Acetate Kinetics in Normal and Ketotic Cows

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Abstract

A solution containing acetate-1-14C was infused into two cows during spontaneous ketosis, then later when each was normal. A series of blood samples was taken, and radiochemical analyses were performed for plasma acetate and glucose, and blood CO2. The fraction of plasma glucose-carbon derived from plasma acetate-carbon was 6% in all four experiments. The fraction of blood CO2-carbon derived from plasma acetate-carbon was 33% in three experiments and 64% in one normal. The entry rates in normal cows were about 25-30% higher than previous estimates of ruminal production rates of acetate; this agrees with a previous estimate of endogenous acetate production in fed sheep. The lower entry rates during ketosis probably reflect lower ruminal production rates, as these two cows had exhibited inappetence for several days.

Acetic acid is a major product of ruminal fermentation (8). The entry rate of acetate into the pool sampled by plasma acetate has been measured by isotope dilution in sheep and cattle (1, 2, 6, 7, 12-14, 16). The relative distribution of 14C from acetate 14C among various products, e.g., CO2 and glucose, has been studied in normal and spontaneously ketotic cows (11). This paper reports acetate entry rates in two lactating cows during spontaneous ketosis and later when recovered, i.e., normal. Also, the fractions of blood CO2-carbon and plasma glucose-carbon derived from plasma acetate-carbon were estimated.

Methods

Two Holstein cows were purchased when first diagnosed by practicing veterinarians to have uncomplicated, primary spontaneous ketosis. Both had a history of inappetence for a few days. Two experiments were performed on each cow: the first on the day after diagnosis, the second a few weeks later when the cow appeared normal (Table 1).2 The cows were offered about 7 kg alfalfa hay and 5 kg corn silage twice a day, plus 1 kg grain concentrate (16% protein) per 3 kg milk produced.

Teflon catheters (1.2 mm ID) were placed in both jugular veins. A sterile, pyrogen-free saline solution containing acetate-1-14C (sodium salt, Volk Radiochemical, Skokie, Illinois), 10 µg/ml, was infused through one catheter at 0.191 ml/min by a Harvard pump (Model 600-900) for four hours following a primary dose of 1.0 ml. A series of blood samples was taken from the other catheter for the radiochemical assay of acetate (2), CO2 (1), and glucose (3, 10). Plasma acetone-plus-acetoacetate was estimated with nitroprusside (10). Radioactivity was determined on planchets of infinite thickness, using a low-background (2-cpm) Geiger detector (Tracelab, Waltham, Massachusetts).

The entry rate of acetate was calculated by simple isotope dilution: the infusion rate (µg/min) was divided by the mean plasma acetate specific activity (µg/g atom carbon) during the last three hours of infusion to give the entry rate (g C/min). The transfer quotients were calculated by dividing the mean

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1 In addition to the two cows reported on here, another two cows were subjected to comparable experiments early in 1964. Radiochemical analysis of the blood CO2 was performed while the experiments were in progress, and plasma was frozen for subsequent analysis of glucose, acetate, and acetoacetate. Attempts four to nine months later to isolate acetoacetate according to the method of Bergman et al. (4) were unsuccessful. Subsequently, we have found through repeated quantitative assays that acetoacetate disappears from plasma samples stored at -20 C at a variable rate, 5-40% per day. Acetate, on the other hand, appears to increase in frozen plasma samples. The data reported here were obtained before July, 1964. When the other plasma samples from the 1964 experiments were analyzed in 1966, values of plasma acetate concentration were 5-20 mEq/liter and the specific activities were very low. For example, a plasma sample which had values of 0.51 mEq/liter and 3.58 µg/g a C in 1964 had corresponding values of 13.2 mEq/liter and 0.13 µg/g a C in 1966; these changes in concentration and specific activity indicate a 26-fold increase of acetate in the frozen plasma sample.

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### TABLE 1
Data on cows used for acetate-¹⁴C experiments

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Date</th>
<th>Cow no.</th>
<th>Body weight (kg)</th>
<th>Plasma glucose (mg/100 ml)</th>
<th>Plasma ketones (units)</th>
<th>Plasma acetate (mEq/liter)</th>
<th>Milk yield (kg/day)</th>
<th>Fat test (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2/9/64</td>
<td>K14</td>
<td>413</td>
<td>53</td>
<td>4</td>
<td>0.51</td>
<td>8</td>
<td>8.7</td>
</tr>
<tr>
<td>II</td>
<td>2/25/64</td>
<td>K14</td>
<td>414</td>
<td>53</td>
<td>0</td>
<td>0.50</td>
<td>12</td>
<td>5.5</td>
</tr>
<tr>
<td>III</td>
<td>2/15/64</td>
<td>K17</td>
<td>476</td>
<td>45</td>
<td>7</td>
<td>1.09</td>
<td>14</td>
<td>5.1</td>
</tr>
<tr>
<td>IV</td>
<td>3/11/64</td>
<td>K17</td>
<td>472</td>
<td>65</td>
<td>0</td>
<td>0.48</td>
<td>12</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Plasma acetone + acetoacetate: 1 unit = approximately 10 mg/100 ml acetone.

Specific activities (µg/g a C) of blood CO₂ and plasma glucose during the last hour of infusion by the mean plasma acetate specific activity.

### Results

Typical primary data are presented in Fig. 1. The estimates of plasma acetate specific activity were more variable than the measurements of CO₂ or glucose. Asymptotes were reached after two hours for CO₂ and after three hours for glucose. Mean specific activities are given in Table 2, together with the derived data.

Only 6% of plasma glucose-carbon was derived from acetate-carbon. About one-third of blood CO₂-carbon was derived from plasma acetate-carbon in three experiments, about two-thirds in one normal. The acetate entry rates ranged from 1,900-4,300 g/day. In each cow the entry rate was clearly lower during ketosis than when normal.

### Discussion

Previous comparable estimates of the CO₂-carbon derived from plasma acetate-carbon have dealt with expired CO₂ and ranged in fed cows and sheep from 35-55% (1-4). The estimates of Lindsay and Ford (14) and Ford and Lindsay (7) were calculated in a way, if I follow them correctly, which would give results half those calculated here with specific activity expressed in gram atoms of carbon; they obtained values of 11-26% in fed sheep, 7-11% in undernourished ketotic pregnant sheep (7, 14).

Previous estimates of plasma glucose-carbon from plasma acetate-carbon have not been found in the literature for ruminants, though preliminary cross-labeling experiments of Aninson and White (2) indicated specific activities of plasma glucose which were 1.3-5.5% those of plasma acetate after infusing labeled acetate for 80-160 minutes.

In previous experiments involving the single intravenous injection of acetate-¹⁴C, both the specific activities and the total radioactivity recovered in expired CO₂ and plasma glucose were greater than normal during spontaneous ketosis (11). It was concluded that the proportion of metabolized acetate proceeding to CO₂ and glucose, by way of the citric acid cycle, was increased during spontaneous ketosis (11). This did not imply that the absolute amount of acetate reaching these products was increased, for that could not be determined without measuring the entry rate and assuming an equal removal (utilization) rate. In the present experiments, the entry rate of acetate was less during spontaneous ketosis in each cow than when the cow had recovered (Table 2). Although only two cows were studied, the difference was clear-cut, the entry rates during
ketosis being only 55 and 73% of the corresponding normal value. Low acetate entry rates have been reported previously in undernourished and starved sheep (1, 7, 14). The low entry rates in these two ketotic cows were probably mainly due to a decrease in ruminal production of acetate (5, 15), since these cows had a history of inappetence. The lower entry-utilization rate (Table 2), acting against the higher proportion of the over-all acetate utilized being used for the formation of CO₂ and glucose (11), could account for the lack of an increase in the fractions of CO₂ and glucose being derived from acetate during ketosis (Table 2).

Although previous estimates of acetate entry rates by continuous tracer infusion in lactating cows have not been found in the literature, there are estimates by single tracer injections (13) and reports of acetate production in the rumen (8, 9). Hungate et al. (8), using an in vitro method, found ruminal acetate production rates of 1.38, 1.48, 1.54, and 2.27 mole/hour in four lactating cows (i.e., 2,000-3,300 g/day). Knox et al. (9), using isotope dilution in the rumen, estimated acetate production to be 23 mmole/min in a cow, i.e., 2,000 g/day. The present estimates of entry rate in normal lactating cows are higher, 3,100-4,300 g/day. This comparison suggests an endogenous acetate production of about 1,000 g/day in lactating cows, i.e., 25-30% of the entry rate. Anison and White (2) previously estimated that endogenous acetate production accounts for about 25% of the acetate entry rate under conditions of normal acetate absorption in sheep.

Lee and Williams (13) also suggested that endogenous acetate production accounts for as much as 25% of the acetate turnover in cattle. They compared estimates of acetate turnover obtained with single tracer injections into a growing steer, three dry-open cows, and two lactating cows with various estimates of ruminal acetate production in steers, goats, and sheep. Their four values for acetate turnover in the lactating cows were 1,300-2,000 g/day, i.e., were generally lower than the ruminal acetate production of 2,000-3,300 g/day reported for lactating cows (8, 9); this comparison would indicate no endogenous acetate production. The difference between the results of Lee and Williams (13) and the present data (Table 2) may lie between animals or in the methods, especially the modes of tracer administration and isolations of plasma acetate. Kinetic data derived following single tracer injection may be overestimated for several reasons. A major problem arises if part of the tracer dose leaves the pool before mixing is complete, so that the rate of isotope dilution does not solely represent the entry of nonradioactive substances into the pool. This factor appears negligible in large pools which turn over slowly (e.g., calcium); its effect is debated regarding some pools of intermediate size (e.g., glucose), but it clearly becomes important in small pools which turn over readily, like the acetate pool. This has led to the more common use of continuous tracer infusion for the study of such pools. Two direct comparisons of the two modes of tracer administration in a cow gave expected results of acetate turnover estimated by single injection being greater than that estimated by continuous infusion (12). It is surprising that the estimates of acetate turnover in lactating cows obtained by Lee and Williams (13), using single tracer injections, were smaller than those obtained by us with continuous infusions (Table 2).

Another problem, which affects both single injection and continuous infusion methods, is
that of recycling, i.e., "C leaving the acetate pool then returning to it. This tends to raise the plasma acetate specific activity. Following a single injection, the effect of recycling on the estimated entry rate is complex and depends partly on the method of calculation. If a single exponential function is assumed, recycling tends to increase the pool size and to decrease the fractional turnover rate. These tendencies may cancel out, though usually the former predominates, so that the entry rate becomes erroneously high. During a continuous infusion, however, the effect of recycling is to make the estimated entry rate erroneously low. Excessive recycling, e.g., via longer-chain fatty acids (2), might be expected in highly productive cows mobilizing body fat or during spontaneous ketosis. Thus, the estimates of acetate entry rate in our ketotic cows may be erroneously low (Table 2).

A paradoxical situation arises in the estimation of endogenous acetate production as the difference between acetate entry rate and ruminal acetate production. This difference would be decreased by an actually higher endogenous acetate production exacerbating the error due to recycling, i.e., decreasing the acetate entry rate estimated by continuous infusion. The variables which influence acetate turnover and its estimation clearly invite further study.

References