Acetate Metabolism in the Ruminant*

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(Received for publication, July 26, 1963)

Biochemists have long recognized the central role of acetate in the energy metabolism of most forms of life. But whereas for most organisms the supply of acetate, or rather its active form, acetyl coenzyme A, is almost entirely endogenous, ruminant animals have a large exogenous supply of acetate, which is produced in the rumen by microbial fermentation of ingested foodstuffs. In the ruminant, acetate, along with the other short chain fatty acids, particularly propionate and butyrate, provides the chief source of energy for the animal.

Tolerance tests (1-3) have shown that the ruminant can metabolize the imposed load of large amounts of acetate. Previous isotope studies (4-7) indicate that the body pool of acetate turns over very rapidly and that the turnover rate, or acetate flux, when expressed as calories, represents a considerable proportion of the total energy requirement of the animal.

As part of a continuing study of energy metabolism in ruminants we have used W-acetate to study the size and rate of metabolism of the acetate pool in fed sheep. To understand more fully the role of acetate in the energetics of the whole animal, we have examined the metabolism of both the methyl and the carboxyl carbons, and also the metabolism of acetate infused into a rumen vein as opposed to a jugular vein.

EXPERIMENTAL PROCEDURE

Owing to the rapid turnover of body acetate, the method of a single injection of isotope, as used in early turnover studies (6), is inadequate because there is extensive metabolism before mixing is complete (4). Therefore, we have again used continuous infusion (7).

During and following infusion of W-acetate, blood samples were collected from the jugular vein and analyzed for amount and specific activity of acetate. Expired gas was continuously collected, and frequent samples were analyzed for the amount and specific activity of CO₂.

The values obtainable by such a technique are:

(a) From the blood analyses

Turnover rate (the amount of acetate disappearing from the acetate pool), which is equal to

\[
\text{Infusion rate (d.p.m. per minute)} / \text{Specific activity of blood acetate (d.p.m. per meq)}
\]

Turnover time (the time taken for the body to remove from the pool an amount of acetate equal to the total present), which is equal to

\[
\frac{\text{Half-life}}{\ln 2}
\]

Half-life of acetate, which was calculated from the fall in specific activity of the blood acetate after infusion was stopped

Pool size (the amount of acetate present in the body, exclusive of the rumen, at any given time), which is equal to

\[
\text{Turnover rate (meq per minute)} \times \text{turnover time (minutes)}
\]

Acetate space (the volume of fluid in which the acetate pool is dissolved), which is equal to

\[
\frac{\text{Pool size (meq)}}{\text{Concentration (meq per liter)}}
\]

(b) From analyses of expired ¹⁴C₀₂

Percentage of acetate appearing as CO₂, which is equal to

\[
\frac{\text{Expired } ^{14}\text{C}}{\text{Infused } ^{14}\text{C}} \times 100
\]

Percentage of CO₂ derived from acetate, which is equal to

\[
\frac{\text{Specific activity of } ^{14}\text{C }\text{O}_2 \times 2}{\text{Specific activity of blood acetate}} \times 100
\]

Half-life of intermediates between acetate and CO₂, which was calculated from the fall in specific activity of expired ¹⁴CO₂ after infusion was stopped.

Seven mature grade sheep, both ewes and whethers, were used for a total of nine infusions. When the same sheep was used for two infusions, a recovery period of about 6 months was allowed between experiments. All animals were housed indoors in individual cages and were fed once daily a maintenance ration of mixed clover and alfalfa hay.

Of the nine experiments reported here, five infusions were of acetate-¹⁴C into the jugular vein, two were of acetate-2-W into the jugular vein, and two were of acetate-¹⁴C into the rumen vein.

The essential features of the experimental conditions for each infusion are shown in Table I. In one case (Experiment E) the animal was fed during the course of the infusion; thus CO₂...
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The musculature was divided and the rumen exposed. A slightly oblique, dorsal to ventral incision, 20 cm long, was made in the left side, about 5 to 10 cm posterior to the last rib. The catheter was then inserted approximately 3 cm through an 11-gauge hypodermic needle inserted on the day before the surgical procedure was necessary. Under local anesthetic (3% procaine) a slightly oblique, dorsal to ventral incision, 20 cm long, was made in the left side, about 5 to 10 cm posterior to the last rib. The musculature was divided and the rumen exposed. The catheter was then inserted approximately 3 cm through a split made in the wall of one of the major branches of the rumen vein. It was tied in place with a nylon suture that occluded the branch into which the catheter had been inserted. The muscles were then sewn together so that two or three loops of catheter were left below the surface, i.e., against the rumen wall. The skin was then sutured. Rumen vein blood can be readily withdrawn through such a catheter. In order to keep the tube open, it was found necessary to continuously infuse 0.09% NaCl solution slowly through it. It could be kept open without difficulty for up to 3 days, and infusions of labeled acetate were not made until the animal had recovered and regained its appetite (3 to 4 days).

Blood samples, 15 ml, were withdrawn through an indwelling catheter that was inserted into the jugular vein as described above. To collect expired CO₂, a tracheal fistula was constructed approximately 7 days before the infusion by exposing the trachea through a 5-cm incision along the midline of the neck, 1.5 cm was removed from one tracheal ring, and a stainless steel tube (1.3 cm in external diameter) was inserted. This tube was removed, cleaned, and replaced daily. To collect expired gases, the metal tube was removed and a Magill catheter (a rubber tube with an inflatable bulb) was inserted in its place. When the bulb was inflated, all respired gases, and only respired gases, passed through the tube; contamination of expired air by rumen gases was thus prevented. A respiratory valve was connected, and the expired gases were passed through a meter that simultaneously measured the volume and gave a continuous aliquot (0.2%) that was collected in a football bladder.

In the early experiments the blood samples were analyzed for acetate by the method of Sabine and Johnson (8), but in later studies the method of Davis and Brown (9) was used. The latter method involves chromatography of a protein-free filtrate of the blood on a silicic acid column with the use of chloroform and butanol as eluents. For ¹⁴C determinations, aliquots of the column effluent were counted in a liquid scintillation counter. The percentage of CO₂ in the expired air was determined with a Haldane gas analysis apparatus. The specific activity was determined by the method of Oppermann et al. (10), which involves absorption of the CO₂ in Primene, titration of the Primene-CO₂ complex and liquid scintillation counting. Acetate-¹⁴C was synthesized from Ba¹³CO₃ by carbonation of methyl magnesium iodide. Acetate-²¹⁴C was obtained commercially.

RESULTS

A typical record of the level and specific activity of blood acetate found during the course of an infusion is shown in Table I, taken from Experiment A. In all experiments, equilibrium values were reached before the first blood sample was taken (30 minutes). The rate of fall of the specific activity of blood acetate during the exponential part of the curve (about 3 minutes) after infusion was stopped is plotted in Fig. 1. For the

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sleep No.</th>
<th>Route of infusion</th>
<th>Label</th>
<th>Weight of animal</th>
<th>Infusion rate</th>
<th>Infusion time</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2525</td>
<td>Jugular vein</td>
<td>¹⁴C</td>
<td>44</td>
<td>10.29</td>
<td>300</td>
</tr>
<tr>
<td>B</td>
<td>2502</td>
<td>Jugular vein</td>
<td>¹⁴C</td>
<td>41</td>
<td>7.60</td>
<td>401</td>
</tr>
<tr>
<td>C</td>
<td>3070</td>
<td>Jugular vein</td>
<td>¹⁴C</td>
<td>37</td>
<td>10.08</td>
<td>721</td>
</tr>
<tr>
<td>D</td>
<td>2941</td>
<td>Rumen vein</td>
<td>¹⁴C</td>
<td>35</td>
<td>9.71</td>
<td>482</td>
</tr>
<tr>
<td>E</td>
<td>2525</td>
<td>Rumen vein</td>
<td>¹⁴C</td>
<td>45</td>
<td>9.72</td>
<td>783</td>
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<tr>
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<td>48</td>
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<td>606</td>
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<tr>
<td>G</td>
<td>2488</td>
<td>Jugular vein</td>
<td>²¹⁴C</td>
<td>37</td>
<td>5.73</td>
<td>544</td>
</tr>
<tr>
<td>H</td>
<td>3043</td>
<td>Jugular vein</td>
<td>¹⁴C</td>
<td>30</td>
<td>5.95</td>
<td>480</td>
</tr>
<tr>
<td>I</td>
<td>3212</td>
<td>Jugular vein</td>
<td>¹⁴C</td>
<td>41</td>
<td>2.97</td>
<td>479</td>
</tr>
</tbody>
</table>

CH₃¹⁴COOH in blood during infusion of ¹⁴C-acetate (Experiment A)

<table>
<thead>
<tr>
<th>Time of infusion</th>
<th>CH₃¹⁴COOH</th>
<th>Specific activity</th>
<th>Turnover rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>meq/liter</td>
<td>¹⁰⁶ d.p.m./meq</td>
<td>meq/min</td>
</tr>
<tr>
<td>0</td>
<td>1.33</td>
<td>3.33</td>
<td>3.2</td>
</tr>
<tr>
<td>30</td>
<td>1.09</td>
<td>2.32</td>
<td>4.4</td>
</tr>
<tr>
<td>60</td>
<td>1.05</td>
<td>2.30</td>
<td>4.5</td>
</tr>
<tr>
<td>90</td>
<td>0.90</td>
<td>1.90</td>
<td>5.4</td>
</tr>
<tr>
<td>180</td>
<td>0.89</td>
<td>2.40</td>
<td>4.3</td>
</tr>
<tr>
<td>240</td>
<td>0.73</td>
<td>2.80</td>
<td>3.7</td>
</tr>
<tr>
<td>300</td>
<td>0.90</td>
<td>2.34</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Average 60-300

FIG. 1. Fall in specific activity of blood acetate after infusion of ¹⁴C-acetate was stopped. Line computed by method of least squares. The symbols represent experiments, as follows: ●, B; ○, C; X, D; △, F; □, G; ▲, H; and ∧, I.
nine experiments performed, this rate of fall was not significantly affected by the site of labeling or the route of infusion. The average half-life of body pool acetate was 1.3 minutes, which corresponds to a mean turnover time of 1.9 minutes (Table III).

Fig. 2 shows the specific activity of the expired $^{14}$CO$_2$ during and after infusion. One example has been taken from each type of infusion. The lower level in Experiment F is merely a reflection of the lower rate of infusion of the labeled acetate (Table I).

The recovery of $^{14}$C in the expired air is a measure of the acetate oxidized during the course of the experiment. Table IV, taken from Experiment D, is a typical example. The concurrent figures represent the amount of label recovered during the preceding time period as a percentage of label infused during that time period, whereas the cumulative recovery is the total recovery as a percentage of the total infused from the start of the infusion. The rate at which $^{14}$C was expired as CO$_2$, expressed as a percentage of the $^{14}$C infused during the preceding time period (i.e. concurrent data), is plotted in Fig. 3 against time after start of infusion. Data from all six experiments having continuous CO$_2$ collection are plotted on the same graph. No difference is apparent between C$_1$- and C$_2$-labeled acetate, nor between ruminal and jugular vein infusion.

Cumulative values of $^{14}$CO$_2$ expired expressed as a percentage of total $^{14}$C infused are plotted in Fig. 4 against time of infusion to show the total percentage of acetate the animal has oxidized and expired at any given time after infusion was started. In this plot also, there appears to be no effect of site of labeling or of infusion on rate of acetate oxidation.

CO$_2$ collections were continued for some hours after infusion

**Table III**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Animal No.</th>
<th>Blood acetate</th>
<th>Mean blood acetate specific activity</th>
<th>Turnover rate</th>
<th>Half-life</th>
<th>Turnover time</th>
<th>Acetate pool</th>
<th>Acetate space</th>
<th>CO$_2$ from acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2525</td>
<td>0.90</td>
<td>2340</td>
<td>4.4</td>
<td>1.0</td>
<td>1.5</td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>B</td>
<td>2502</td>
<td>0.70</td>
<td>2220</td>
<td>4.5</td>
<td>1.0</td>
<td>1.5</td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>C</td>
<td>3070</td>
<td>0.73</td>
<td>2580</td>
<td>3.8</td>
<td>1.8</td>
<td>2.6</td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>D</td>
<td>2941</td>
<td>0.91</td>
<td>2941</td>
<td>3.9</td>
<td>1.8</td>
<td>2.6</td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>E</td>
<td>2525</td>
<td>0.91*</td>
<td>2470</td>
<td>3.9</td>
<td>1.8</td>
<td>2.6</td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>F</td>
<td>2502</td>
<td>1.01</td>
<td>1710</td>
<td>5.7</td>
<td>1.8</td>
<td>2.6</td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>G</td>
<td>2488</td>
<td>0.69</td>
<td>2060</td>
<td>2.2</td>
<td>1.3</td>
<td>1.9</td>
<td></td>
<td></td>
<td>36</td>
</tr>
<tr>
<td>H</td>
<td>3043</td>
<td>0.55</td>
<td>3043</td>
<td>2.6</td>
<td>1.4</td>
<td>2.0</td>
<td></td>
<td></td>
<td>50</td>
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<tr>
<td>I</td>
<td>2212</td>
<td>0.74</td>
<td>2416</td>
<td>2.5</td>
<td>1.4</td>
<td>2.0</td>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

* Before feeding.
† After feeding.

![Fig. 2](http://www.jbc.org/) Specific activity of expired $^{14}$CO$_2$ during and following infusion of $^{14}$C-acetate. Experiment B is represented by O-O; Experiment E, by X-X; Experiment F, by Δ-Δ.
TABLE IV
Proportion of CH₃COOH metabolized to CO₂ during infusion of ¹⁴C-acetate (Experiment D)

<table>
<thead>
<tr>
<th>Time of infusion</th>
<th>Infused ¹⁴C appearing as ¹⁴CO₂</th>
<th>Concurrent</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.4</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>8.9</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>35.3</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>57.0</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>59.7</td>
<td>35.9</td>
<td></td>
</tr>
<tr>
<td>330</td>
<td>62.1</td>
<td>40.7</td>
<td></td>
</tr>
<tr>
<td>390</td>
<td>80.9</td>
<td>46.9</td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>68.6</td>
<td>49.8</td>
<td></td>
</tr>
<tr>
<td>483</td>
<td>71.9</td>
<td>51.3</td>
<td></td>
</tr>
</tbody>
</table>

A summary of the results obtained from all of the infusions is given in Table III.

DISCUSSION

1. Essig, Norton, and Johnson (7) reported a half-life of 2 minutes for acetate in fed sheep. Annison and Lindsay (6) suggested a half-life of 3 to 4 minutes, which they estimated after a single injection into a sheep that had been deprived of food. By means of a single injection of labeled acetate superimposed upon a continuous infusion, Lee and Williams (11) obtained values of 1.6 and 3.8 minutes for the half-life of acetate in two dairy cows 3 hours after feeding.

The present finding in seven experiments with fed sheep of a turnover time averaging about 2 minutes is in the same range as the previous data of Essig et al. (7). The fact that the rectilinear decrease in specific activity of blood acetate after infusion, from which the half-life is calculated, does not hold beyond about 3 minutes means that recycling of labeled acetate is occurring. This labeled acetate could be coming into the peripheral blood from the breakdown of compounds synthesized from the infused labeled acetate since the infusion began, or from a part of the acetate pool not in rapid equilibrium with the rest. The effect of recycling is to increase the estimate of the half-life and hence of turnover time.

Recycling via the pathway, acetate to CO₂ to bicarbonate in saliva to acetate synthesis in the rumen, is a possibility, but on the basis of the data of Kleiber et al. (12) it may be assumed that recycling of ¹⁴CO₂ contributes little during the several hours of infusion.

2. The turnover rate of acetate disappearing from the acetate pool was found to average 5.2 meq per hour per kg of body weight. The values found varied over a 2-fold range from 3.6 to 7.3 meq per hour per kg of body weight (Table III). The values found are perhaps correlated (not significant at 5% level) with the concentration of acetate in the blood and thus presumably with the over-all metabolic state of the animal. Evidence for this is the sharp rise in both acetate concentration and acetate turnover rate found immediately following feeding (Experiment E, Fig. 2 and Table IV). The rate found in these experiments is lower than that found by Essig et al. (7), which was approximately 10 meq per minute per sheep, but their animals had higher levels of blood acetate and would be expected to be utilizing acetate at a greater rate. Comparison with the work of Annison and Lindsay (6) is difficult owing to the 8-fold range of rates found in their experiments. An interesting comparison can be made with the results on cattle of Davis et al. (4), who used a similar experimental technique and found an acetate turnover rate for a 160-kg brown Swiss steer calf after feeding of 7.0 meq per hour per kg of body weight. Their method for calculating this rate differs from ours, and when our method is used, their data indicate a turnover rate of 4.2 meq per hour per kg of body weight. Lee and Williams (11), with their combination of two techniques, in two trials with dairy cows after feeding found values ranging from 1.2 to 2.1 meq per hour per kg of body weight.

3. The acetate pool varied over more than a 2-fold range from 3.8 to 10.3 meq per animal, but showed no clear association with other variables.

4. The acetate space corresponds to approximately 20% of the body weight and thus approximates the extracellular volume. In these experiments acetate space and acetate pool refer to only that volume of body fluid through which the injected ¹⁴C-acetate readily diffuses (acetate space) and that amount of acetate with which it readily comes into equilibrium (acetate pool).
5. While an equilibrium level for specific activity of blood acetate during continuous infusion of labeled acetate is reached very rapidly, the specific activity of the expired CO₂ does not reach equilibrium before about 4 hours (Fig. 2). This finding indicates that the turnover rate of some compound or compounds synthesized by the body from pool acetate is far slower than the turnover rate of pool acetate itself, and that much of the acetate absorbed is converted to other compounds, which are then burned by the body to CO₂.

6. In one experiment saliva was collected as it dripped from the animal's chin, and it was found that the HCO₃⁻ was labeled, but at a lower specific activity than the CO₂ of the expired air. Since the HCO₃⁻ of the saliva becomes labeled, the HCO₃⁻ of the rumen must also eventually be labeled.

7. The percentage of conversion into respiratory CO₂ of the acetate being infused was found to increase with the length of time of infusion and in general to reach a plateau level of about 70% at about 5 hours after the start of infusion (Fig. 3). That is, in the steady state, about 70% of the pool acetate is being converted to CO₂ and presumably then the other 30% is being used for the synthesis of compounds with even longer half-lives.

8. The percentage of the total radioactivity infused that was expired as ¹⁴CO₂ increased, as shown in Fig. 4, and reached 50% by 10 hours.

9. The proportion of the expired CO₂ that is derived (directly or indirectly) from the oxidation of the acetate pool varied from 36 to 55% (Table III) and does not appear to be influenced by either the site of infusion or the location of label in the acetate infused. These data were obtained as follows. The mean plateau values for the specific activity of expired CO₂ (e.g. 350 d.p.m. per μg for Experiment F, Fig. 2) multiplied by 2 (to account for the fact that only 1 carbon of the acetate was labeled) and then by 100 were divided by the mean values for the specific activity of blood acetate (e.g. 1380 d.p.m. per μg for Experiment F, giving 51% of CO₂ from acetate for this experiment). These data indicate that about 40 to 50% of the carbon burned by the sheep comes from acetate. The fact that the values are this low indicates that acetyl-CoA derived from glucose via pyruvate, from butyrate, or from propionate via glucose is not in equilibrium with the body acetate pool and thus that the data truly represent the proportion of the energy burned by the animal that is derived from rumen-produced acetate.

In the case of Experiment F, the value of 50% for the CO₂ derived from acetate was obtained from the plateau value for the specific activity of ¹⁴CO₂, which was reached after feeding, and the blood acetate specific activity, which was reached before feeding. This was done because of the lag of approximately 4 to 5 hours in reaching a plateau in CO₂ specific activity after a constant value had been reached in blood acetate specific activity (Fig. 2). Presumably, a drop in CO₂ specific activity would occur some hours after the drop in blood acetate specific activity, which occurred after feeding.

10. Both the carboxyl carbon and the methyl carbon of the acetate molecule are metabolized. If, however, the 2 carbons of acetate are metabolized at different rates, this should be revealed in the rate at which ¹⁴CO₂ is expired. In the present studies with continuous infusion there does not appear to be any consistent difference in time required to reach a constant percentage of the infused ¹⁴C-acetate appearing as expired CO₂ (Fig. 3) or for the specific activity of expired ¹⁴CO₂ to reach a plateau (Fig. 2; [2 specific activity] versus D and E[1 specific activity]). Kleiber et al. (12) and Black and Kleiber (13) found that after a single injection of acetate-1,²⁻¹⁴C into a lactating cow, the peak of specific activity in the expired CO₂ was reached in 6 minutes, whereas after injection of acetate-2,²⁻¹⁴C this peak was not reached until 12 minutes and was only half the height. A difference of only 6 minutes would not be detectable in the present constant infusion experiments.

11. The half-life as determined from the rate of decrease of ¹⁴C in expired CO₂ immediately after infusion was stopped was about 90 minutes. This result would mean a turnover time of about 130 minutes for the limiting rate of the compounds between acetate and CO₂. On the other hand, the half-life required to reach a plateau value for the radioactivity of expired ¹⁴CO₂ (Fig. 2) was of the order of 150 minutes (i.e. a turnover time, acetate to CO₂, of about 200 minutes). This indicates rate-limiting steps beyond acetate that are not involved in the direct breakdown to CO₂ of the products synthesized from acetate.

SUMMARY

1- or ²⁻¹⁴C-Acetate was infused into either the ruminal or jugular veins of fed sheep for periods up to more than 10 hours. Calculations of the rate and extent of acetate metabolism in vivo gave the following mean results: turnover rate, 5.2 meq per hour per kg of body weight; half life of pool acetate, 1.3 minutes; turnover time of acetate pool, 1.9 minutes; acetate pool size, 6.6 meq per sheep; acetate space, 20% of body weight; oxidation of injected ¹⁴C-acetate to CO₂, 56% in 10 hours (thus 56% of the acetate coming into the pool); derivation from acetate of expired CO₂ about 50%.

There were no significant differences between the peripheral and ruminal vein infusions of ¹⁴C-acetate nor between C₁ and C₂-labeled acetate in the data computed.

The data indicate the conversion of much of the pool acetate into other compounds before oxidation, the turnover time from acetate to CO₂ being of the order of 3 hours, in contrast to the acetate pool turnover time of 2 minutes.

Acknowledgment—The authors are very grateful to Dr. H. W. Norton for the mathematical analysis of the data and for aid in its interpretation.

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