tr>
<td>IV</td>
<td>860</td>
<td>544</td>
<td>4.8</td>
<td>2-C\textsubscript{14}</td>
</tr>
<tr>
<td>VII</td>
<td>65</td>
<td>470</td>
<td>1.1</td>
<td>2-C\textsubscript{14}</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

The specific activities of the amino acids are listed in Tables II and III for trials with propionate-1-C\textsubscript{14} and propionate-2-C\textsubscript{14}, respectively. The specific activities, microcuries of C\textsubscript{14} per gm. atom of carbon, are expressed relative to the injected dosages, microcuries injected per kilo of body weight, to compensate for differences in body size of the cows and the amounts of C\textsubscript{14} injected.

The specific activity of the amino acids provides an index of their relationship to propionate. One may assume that, among the compounds synthesized during the same interval, the greater the specific activity the more important the role of propionate as a precursor. Among the amino acids, the specific activity was greatest for serine or aspartic acid in the 3 hour milk samples for each cow injected with propionate-C\textsubscript{14}. Lactose\textsuperscript{1} from the same milk samples had specific activities no greater than those in serine or aspartic acid at 3 hours after injection, and the average specific activities for all three compounds were approximately equal during the 34 hour experimental period. These results demonstrate that, quantitatively,

\textsuperscript{1} The specific activities of lactose samples from propionate Trials I to IV have been published earlier (12). Subsequent to publication it was found that Lactose 1, 2, and 4 of propionate Trial I contained small amounts of foreign material. The specific activity changed slightly after purification and recrystallization. The corrected values are as follows: Lactose 1, 1.26; Lactose 2, 1.17; Lactose 4, 0.18. The specific activities of lactose samples in propionate Trial VII, not reported previously, were as follows: Lactose 1, 5.90; Lactose 2, 5.00; Lactose 3, 1.20. All specific activities are expressed as microcuries of C\textsubscript{14} per gm. atom of carbon per unit of injected dose (microcuries injected per kilo of body weight) as explained in the text.
TABLE II
Specific Activity* of Amino Acids from Casein after Intravenous Injection of Propionate-1-\(^{14}\)C

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Time after injection</th>
<th>Glutamic acid</th>
<th>Aspartic acid</th>
<th>Alanine</th>
<th>Serine</th>
<th>Glycine</th>
<th>Proline</th>
<th>Arginine</th>
<th>Leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>hrs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.84</td>
<td>1.36</td>
<td>0.90</td>
<td>1.06</td>
<td>0.72</td>
<td>0.122</td>
<td>0.209</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.642</td>
<td>0.85</td>
<td>0.87</td>
<td>0.745</td>
<td>0.687</td>
<td>0.131</td>
<td>0.210</td>
<td>0.002</td>
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<td>23</td>
<td>0.268</td>
<td>0.30</td>
<td>0.30</td>
<td>0.29</td>
<td>0.335</td>
<td>0.035</td>
<td>0.075</td>
<td>0.006</td>
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<td>34</td>
<td>0.103</td>
<td>0.150</td>
<td>0.182</td>
<td>0.145</td>
<td>0.174</td>
<td>0.019</td>
<td>0.042</td>
<td>0.002†</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>2.99</td>
<td>5.72</td>
<td>2.35</td>
<td>3.44</td>
<td>2.71</td>
<td>0.42</td>
<td>0.435</td>
<td></td>
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<tr>
<td></td>
<td>10</td>
<td>0.629</td>
<td>0.918</td>
<td>0.78</td>
<td>0.87</td>
<td>1.03</td>
<td>0.17</td>
<td>0.28</td>
<td>0.003‡</td>
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<td>23</td>
<td>0.127</td>
<td>0.165</td>
<td>0.153</td>
<td>0.262</td>
<td>0.332</td>
<td>0.049</td>
<td>0.072</td>
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<tr>
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<td>34</td>
<td>0.041</td>
<td>0.075</td>
<td>0.069</td>
<td>0.116</td>
<td>0.165</td>
<td>0.022</td>
<td>0.04</td>
<td>0.006‡</td>
</tr>
</tbody>
</table>

* Specific activity expressed as (microcuries per gm. atom of C)/(microcuries injected per kilo of body weight).
† Lysine instead of leucine.
‡ Valine instead of leucine.

TABLE III
Specific Activity of Amino Acids from Casein after Intravenous Injection of Propionate-2-\(^{14}\)C

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Time after injection</th>
<th>Glutamic acid</th>
<th>Aspartic acid</th>
<th>Alanine</th>
<th>Serine</th>
<th>Glycine</th>
<th>Proline</th>
<th>Arginine</th>
<th>Leucine</th>
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<tbody>
<tr>
<td>III</td>
<td>hrs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>7.26*</td>
<td>8.72</td>
<td>2.2</td>
<td>7.15</td>
<td>1.21</td>
<td>0.63</td>
<td>0.11</td>
<td>0.000†</td>
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<td>10</td>
<td>5.51</td>
<td>5.44</td>
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<td>1.98</td>
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<td>0.46</td>
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<td>23</td>
<td>1.04</td>
<td>0.76</td>
<td>0.7</td>
<td>0.94</td>
<td>0.63</td>
<td>0.15</td>
<td>0.14</td>
<td>0.04</td>
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<td>0.3</td>
<td>0.25</td>
<td>0.27</td>
<td>0.43</td>
<td>0.3</td>
<td>0.06</td>
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<tr>
<td>IV</td>
<td>3</td>
<td>3.22</td>
<td>2.61</td>
<td>2.30</td>
<td>4.36</td>
<td>1.34</td>
<td>0.44</td>
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<td>0.17</td>
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<td>0.41</td>
<td>0.41</td>
<td>0.36</td>
<td>0.10</td>
<td>0.08</td>
<td>0.007</td>
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<tr>
<td>VII</td>
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<td>6.88</td>
<td>5.06</td>
<td>10.22</td>
<td>2.16</td>
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<td></td>
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<tr>
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<td>10</td>
<td>5.0</td>
<td>4.11</td>
<td>3.89</td>
<td>4.68</td>
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<td>1.13</td>
<td>1.22</td>
<td>1.43</td>
<td>1.01</td>
<td></td>
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<td></td>
</tr>
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</table>

* Specific activity of 7.26 µc. per gm. atom of C divided by microcuries injected per kilo of body weight = 2734 net c.p.m. in our counting system with BaCO₃ planchets of "infinite thickness."
† Valine instead of leucine.

Propionate is as important a precursor for serine and aspartic acid as it is for lactose. Since lactose is derived largely from blood sugar (17), it appears that, in the cow, this comparison would hold for carbohydrates in
The importance of propionate as a precursor of sugar has been established in phlorizinized dogs (11), in intact rats (18, 19), in sheep (20), and in cows (12). The current study demonstrates its quantitative importance in amino acid synthesis for cows.

The specific activities of glutamic acid and alanine were, on the average, lower than those of the three compounds discussed above. Glycine, proline, and arginine had very low specific activities, while leucine, valine, and lysine had only insignificant levels of \( ^{14} \text{C} \) derived from propionate.

The relatively low specific activity of alanine was especially interesting. If propionate were oxidized to pyruvate, as suggested by Mahler and Huennekens (21), one would not expect alanine to have lower specific activities than other compounds such as serine, aspartic acid, and lactose, especially in the early samples. These results strongly indicate that, in the cow, pyruvate is not on the direct pathway for propionate metabolism. The results observed would not be inconsistent, however, with the metabolism of propionate via succinate (22).

The specific activity among the amino acids and lactose after injection of propionate was quite different from that observed after injection of cows with acetate-\( ^{14} \text{C} \) or glucose-\( ^{14} \text{C} \). When cows were injected with uniformly labeled glucose (23), lactose was formed with the greatest specific activity, thereby reflecting the important role of glucose as a precursor of milk sugar. Among the amino acids, those derived from the glycolytic intermediates, serine and alanine, had approximately equal specific activities but at levels almost 3 times as great as those of aspartic and glutamic acids. These results demonstrated the importance of glucose as a precursor of the 3-carbon amino acids in the cow (16).

When cows were injected with acetate-1-\( ^{14} \text{C} \) or acetate-2-\( ^{14} \text{C} \), glutamic and aspartic acids had the greatest specific activities. Alanine, serine, and lactose were formed with approximately equal specific activities but at levels only one-half to one-third as great as those of glutamic and aspartic acids.

The results obtained after injection of propionate do not fit either of these patterns. One of the amino acids derived from glycolytic intermediates, serine, had a specific activity as great or greater than those of aspartic and glutamic acids, while the other “glycolytic” amino acid, alanine, had lower specific activities. The specific activity of lactose\(^1\) followed more closely the specific activity of serine than that of alanine.

The difference in labeling pattern among the amino acids and lactose after injection of glucose-\( ^{14} \text{C} \); on the one hand, or propionate-\( ^{14} \text{C} \), on the other, demonstrates that carbon from propionate is not converted to glucose prior to its appearance in amino acids. During the normal metabolism of propionate, some \( ^{14} \text{C} \) will appear in glucose and subsequently in amino
acids, but a major part of the transfer must follow a more direct pathway to the amino acids.

The metabolic scheme depicted in Fig. 1 provides a hypothetical basis for interpreting some of the results obtained in tracer studies with cows. Acetate enters the tricarboxylic acid cycle at the level of citrate, while propionate enters at the level of succinate. Carbon from each is distributed subsequently to glutamic and aspartic acids and, via glycolytic intermediates, to alanine and serine and to lactose. The transfer of carbon from glucose could be accounted for by essentially the same pathways but in the reverse direction.

![Diagram of metabolic scheme](image)

**Fig. 1.** Metabolic scheme for transfer of carbon from acetate, propionate, and glucose to amino acids and lactose.

One observation, however, is difficult to explain in terms of this scheme, namely that the specific activity of serine relative to alanine was greater after injection of propionate than after injection of acetate. If propionate and acetate were metabolized via the same cyclic mechanism, then the specific activity of serine relative to alanine should have been the same for both fatty acids. Carbon from either fatty acid, upon entering the cycle, would label oxalacetate in the normal turnover of intermediates, and subsequent distribution of C\textsuperscript{14}, via phospho-enol-pyruvate, to alanine or serine (see Fig. 1) would be independent of the form in which C\textsuperscript{14} was administered, that is acetate or propionate. After injection of acetate-1-C\textsuperscript{14} or acetate-2-C\textsuperscript{14}, the specific activities of alanine and serine were approximately equal in seventeen of the eighteen casein samples on which determinations were made (15). In contrast, after injection of propionate-
1-C\textsuperscript{14} or propionate-2-C\textsuperscript{14} (see Tables II and III), the specific activity of serine always exceeded that of alanine in the 3 hour casein sample and was generally greater in later samples as well. These results cannot be explained by a common tricarboxylic acid cycle for the metabolism of acetate and propionate. There are two explanations which may account for the observed results: (1) There is a more direct pathway from propionate to serine that does not involve the tricarboxylic acid cycle. (2) Propionate is metabolized, in part, via a cycle different from that involved in acetate metabolism. In this case, one could conclude that the cycle largely responsible for propionate metabolism was more closely associated with the formation of serine than alanine.

Data on the labeling pattern revealed by stepwise degradation of various compounds are now being collected and will provide additional information on the relationship between propionate and the amino acids. These data should help to decide between the possibilities listed above.

**SUMMARY**

Five lactating cows were injected intravenously with propionate-1-C\textsuperscript{14} or propionate-2-C\textsuperscript{14}, and the C\textsuperscript{14} level was measured in amino acids recovered subsequently from casein.

The specific activities of aspartic acid, serine, and lactose from the same milk sample were very similar, indicating that propionate is as important a precursor of aspartic acid and serine as it is of carbohydrate.

Alanine had a low specific activity relative to serine, in contrast to the results obtained after injection of glucose-C\textsuperscript{14} or acetate-C\textsuperscript{14}. Our results indicate that propionate is not converted to pyruvate or glucose prior to its conversion to amino acids but probably follows a pathway via succinate. In this latter case, it would be necessary to postulate that succinate enters, at least in part, into a cyclic process distinct from that in which carbon from acetate is distributed to the amino acids of casein.

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**BIBLIOGRAPHY**

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