THE PENTOSE CYCLE AS A PATHWAY FOR GLUCOSE METABOLISM IN INTACT LACTATING DAIRY COWS*

BY ARTHUR L. BLACK, MAX KLEIBER, EARL M. BUTTERWORTH, GEORG B. BRUBACHER,† AND JIRO J. KANEKO‡

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

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The classical scheme of Embden-Meyerhof has been generally accepted as describing the pathway for glucose metabolism in animals, although recent observations have challenged this tenet. A number of in vitro studies based on the differential conversion of specifically labeled glucose to CO₂ have indicated the existence of a pathway in addition to the Embden-Meyerhof pathway for glucose metabolism in several animal tissues. Evidence for an alternate pathway has been obtained with rat liver (1-7) and mammary gland (8, 9), mouse liver (6), and rabbit spleen, testis, and bone marrow (10). Estimates made by various investigators have indicated that in liver slices almost 0 to as much as 50 per cent¹ of the glucose may be metabolized by an alternate pathway (3, 4, 6, 7). In rat mammary gland slices, it was estimated that approximately 60 per cent of the glucose was metabolized along an alternate pathway (8).

In vitro studies with rat muscle (1, 2) and with rabbit brain (10) have indicated that the Embden-Meyerhof pathway accounts for essentially all of the glucose metabolized in these tissues. A similar conclusion has been reached in studies with intact rats (1, 12, 13). The discrepancy between results obtained with the intact rat and rat tissues either indicated an artifact under in vitro conditions or showed that in the intact animal little glucose is metabolized in those tissues which have an alternate pathway for glucose metabolism (1).

Recently Bloom et al. (14) detected the pentose cycle in intact non-lactating rats, based on labeling patterns in glucose after administering specifically labeled ribose and glucose; however, they made no estimate of the quantitative significance of the pathway.

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† Present address, Hoffmann-La Roche and Company, Basel, Switzerland.
‡ Postdoctoral Fellow of the Public Health Service.
¹ See the review by Wood (11) in which he discusses the calculations made by various investigators and the uncertainties in their assumptions which account, in part, for the variation between results.
Earlier studies with the intact cow demonstrated that glucose was rapidly metabolized, and within 35 hours essentially all of an injected dose appeared in the respiratory CO2 (40 per cent) and milk constituents (56 per cent) (15). The nature of the pathways involved in glucose catabolism was not apparent in these studies since the injected material was uniformly labeled.

The present paper reports the results of trials in which two lactating cows were injected intravenously with glucose-6-C\textsuperscript{14} and then, after several weeks, with glucose-1-C\textsuperscript{14}. The transfer of C\textsuperscript{14} from glucose to CO2, to glycerol of milk fat, and to alanine and serine of casein could not be explained by exclusive operation of the Embden-Meyerhof pathway. Estimates based on C\textsuperscript{14} recovery in CO2 and various milk constituents, in each case, indicated that the pentose cycle or a similar pathway played a significant role in glucose catabolism of the intact lactating cow.

**TABLE I**

Data on Experiments with Cows Injected Intravenously with Glucose-1-C\textsuperscript{14} and Glucose-6-C\textsuperscript{14}

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Cow No.</th>
<th>Body weight kg.</th>
<th>Compound injected</th>
<th>Milk yield kg. per day</th>
<th>Stage of lactation wks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>965</td>
<td>594</td>
<td>G-6-C\textsuperscript{14} 2.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>965</td>
<td>611</td>
<td>G-1-C\textsuperscript{14} 1.80</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>V</td>
<td>84</td>
<td>423</td>
<td>G-6-C\textsuperscript{14} 1.96</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>VI</td>
<td>84</td>
<td>425</td>
<td>G-1-C\textsuperscript{14} 1.78</td>
<td>12</td>
<td>7</td>
</tr>
</tbody>
</table>

**EXPERIMENTAL**

*Cows*—Table I summarizes the characteristics of the two lactating Jersey cows used in these studies. Each cow served as its own control by being injected first with glucose-6-C\textsuperscript{14} (G-6-C\textsuperscript{14}) and, after 5 weeks (Cow 965) or 3 weeks (Cow 84), with G-1-C\textsuperscript{14}. In all trials 75 to 98 per cent of the C\textsuperscript{14} was accounted for in the respired CO2 and milk constituents during the first 34 hours of the trial. Within a few days after G-6-C\textsuperscript{14} was injected, the C\textsuperscript{14} level in milk was too low to be detected by our counting equipment and it can be assumed that the amount of C\textsuperscript{14} remaining in the cows at the time of the second experiments, with G-1-C\textsuperscript{14}, was negligible.

*Isotope*—The C\textsuperscript{14}-labeled D-glucose was obtained from the National Bureau of Standards.\textsuperscript{2} The sugars were radiochemically pure, as shown by autoradiograms of samples chromatographed with butanol-acetic acid-

\textsuperscript{2} The authors wish to express their appreciation to Dr. H. S. Isbell, National Bureau of Standards, for his valuable cooperation in preparing millicurie quantities of G-1-C\textsuperscript{14} and G-6-C\textsuperscript{14} for use in these studies.
H₂O (4:4:1) and phenol-H₂O (10:4). The amount of C¹⁴-glucose injected into each cow is shown in Table I.

Samples—Respired CO₂ was collected continuously during the first 3 hours of each trial and then at intervals until 34 hours. The methods used for the collection and C¹⁴ assay of CO₂ have been described (16). Milk was collected periodically during the first 34 hours after isotope injection and was fractionated into its major organic constituents. Casein was precipitated from the skim milk by adjusting the pH to 4.6 with 1 N HCl. The casein was filtered, washed with water, and then redissolved in 1 N NH₄OH. This procedure was repeated and the casein, after the third precipitation, was washed thoroughly with water, followed by alcohol and ether. 5 gm. of dried casein were hydrolyzed, and the alanine, serine, and glutamic acid were recovered separately from ion exchange columns and prepared in crystalline form by methods already described (17).

Glycerol was recovered from the aqueous phase that separated upon acidifying milk fat hydrolysates. It was purified by preparing the tribenzoate derivative according to the method described by Mulliken (18).

Other methods used in this investigation have already been described, including those for combustion of samples and radioassay of the resulting CO₂ (17). Sakami's method was used for the stepwise degradation of serine (19).

Results

Fig. 1 shows the specific activity in the expired CO₂ as a function of time after injecting cows intravenously with G-1-C¹⁴, G-6-C¹⁴, and uniformly labeled glucose (G-U-C¹⁴). After each trial with G-6-C¹⁴, the specific activity of expired CO₂ increased much more slowly and its maximum was only one-half that of the trials in which G-1-C¹⁴ was injected; furthermore, the maximum for G-6-C¹⁴ occurred later (1 to 1.5 hours) than that for G-1-C¹⁴ (0.5 to 0.8 hours). These results demonstrate that C-1 of glucose was oxidized more rapidly than C-6 and indicate that these 2 carbon atoms, in part, followed different metabolic pathways.

The results obtained with G-U-C¹⁴ have been included for comparison (15). In general the rate of appearance of C¹⁴ in CO₂ for G-U-C¹⁴ was intermediate between the results obtained with G-1-C¹⁴ and G-6-C¹⁴.

The specific activities of serine and alanine from casein and of glycerol from milk fat are listed in Table II for different times after injection of G-1-C¹⁴ and G-6-C¹⁴. For each compound the specific activity was greater, in some samples by as much as two times, after G-6-C¹⁴ than it was after G-1-C¹⁴. Thus, the data from milk products, like those of expired CO₂, indicate that C-1 and C-6 of glucose do not follow a common metabolic pathway and that there is some mechanism in addition to the Embden-Meyerhof pathway for glucose metabolism in the cow.
The mean specific activity in alanine (during 34 hours) was 50 per cent greater after G-6-C\textsuperscript{14} than it was after G-1-C\textsuperscript{14} for both cows (see Table V).

**TABLE II**

Specific Activities of Milk Constituents\* after Injecting G-1-C\textsuperscript{14} and G-6-C\textsuperscript{14}

<table>
<thead>
<tr>
<th>After injection (hrs.)</th>
<th>Glycerol</th>
<th>Serine</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G-1-C\textsuperscript{14}</td>
<td>G-6-C\textsuperscript{14}</td>
<td>Ratio, 6.1</td>
</tr>
<tr>
<td>3.3</td>
<td>5.0</td>
<td>12.8</td>
<td>2.6</td>
</tr>
<tr>
<td>9.3</td>
<td>10.9</td>
<td>26.4</td>
<td>2.4</td>
</tr>
<tr>
<td>23.5</td>
<td>3.9</td>
<td>8.6</td>
<td>2.2</td>
</tr>
<tr>
<td>34</td>
<td>1.3</td>
<td>3.8</td>
<td>2.9</td>
</tr>
</tbody>
</table>

\* The data in Table II are from Trials III and IV.

\dagger \lambda_s = \text{microcuries per gm. atom of C per microcurie injected per kilo of body weight.}

In contrast to these results, the Embden-Meyerhof pathway would lead to the same specific activity in alanine (derived from pyruvate) for both...
types of labeled glucose unless the trioses formed at the aldolase stage had different metabolic fates. For example, if the equilibration between phosphoglyceraldehyde and phosphodihydroxyacetone had been slow and the latter (representing C-1,2,3 of glucose) had been preferentially diverted into the pathway of glycerol synthesis, the observed labeling of alanine might be expected. However, such an explanation would not account for the greater recovery of C\textsuperscript{14} in expired CO\textsubscript{2} after G-1-C\textsuperscript{14}. Furthermore, this interpretation of the results obtained with alanine would lead one to expect the C\textsuperscript{14} levels in glycerol to be higher from G-1-C\textsuperscript{14} than from G-6-C\textsuperscript{14} when, to the contrary, these levels were, in fact, 2 to 3 times as great after G-6-C\textsuperscript{14} as they were after G-1-C\textsuperscript{14} (see Table II).

**Utilization of Glucose for Biosynthesis**—The observed results with expired CO\textsubscript{2} and milk products can be explained by the combined operation of the Embden-Meyerhof pathway and an alternate pathway which, for purposes of calculation, we have assumed to be the pentose cycle. Our reasons for deciding that the pentose cycle functions as the alternate pathway are discussed later. To assess the relative importance of the pentose cycle we have assumed that it functions together with the Embden-Meyerhof pathway to provide the major mechanisms for glucose catabolism in the cow. In addition, we assume that pyruvate is formed at equal rates from C-1 and C-6 of glucose along the Embden-Meyerhof pathway but arises only from C-6 of glucose along the pentose cycle since C-1 is lost as CO\textsubscript{2} (Fig. 2). Alanine indicates the C\textsuperscript{14} level in pyruvate, since it can be formed from the latter by transamination. The amount of C\textsuperscript{14} in alanine would be the same after G-1-C\textsuperscript{14} and G-6-C\textsuperscript{14} (per unit C\textsuperscript{14} injected and per liter of milk produced) if the Embden-Meyerhof pathway operated exclusively. Concurrent glucose metabolism in the pentose cycle would result in greater transfer of C\textsuperscript{14} to alanine after G-6-C\textsuperscript{14}, and the difference between results obtained with the two types of labeled glucose would be directly proportional to the quantity of glucose metabolized via the pentose cycle.

Under these conditions our estimate for the quantitative significance of the pentose cycle was derived\textsuperscript{8} for the data obtained with alanine (these data are listed in Table III) as shown below: in Trials III and IV, 100 \times (0.0321 - 0.0178)/0.0321 = 44.5 per cent via pentose cycle; in Trials V and VI, 100 \times (0.0365 - 0.0231)/0.0365 = 36.7 per cent via pentose cycle.

The results from the two cows are in close agreement and indicate that at the site of alanine synthesis about 40 per cent of the glucose molecules had been converted to pyruvate via the pentose cycle. If considerably more glycerol were formed from the C-1,2,3 moiety of glucose than from

\textsuperscript{8} This is essentially the method applied by Abraham et al. to estimate pentose cycle activity during fatty acid synthesis in mammary gland (8).
the C-4, 5, 6 moiety along the Embden-Meyerhof pathway (the data in Table II suggest that it was not), then our calculated values for the pentose cycle would be too high. On the other hand, if pentose arising in the pentose cycle was utilized for nucleotide synthesis, it would have an oppo-

Fig. 2. Hypothetical scheme of glucose metabolism along the Embden-Meyerhof (E-M) pathway and the pentose cycle. Two trioses arise via the E-M pathway, and it is assumed that these are converted to pyruvate in approximately equal quantities. In the pentose cycle, C-1 of glucose is oxidized to CO₂ and a triose is formed from C-4, 5, 6. This triose may mix with E-M intermediates at the phosphoglyceraldehyde or pyruvate level but, in either case, will result in the transfer of C¹⁴ to pyruvate only from C-6 but not C-1 of glucose. Pyruvate may give rise to alanine via transamination or may enter the TCA cycle, where it may be oxidized to CO₂ or converted into other compounds such as glutamic acid.

site influence and make our estimated values for the pentose cycle too low. Since we have no information on the relative magnitude of glycerol or nucleotide (pentose) synthesis from glucose, it is not possible to judge their influence on our calculated values.

Using the same method described above for alanine, we have estimated the quantitative importance of the pentose cycle based on the amounts of C¹⁴ recovered in serine and glycerol (Table III). The results of these cal-
Calculations are summarized in Table IV and indicate that 50 to 65 per cent of the glucose molecules were metabolized along the pentose cycle at the sites of synthesis of glycerol and serine.

**Glucose Oxidation**—The C\(^{14}\) levels in respired CO\(_2\) provide an additional

### Table III
**Recovery of C\(^{14}\) in CO\(_2\) and Milk Constituents during 34 Hours after Injection of Glucose-C\(^{14}\)**

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Position of C(^{14})</th>
<th>Per cent injected C(^{14}) converted to CO(_2) (\times 10^{-1})</th>
<th>Milk constituents* (\times 10^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>G-6-C(^{14})</td>
<td>33</td>
<td>3.21 5.68 37.5</td>
</tr>
<tr>
<td>IV</td>
<td>G-1-C(^{14})</td>
<td>54</td>
<td>1.78 1.98 14.6</td>
</tr>
<tr>
<td>V</td>
<td>G-6-C(^{14})</td>
<td>15</td>
<td>3.65 6.31 36.9</td>
</tr>
<tr>
<td>VI</td>
<td>G-1-C(^{14})</td>
<td>49.7</td>
<td>2.31 2.74 18.2</td>
</tr>
</tbody>
</table>

* The per cent of injected C\(^{14}\) converted to milk constituents is expressed per liter of milk produced during the 34 hour period. This expression minimizes the influence of variations in milk production on the relative amounts of C\(^{14}\) transferred to milk constituents from G-1-C\(^{14}\) and G-6-C\(^{14}\).

### Table IV
**Quantitative Estimate of Glucose Metabolized via Pentose Cycle**

<table>
<thead>
<tr>
<th>Calculation based on C(^{14}) recovered per liter of milk in</th>
<th>Data from Trials III and IV Trials V and VI</th>
</tr>
</thead>
</table>
| Alanine
   | 44* 37 |
| Serine
   | 65 57 |
| Glycerol
   | 61 51 |

* The method for estimating the quantitative importance of the pentose cycle is discussed in the text.
specific activity of alanine would also be proportional to the amount of \( ^{14} \text{O}_2 \) arising from G-1-C\(^{14} \) in the TCA cycle.

As shown in Fig. 2, \( \text{CO}_2 \) would arise from C-6 of glucose only in the TCA cycle but could arise from C-1 of glucose during metabolism in either the pentose cycle or the TCA cycle. By using these conditions, the data from Trials III and IV provide the following indication of the importance of the pentose cycle. In Trial III, after G-6-C\(^{14} \) was injected, the mean specific activity of alanine was 4.18 during the time that 33 per cent of the injected C\(^{14} \) was oxidized to \( \text{CO}_2 \). When the same cow was injected with G-1-C\(^{14} \) (Trial IV), 54 per cent of the C\(^{14} \) was oxidized to \( \text{CO}_2 \), but the mean specific activity of alanine during this same period was only 2.84. From these data it may be estimated that \( 2.84/4.18 \times 33 = 22.4 \) per cent of the C\(^{14} \) injected as G-1-C\(^{14} \) was oxidized to \( \text{CO}_2 \) by way of the TCA cycle in Trial IV.\(^4 \) Since 54 per cent of the C\(^{14} \) was recovered in \( \text{CO}_2 \), it appears that \( (54 - 22.4)/54 = 59 \) per cent of the \( ^{14} \text{O}_2 \) from G-1-C\(^{14} \) arose via the pentose cycle. The same calculations based on the data collected in Trials V and VI indicated that 80 per cent of C-1 of glucose was converted to \( \text{CO}_2 \) via the pentose cycle.

Previous studies have demonstrated that, in the cow, glutamic acid of casein is derived from \( \alpha \)-ketoglutarate in the TCA cycle (20). Thus the C\(^{14} \) level in C-1 of glutamic acid (Table V) should correspond closely to the C\(^{14} \) level of \( \text{CO}_2 \) arising in the TCA cycle. From these data one can make an independent estimate of the relative importance of the pentose cycle in the oxidation of C-1 of glucose to \( \text{CO}_2 \). The method used for these calculations was the same as those discussed above for alanine, and the results are summarized in Table V. The estimates calculated from the specific activities of glutamic acid C-1 are in close agreement with those based on alanine and indicate that 56 to 75 per cent of the \( \text{CO}_2 \) from glucose C-1 was formed via the pentose cycle.

These values, which express the amount of \( \text{CO}_2 \) from C-1 of glucose formed via the pentose cycle, do not directly indicate the relative amount of glucose metabolized along this pathway, since the calculations neglected the amount of C\(^{14} \) utilized for biosynthesis along the Embden-Meyerhof and TCA cycle pathways. In the pentose cycle, C\(^{14} \) from G-1-C\(^{14} \) is con-

\(^4 \) For these calculations we assume that the relationship between alanine and the pyruvate pool and between the TCA cycle and the pyruvate pool is the same in Trials III and IV. In other words, a given specific activity in the pyruvate pool \( (X) \) will result in the appearance of alanine in casein with a specific activity \( A \) \( (X) \) where \( A \) is the dilution factor. \( A \) will be approximately constant for a given cow on a given ration. When the calculations are based on results collected over a 34 hour period, variations in \( A \), due to fluctuations in food intake, tend to average out. The same cow was used in Trial III and Trial IV and its ration was the same during both trials. The same conditions apply for Trials V and VI.
verted only to CO2, whereas in the Embden-Meyerhof, TCA cycle pathway this C14 is converted to amino acids, fatty acids, glycerol, etc., as well as to CO2. The amount of C14 entering these non-lactose milk constituents would be approximately equal to the total C14 injected as G-1-C14 minus the C14 in lactose and respired CO2. In Trial IV, 54 per cent of the injected C14 was in CO2 and 40.7 per cent in lactose; thus, 5.3 per cent can be assumed to have entered glycerol, fatty acids, amino acids, etc. In Trial VI the corresponding values were 49.7 per cent to CO2 and 41.4 per cent to lactose, leaving 8.9 per cent to go into compounds other than lactose.

In Trial IV we estimated that 41 per cent (38 to 44 per cent, Table V) of the CO2 from glucose C-1 arose in the Embden-Meyerhof, TCA cycle pathway which would be equivalent to 22 per cent (0.41 X 54 per cent) of the injected C14. An additional 5.3 per cent of the C14 went into compounds synthesized from the Embden-Meyerhof pathway and TCA cycle intermediates. Thus it appears that 27.3 per cent (22 + 5.3 per cent) of the injected glucose followed the Embden-Meyerhof pathway, whereas 32

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TABLE V

<table>
<thead>
<tr>
<th>Glucose C-1 Arising via Pentose Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Glutamic acid C-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Alanine</th>
<th>Glucose C-1 to CO2 via pentose cycle</th>
<th>Glutamic acid C-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λα</td>
<td>per cent</td>
<td>λα</td>
</tr>
<tr>
<td>III</td>
<td>4.18</td>
<td>59</td>
<td>1.78</td>
</tr>
<tr>
<td>IV</td>
<td>2.84</td>
<td>80</td>
<td>1.28</td>
</tr>
<tr>
<td>V</td>
<td>3.74</td>
<td>80</td>
<td>1.05</td>
</tr>
<tr>
<td>VI</td>
<td>2.48</td>
<td>80</td>
<td>0.865</td>
</tr>
</tbody>
</table>

* $\lambda_α = \frac{1}{\alpha} \sum_{t=0}^{24} \lambda_α \Delta t$, where $\lambda_α$ is the specific activity in microcuries per gm. atom of C per microcurie injected per kilo of body weight. The specific activity of the milk constituent from each milk sample was multiplied by the time period of milk formation, and the resulting products summed for the 34 hour experimental period.

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5 It is assumed that most of the glucose incorporated into lactose was utilized without passing along either the Embden-Meyerhof pathway or the pentose cycle. Degradation of glucose from lactose shows that 95 per cent of the C14 was present in C-1 or C-6 when G-1-C14 or G-6-C14, respectively, was injected (Butterworth, E. M., unpublished data).
per cent was metabolized in the pentose cycle. Of the glucose catabolized by the two pathways, $\frac{32}{59.3} = 54$ per cent followed the pentose cycle. The same methods applied to the data from Trial VI indicated that 65.7 per cent of the glucose catabolized entered the pentose cycle.

These values, based on CO$_2$ production from G-1-C$^{14}$, indicate that 54 to 66 per cent of the glucose catabolized entered the pentose cycle. They are in close agreement with the results based on C$^{14}$ levels in milk constituents, which indicated that 40 to 65 per cent of the glucose catabolized had passed via the pentose cycle.

$C^{14}$ Distribution in Serine—The distribution of C$^{14}$ in the serine synthesized after injection of G-1-C$^{14}$ and G-6-C$^{14}$ is shown in Table VI. The

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Compound injected</th>
<th>Time of sample</th>
<th>C$^{14}$ distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hrs.</td>
<td>C-1 per cent</td>
</tr>
<tr>
<td>IV</td>
<td>G-1-C$^{14}$</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>VI</td>
<td>&quot;</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>VI</td>
<td>&quot;</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>IV</td>
<td>&quot;</td>
<td>34</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>G-6-C$^{14}$</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>V</td>
<td>&quot;</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>V</td>
<td>&quot;</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>III</td>
<td>&quot;</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>V</td>
<td>&quot;</td>
<td>34</td>
<td>10</td>
</tr>
</tbody>
</table>

C$^{14}$ was located predominantly in C-3 of serine after injection of either type of labeled glucose. This labeling pattern excludes the Entner-Doudoroff scheme (21) and any similar mechanism from consideration as the alternate pathway in the cow. The Entner-Doudoroff pathway results in the conversion of C-1 of glucose to the carboxyl of pyruvate which would lead to greater labeling in C-1 of serine. The low C$^{14}$ level in C-1 of serine also shows that CO$_2$ fixation was of minor importance in C$^{14}$ transfer to serine and thus can be neglected without introducing serious errors in our estimation of pathways for glucose metabolism.

DISCUSSION

Isotope studies in intact animals seldom delineate pathways of metabolism but more often serve as a basis for deciding between various possibilities. In the present study with G-1-C$^{14}$ and G-6-C$^{14}$, the results are
clearly inconsistent with exclusive operation of the Embden-Meyerhof pathway, although it is quite probable that this pathway accounts for a considerable part of the glucose catabolism in the cow. All of our results could be explained by postulating a combined operation of the Embden-Meyerhof pathway and pentose cycle in glucose catabolism in the cow.

Among the various alternate pathways proposed for glucose catabolism, the pentose cycle is the only one for which there is a substantial body of supporting evidence in animal tissues. Two enzymes of the pentose cycle, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, are widely distributed in animal tissues and are found in high concentration in rat mammary gland (22). The levels of these dehydrogenases in rat mammary gland were found to increase 20- to 60-fold during lactation (23), which suggests a relationship between the pentose cycle and milk formation. These dehydrogenases have also been measured in sheep mammary tissue but apparently undergo smaller increases in activity during lactation than in rat mammary gland (9).

The disappearance of ribose 5-phosphate has been measured in several animal tissues in vitro and was found to be greatest in lactating mammary gland, especially during later stages of lactation (22). This measurement reflects, in part, pentose breakdown and indicates the presence of enzymes associated with the pentose cycle. Peeters et al. (24) have detected a ketohexose in colostrum and in mammary gland tissue from cows, which, according to chromatographic methods, appears to be sedoheptulose. For these reasons it appears most probable that a pathway similar to, if not identical with, the pentose cycle is responsible for part of the glucose metabolism in the cow.

The serine, alanine, and glycerol recovered from milk were probably synthesized to a large extent in liver and mammary gland, and the values calculated from their mean specific activities represent principally the influence of the pentose cycle on glucose metabolism in these organs. However, the quantitative significance of the pentose cycle estimated from alanine (40 per cent) was somewhat smaller than the estimate based on serine or glycerol (50 to 65 per cent).

One explanation for the lower result obtained in the case of alanine would be that alanine reflects a more general picture of glucose metabolism in the cow as a whole, whereas serine and glycerol reflect more closely metabolic pathways of a special organ such as mammary gland and liver. Studies in vitro indicate that, in muscle, glucose is metabolized predominantly, if not exclusively, by the Embden-Meyerhof pathway (2, 25). It seems probable that a part of the lactate produced in muscle mixes with lactate and pyruvate in liver (and mammary gland) and thereby modifies the specific activity of the pyruvate (and, in turn, alanine) produced in
those organs after injection of specifically labeled glucose. Under these conditions, the specific activity of alanine would be influenced by the pathway of glucose catabolism, not only in liver and mammary gland but in muscle as well.

Phosphodihydroxyacetone, the precursor of glycerol (26), and phosphoglyceric acid, the probable precursor of serine (27), arise at a higher level in the Embden-Meyerhof pathway than pyruvate and would not be expected to mix so largely with metabolites from other tissues, especially those from muscle. Under these conditions, the calculations based on the specific activities of glycerol and serine would represent more closely (than those based on alanine) the influence of the pentose cycle on glucose metabolism in liver and mammary gland. These values indicated that 50 to 65 per cent of the glucose had been metabolized via the pentose cycle. It is interesting that these values are in the same range as that derived by Abraham et al. (8) from in vitro studies with rat mammary gland, in which it was estimated that 60 per cent of the glucose had been metabolized by the pentose cycle.

Differences in the metabolic behavior of different parts of the body may decrease the validity of our assumptions used in making these calculations. For example, when the CO₂ produced from glucose metabolized along the Embden-Meyerhof pathway does not arise in the same TCA cycle as the α-ketoglutarate from which glutamic acid of casein is formed, then our estimate for the per cent Cl⁴O₂ from glucose-1-C⁴ arising in the TCA cycle or pentose cycle may be in error. This problem arising from the inhomogeneity of the system is not necessarily avoided at the tissue slice or even single cell level, since the discrete distribution of enzymes within the cell may lead to metabolic pools that equilibrate with their surroundings at different rates.

The agreement between results calculated from five different sets of data and the agreement between results for the two cows lend strength to the conclusion that the pentose cycle plays a major role in the glucose catabolism of the lactating cow. It appears that at least 40 per cent of the glucose catabolism occurs via the pentose cycle, but in specific organs such as liver or mammary gland 60 per cent or more of the glucose may be catabolized via the pentose cycle.

**SUMMARY**

Glucose catabolism was studied with intact lactating dairy cows injected intravenously with glucose-1-C⁴ and glucose-6-C⁴. The recovery of C⁴

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6 It is recognized that there may be some mixing between these compounds and pyruvate in liver and mammary gland during reverse glycolysis, but this would probably have a relatively smaller influence on the specific activities of serine and glycerol compared with the effect on alanine.
was greater in the expired CO₂ after glucose-1-C¹⁴ but was smaller in alanine, and serine from casein, and glycerol from milk fat, than the corresponding results after glucose-6-C¹⁴. These results are inconsistent with the exclusive operation of the Embden-Meyerhof pathway and suggest an additional pathway of glucose metabolism through the pentose cycle.

The C¹⁴ levels in alanine, serine, and glycerol were used to estimate the quantitative importance of the pentose cycle in glucose catabolism. In each case the results indicated that one-half to two-thirds of the glucose molecules catabolized had entered the pentose cycle. Calculations based on C¹⁴ levels in respired CO₂ gave similar results. From these studies, involving assumptions, the limitations of which have been discussed in the text, we conclude that a non-Embden-Meyerhof pathway, which is probably the pentose cycle, has a major role in glucose metabolism in the lactating cow.

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BIBLIOGRAPHY

THE PENTOSE CYCLE AS A PATHWAY FOR GLUCOSE METABOLISM IN INTACT LACTATING DAIRY COWS

Arthur L. Black, Max Kleiber, Earl M. Butterworth, Georg B. Brubacher and Jiro J. Kaneko


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