GLUCOSE METABOLISM IN DAIRY CATTLE AND THE EFFECT OF ACETATE INFUSION

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SUMMARY

Glucose turnover rate, glucose pool size, and glucose space were determined in three nonlactating Holstein cows 2-4 hr after feeding, using two isotope dilution techniques. Turnover rates obtained by the single injection and constant infusion methods were 1.46 and 1.12 mg/min/kg body wt, respectively. The mean pool size, when expressed on a unit weight basis (mg/kg body wt), and mean turnover rate were significantly higher (P < 0.05) as determined by the single injection procedure. Comparison of the ruminant turnover rate data to nonruminant data lends further support to the contention that glucose is quantitatively less important in ruminant metabolism. Evidence of diminished glucose utilization was obtained during the intravenous infusion of unlabeled acetate (approximately 1 Mol/hr). Glucose turnover rates obtained during acetate infusion were only 75 and 79% of the preinfusion rates. These data are interpreted as suggesting the preferential utilization of acetate by the peripheral tissues during intervals of increased acetate availability.

The importance of glucose in ruminant metabolism has been a subject of speculation owing to the persistent low blood glucose concentration, the inability to absorb significant quantities from the digestive tract, and the apparent reduced uptake of glucose by the tissues, compared to that found in the nonruminant (17, 19, 20). Only in the last few years, however, through the use of carbon-labeled glucose have utilization rates been investigated at normal circulating concentrations of blood glucose. Baxter et al. (5) first employed the single-injection isotope dilution technique in ruminant studies. Using lactating dairy cows, they obtained estimates for turnover rate and pool size, and followed the partitioning of glucose into oxidative and synthetic metabolism. More recent studies have been conducted employing either the single-injection or constant-rate infusion isotope dilution procedures and a wide range of data have been obtained, depending upon the technique employed and the nutritional and physiological condition of the animal (3, 6, 7, 12-14). From these observations it seemed apparent that the ruminant animal utilized less glucose than did the nonruminant, although this could not be stated with assurance, because of the varied experimental protocols which have been employed.

The present studies of glucose turnover were undertaken with open, nonlactating dairy cattle under conditions such that comparison to the available nonruminant data would be possible. These turnover rates were obtained, using both isotope dilution techniques, to permit direct comparison of normal turnover rates obtained using these techniques in cattle. The glucose turnover rates prevailing during increased availability of acetate were studied, using constant-infusion techniques.

EXPERIMENTAL PROCEDURE

Three open, nonlactating Holstein dairy cows were employed in the experiments to be reported. They were maintained on an ad libitum hay, limited-grain diet and were fed twice daily. They were accustomed to the experimental routine prior to the initiation of the experiments. All experiments were conducted 2-4 hr after the AM feeding.

The two isotope dilution procedures employed were performed in the following manner: In single-injection experiments, a polyethylene catheter (Clay-Adams no. 206) was placed upwards (4-6 in.) in the left jugular vein on the evening preceding the experimental day, except in Experiments 1 and 2, in which they...
were placed about 1 to 1.5 hr before the experiment was begun. When placed the preceding evening, the catheters were filled with dilute heparin solution, clamped, coiled, and placed in small cloth pouches (3 x 4 in.) which had been cemented to the skin of the animal in the area where the catheter passed into the vein. At the start of the experiment a known amount of glucose-C\(^{14}\) (UL), dissolved in sterile saline, was rapidly injected and thoroughly washed in with dilute heparin solution. Blood samples were withdrawn at frequent intervals throughout the duration of the 3-hr experiments.

In constant-infusion experiments, polyethylene catheters were placed in both jugular veins the evening preceding the experimental day. The left side catheter was inserted upwards, 4-6 in., and was used for withdrawing samples. Either one or two catheters were inserted downwards, 6-8 in., in the right jugular vein and were used for infusing glucose-C\(^{14}\) (UL) and acetate solutions. In each instance the catheters were treated as previously described. At the start of an experiment a priming dose of glucose-C\(^{14}\) (UL) was rapidly injected and immediately followed by the constant infusion (12.6 ml/hr) of the same glucose-C\(^{14}\) (UL) solution for periods of time extending up to 6 hr.

All constant infusion experiments except no. 8 were extended to evaluate the effect of exogenous acetate infusion upon glucose turnover rate. The procedure employed was as follows: The constant infusion experiments were performed as described, usually for 180 min, after which approximately one mole per hour of an acetate solution, prepared from equimolar quantities of acetic acid and sodium acetate, was infused while maintaining the constant infusion of labeled glucose. Throughout the constant-infusion experiments blood samples were withdrawn at frequent intervals for glucose and acetate analyses.

The large blood samples (75-100 ml) withdrawn for glucose isolation were heparinized and sodium fluoride (100-125 mg) was added to inhibit glycolysis. To further inhibit glycolysis the samples were kept chilled until laboratory analyses were carried out. Plasma was obtained by centrifugation and the plasma proteins precipitated by the barium hydroxide-zinc sulfate method (22). Smaller blood samples (20 ml), drawn for whole blood glucose and acetate analyses, were immediately added to the precipitating reagents. Plasma and whole blood glucose concentrations were estimated by the iodometric titration procedure of Somogyi (23). Acetate concentration was determined in 50-ml aliquots of whole blood filtrate by a modified Wiseman-Irvin method (29), as used by Lee and Williams (15). In all cases, duplicate analyses were performed.

Plasma glucose was isolated as the osazone derivative. The osazones were prepared and purified by a procedure adapted from that described by Baxter et al. (5) and Searle and Chaikoff (21). The purified dry osazones were accurately weighed (2.4 mg) and combusted to C\(^{14}\)O\(_2\) according to the procedure described by Van Slyke et al. (25) in a specially prepared combustion train containing the components described by Jeffay and Alvarez (10). The evolved C\(^{14}\)O\(_2\) was trapped in an ethanol-amine-ethylene glycol monomethylether (1:2 v/v) solution (10) and 3-ml aliquots were combined with 15 ml of a scintillation solution made up of toluene-ethyleneglycol monomethylether (2:1 v/v) containing 5.5 g PPO (2,5-diphenyloxazole) and 50 mg POPOP [2,2-p-phenylenebis (5-phenyloxazole)] per liter. Each individual osazone sample was combusted in quadruplicate and duplicate aliquots of each were counted for a minimum of 4,000 total counts in a liquid scintillation spectrometer.

RESULTS

The specific activity data obtained between 30-180 min in the single-injection experiments were considered in calculating the best-fitting straight line by the method of least squares. These data have been plotted on a logarithmic axis against time and are shown in Figure 1. For calculation of the parameters of glucose utilization, according to Zilversmit et al. (30), it was assumed that mixing had been completed by 30 min, recycling of C\(^{14}\) back into the glucose pool was not appreciable through 180 min, and that a first-order dilution process applied. In the constant-infusion experiments, the specific activity data obtained between 60-180 min were employed in calculating the parameters of utilization. These data were fitted to the equation developed by Steele et al. (24), and the values for the turnover rate and pool size so calculated.

Results obtained by the individual isotope dilution techniques are summarized in Tables 1 and 2. When the same animal was used for several experiments within an individual procedure the turnover rates were quite similar, more so than individual animal data obtained between experimental techniques. Values for the parameters of glucose utilization obtained by the two techniques differed, as revealed by comparing the mean values obtained. A sig-
significantly higher value was given by the single-injection technique for the glucose turnover rate (P < 0.05), the mean value being 1.46 mg/min/kg or 0.42 g/hr/kg. There was no statistically significant difference observed for the pool size. However, when pool size was considered on a unit weight basis (mg/kg body wt), results of the two techniques were found to differ significantly (P < 0.05). The average plasma glucose concentration was significantly higher (P < 0.05) in the single-injection studies than the corresponding concentration observed in the constant infusion studies. Annilson and White (3) recorded some correlation between the turnover rate and plasma glucose concentration and suggested that the higher glucose concentration observed during their single-injection studies was partially responsible for the higher turnover rates obtained. Although too few experiments were available to establish such a relationship in the present studies, a similar correlation could partially account for the differences in turnover rate observed. There was no observable difference between experimental techniques in the volume of distribution of the glucose pool (glucose space) nor in the turnover time, although there was a tendency (nonsignificant) for the turnover time to be longer in the constant infusion studies.

Within experimental procedures the turnover rates, calculated on a metabolic weight basis (11), can be compared to similar results obtained in fasted (16 hr) nonruminants and fed sheep. Typical values obtained by the single-injection procedure for man (18), dog (9, 21), rat (4), and sheep (3, 13) were 0.32, 0.40, 0.80, and 0.28-0.37 g/hr/kg, respectively. Typical turnover rates obtained by the constant-infusion procedure for the dog (24) and sheep (6, 13) were 0.31 and 0.22-0.30 g/hr/kg, respectively. In the present studies with fed, dry, open cattle, turnover rates of 0.42 and 0.33 g/hr/kg were obtained by the single-injection and constant-infusion procedures, respectively. Values for fasted nonruminants and fed sheep are quite similar to results of the present studies with fed cattle, when compared within experimental techniques.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Summary of data obtained by the single-injection technique</th>
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<tbody>
<tr>
<td></td>
<td>Experiment</td>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>Animal</td>
<td></td>
</tr>
<tr>
<td>Body wt (kg)</td>
<td>H-312</td>
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<tr>
<td>Plasma glucose cone (mg/100 ml)</td>
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<tr>
<td>Total CPM injected × 10⁶</td>
<td>4.9</td>
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<tr>
<td>Specific activity at t₀ (CPM/mg plasma glucose)</td>
<td>4.56</td>
</tr>
<tr>
<td>Half-time (t½) (min)</td>
<td>78.0</td>
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<tr>
<td>Turnover time (min)</td>
<td>112</td>
</tr>
<tr>
<td>Pool size (grams)</td>
<td>80.7</td>
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<tr>
<td>Pool size relative to body wt (mg/kg)</td>
<td>138.1</td>
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<tr>
<td>Turnover rate (mg/min)</td>
<td>718.5</td>
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<tr>
<td>Turnover rate relative to body wt (mg/min/kg)</td>
<td>1.23</td>
</tr>
<tr>
<td>Glucose space (% body wt)</td>
<td>16.3</td>
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</table>
The effect of increased availability and raised concentration of blood acetate upon the glucose turnover rate were examined by infusing unlabeled acetate solution while maintaining the constant infusion of labeled glucose. Experiments 5 and 6 were extended 180 min, during which unlabeled acetate was infused. New glucose turnover rates were calculated and compared to the rates prevailing prior to acetate infusion (Table 3). The specific activity data for Experiment 6 is shown in Figure 2.

As a result of the exogenous infusion of acetate there was an increase in the plasma glucose specific activity and in the whole blood glucose and acetate concentrations. The increased plasma glucose concentration observed was significant in both Experiment 5 (P < 0.05) and Experiment 6 (P < .10). After the plasma glucose specific activity had obtained a new and relatively constant equilibrium level, recalculation of the turnover rate gave values which were only 78 and 75% of the original preinfusion turnover rates observed in the two individual experiments.

Experiment 7 was performed in the following manner: The constant infusion was performed for 160 min, after which labeled glucose infusion was discontinued. Throughout the 163-260-min interval and 268-400-min interval, during which acetate was infused (1.44 millimole/hr/kg), the decline in plasma glucose specific activity was followed. The turnover times of the individual intervals, with and without acetate infusion, were calculated from the slope of plasma glucose specific activity decline (Table 4). There was an increase (5%) in the turnover time, coincident with acetate infusion. A decreased exit rate was indicated by a decreased rate of decline in blood glucose radioactivity per milliliter. In addition to the increased blood acetate concentration resulting from acetate infusion, there was a slight but statistically nonsignificant increase in plasma glucose and whole blood glucose concentrations.

The data obtained in Experiments 9 and 10 did not allow calculation of glucose turnover rates during acetate infusion. Insufficient time was allowed during acetate infusion to permit attaining the new and relatively constant levels of plasma glucose specific activity necessary for such calculations. However, from data obtained it is evident that the infusion of acetate solution resulted in an altered glucose utilization. The responses elicited in the two experi-

### TABLE 3

| Expt. no. | Interval | Body wt (kg) | Plasma glucose conc (mg/100 ml) | Whole blood conc Glucose (mg/100 ml) | Acetate infusion (mM/hr/kg) | Turnover rate (g/hr/kg)
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<th></th>
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<tbody>
<tr>
<td>5</td>
<td>1</td>
<td>627</td>
<td>76.1</td>
<td>53.1</td>
<td>12.4</td>
<td>0.264</td>
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<tr>
<td></td>
<td>2</td>
<td>79.0</td>
<td>58.9</td>
<td>18.8</td>
<td>1.56</td>
<td>0.209</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>531</td>
<td>68.2</td>
<td>52.5</td>
<td>6.7</td>
<td>0.313</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>71.5</td>
<td>57.8</td>
<td>11.8</td>
<td>1.80</td>
<td>0.234</td>
</tr>
</tbody>
</table>

*Intervals 1 and 2 represent acetate preinfusion and infusion intervals of 180 min each.*
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![Graph showing plasma glucose specific activity during the constant rate infusion of glucose-C\textsuperscript{14} and the superimposed infusion of acetate.]

**Fig. 2.** Plasma glucose specific activity during the constant rate infusion of glucose-C\textsuperscript{14} and the superimposed infusion of acetate.

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Interval</th>
<th>Whole blood cone</th>
<th>Glucose turnover time (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0-160 min Glucose-C\textsuperscript{14} inf.</td>
<td>56.0</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>163-260 min Saline inf.</td>
<td>...</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>268-400 min 1.44 mM/hr/kg Acetate inf.</td>
<td>60.2</td>
<td>10.5</td>
</tr>
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</table>

**TABLE 4**

Effect of exogenous acetate on glucose turnover time and blood glucose and acetate concentration

ments were somewhat different. In each experiment acetate infusion (1.58 and 1.70 millimole/hr/kg, respectively) gave rise to a slight increase in plasma glucose concentration (P < .10), as well as an increased whole blood glucose and acetate concentration. In Experiment 9 plasma glucose specific activity did not increase; whereas, in Experiment 10 there was a marked increase which continued even after the cessation of acetate infusion. It should be noted that during the latter stages of Experiment 9 the animal became excited due to the strain imposed by malfunctioning respiration equipment; this may have been partially responsible for the divergent results obtained in these two experiments.

During Experiment 9 the oxidation of glucose was followed by continuously monitoring the expired C\textsuperscript{14}O\textsubscript{2} employing the respiration equipment and procedure described by Williams (26). The ratio $\frac{\mu\text{c/min expired}}{\mu\text{c/min infused}} \times 100$ may be considered indicative of the fraction of glucose turned over which was directed toward oxidative metabolism. The ratio thus obtained for the interval 108-190 min was $0.640 \times 100 = 38.6$, and indicates that 39\% of the glucose pool which turned over was utilized in oxidative metabolism. This represents approximately 0.13 g/hr/kg\textsuperscript{78} or 160 g/hr of glucose being oxidized in this experiment. This value of 39\% agrees quite well with values reported by others for ruminants (5, 6).

**DISCUSSION**

Results presented clearly illustrate a rapid and large turnover of glucose in the ruminant,
even though relative to nonruminants there is a persistent low blood glucose concentration. Assuming that this system, as measured 2-4 hr after feeding, had operated at this rate for 24 hr, in excess of 1,000 g would have been made available to the tissues for synthetic and oxidative utilization.

Through the use of open, nonlactating dairy cattle the turnover rate data were conveniently compared to nonruminant data free from the incalculable utilization for milk synthesis and reproduction. Comparison of these data on a metabolic weight basis clearly demonstrates the similarity in turnover rates obtained. Good agreement between these data and other ruminant data was also observed. These findings without further interpretation suggest glucose to be no less important to ruminant species; however, it must be recalled that nonruminant experiments were invariably conducted on post-absorptive (fasted 16 hr) animals. Data presented here suggest that the fed ruminant metabolizes glucose at a rate comparable to the post-absorptive nonruminant, and that this may be partially a result of acetate absorption in the ruminant. This study, although indicating the lesser quantitative importance of glucose, demonstrates the qualitative importance of glucose in the over-all metabolic economy of the ruminant.

It is of interest to compare the glucose oxidation and turnover rate data obtained with dry cows in the present study to data for lactating cows (5, 12, 14). Actual glucose turnover rates (g/hr/kg~24~) were about 40% greater in lactating animals and although the fraction of glucose utilized for oxidative purposes, expressed as a percentage of the total hourly turnover rate, was of the same order of magnitude, this represents considerably larger hourly quantities in lactating animals. Obviously, some of this difference arises because of different experimental conditions; however, the magnitude probably reflects the important contribution of glucose to milk synthesis. These data suggest that the process of milk synthesis is no more efficient in the utilization of glucose carbon than the general bodily metabolism.

Isotope dilution procedures have been used extensively to estimate glucose turnover rates in ruminant and nonruminant animals. The higher estimates obtained by the single-injection procedure have been attributed to limitations inherent in the straightforward mathematical treatment of the specific activity curve obtained (24). Precise reasons for the divergent results obtained cannot be advanced. It seems likely, though, that some portion of the difference resides with the constant-rate infusion procedure, as has been suggested (15). The close agreement of estimates obtained within the individual techniques supports the contention that for comparative purposes either technique is useful.

Acetate utilization in ruminants is rapid at normal circulating concentrations and appears to be dependent upon acetate availability (2, 8, 16, 19, 20). The acetate infusion rates employed in these studies did not result in unphysiological peripheral concentrations and indicated a rapid utilization comparable to that observed in other studies (2, 8). During this interval of increased acetate utilization, there was a reduction in glucose entry into the pool, as evidenced by the observed increase in glucose specific activity. Similarly, the exit of glucose from the pool was depressed, since a marked decrease in pool size was not recorded. In fact, the increased blood concentration suggests an increased pool size. Such a decreased exit rate is also indicated by the turnover time data of Experiment 7. The simplest interpretation consonant with these findings is that acetate was being preferentially utilized when its availability was increased, and that glucose was at least one of the metabolites whose utilization was diminished. The observable manifestations of this decreased glucose utilization, the increased glucose specific activity, and the increase in blood glucose concentration suggest that the primary effect is in peripheral tissues and that subsequent to and accompanying the decreased exit rate was a decreased entry rate, both of which persisted until a new equilibrium between entry and exit of glucose was attained. Such glucose-sparing effects are of great importance in ruminants in which most glucose arises via gluconeogenesis.

The possibility that long-chain fatty acids would exert a similar peripheral effect can only be speculated upon, although these results with acetate are suggestive. The demonstrated glucose-sparing effect of acetate, when considered in the light of the recently reported (27, 28) Growth Hormone-induced augmentation of acetate and long-chain fatty acid metabolic rates, suggests that Growth Hormone should depress the rate of glucose metabolism. In actual fact, one study (1) in dogs demonstrated an increased rate of glucose metabolism. From this, it follows that Growth Hormone affects glucose metabolism independently of fatty acid mobilization, or the less likely possibility which should be investigated, that ruminant glucose metabolism responds differently to Growth Hormone.
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REFERENCES


