Model to Examine Pathways of Carbon Flux from Lactate to Glucose at the First Branch Point in Gluconeogenesis*

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William G. Blackard‡ and John N. Clore
With the technical assistance of Julie Stillman
From the Department of Medicine, Medical College of Virginia, Richmond, Virginia 23298

The first branch point in gluconeogenesis occurs at the conversion of pyruvate to oxaloacetate. To determine the amount of lactate carbon reaching glucose via the direct pyruvate carboxylase pathway versus the tricarboxylic acid cycle, adult rat hepatocytes in primary culture were incubated for 2 h with one of the following isotopic substrates: [1-14C]lactate, [U-14C]lactate, or [1,2-14C]acetate. Production of 14CO2 and [14C]glucose from each substrate was assessed. The amount of lactate carbon 2 and 3 incorporated into glucose or oxidized to CO2 was determined by subtracting values using [1-14C]lactate from those using [U-14C]lactate. After quantitation of CO2 formed from carbons 2 and 3 of lactate, the amount of these carbons incorporated into glucose via the tricarboxylic acid cycle can be determined by simple proportionality from the ratio of label incorporated into glucose or CO2 from [1,2-14C]acetate. The remaining carbons 2 and 3 of lactate incorporated into glucose are derived from the pyruvate carboxylase pathway directly. Ethanol which on oxidation provides NADH and acetate decreased lactate oxidation and enhanced the pyruvate carboxylase pathway. Glucagon increased carbon flux through both pathways but primarily through the pyruvate carboxylase pathway. In summary, a simple model is presented to examine carbon flux from lactate via the pyruvate carboxylase and tricarboxylic acid pathways during gluconeogenesis.

Lactate is a major substrate for hepatic gluconeogenesis (1). Gluconeogenesis from lactate depends on its oxidation to pyruvate with subsequent conversion to oxaloacetate (Fig. 1). This latter step occurs by a branch point reaction which is the subject of this investigation. Oxaloacetate is then converted by phosphoenolpyruvate carboxykinase to phosphoenolpyruvate which is subsequently converted to glucose by reversal of glycolysis utilizing two additional bypass steps.

An important metabolic switch exists at pyruvate with pyruvate flux to oxaloacetate occurring either by the pyruvate carboxylase pathway or by the tricarboxylic acid (TCA) cycle after decarboxylation to acetyl-CoA by pyruvate dehydrogenase. Dilution and exchange reactions occurring in the TCA cycle result in significant underestimation of lactate carbon incorporation into glucose via that cycle (2). Thus it is important to determine the amount of lactate carbon traversing the TCA cycle en route to glucose and the conditions which may determine the trafficking of lactate carbon through the cycle. Factors influencing which of the two pathways are utilized include pyruvate carboxylase and pyruvate dehydrogenase enzyme mass, concentration of enzyme cofactors (biotin and thiamine), concentration of substrates controlling enzyme activity (acetate), presence of hormones (glucagon, epinephrine, T3), and the energy requirements of the cell.

These studies describe an in vitro model for estimating flux of lactate carbon atoms through the direct pyruvate carboxylase pathway and the TCA pathway during gluconeogenesis. However, the entry point (via pyruvate dehydrogenase or pyruvate carboxylase) of lactate carbon into the TCA cycle cannot be determined by the model.

EXPERIMENTAL PROCEDURES

Materials

The following isotopes were purchased from ICN: [U-14C]lactate, 89 mCi/mM in 70% alcohol; [1-14C]acetate, 52 mCi/mM in 70% ethanol; [1,2-14C]acetate, 50 mCi/mM in 70% ethanol; NaH14CO3, 567 mCi/mM in aqueous solution; [1-14C]alanine, 56.8 mCi/mM in 2% ethanol. Crystalline zinc glucagon was a gift from Eli Lilly.

Methods

Preparation of Cultured Hepatocytes—Male Sprague-Dawley rats weighing 200–250 g were anesthetized with ether, and the liver was perfused with 0.5% collagenase, which reduces the organ to a suspension of mostly single cells. Parenchymal cells were suspended in culture medium after separation from debris and other cell types by low speed centrifugation. Aliquots of the cell isolate containing 3.5 × 106 cells in a total volume of 3 ml were plated without delay in 60 × 15-mm plastic tissue culture dishes previously coated with calskin collagen. The plates were swirled carefully to ensure even distribution of cells over the surface of the plate and were then incubated under 5% CO2, 95% air at 37°C. Within 4 h, the cells had attached to the collagen surface and by 12–24 h, they had formed a confluent monolayer. Culture medium was changed every 24 h and consisted of Eagle’s minimal essential medium vitamin mix plus amino acids and salts of Hanks’ medium 199 with the following modifications: ornithine (20 mg/liter) was substituted for arginine, hydroxyproline and Fe(NO3)3 were omitted, and NaHCO3 was added at a concentration of 1.25 g/liter. In order to examine possible differences in gluconeogenesis in “periportal” and “perivenous” hepatocytes, cells were cultured as described by Probst et al. (3). During the initial 24 h following isolation, hepatocytes were grown in the presence of 10–4 M insulin. Perivenous cells were incubated for an additional 24 h with 10–5 M insulin, whereas perportal cells were incubated in media containing 10–4 glucagon and 0.5 × 10–5 M insulin for the second 24-h period. Each experiment was carried out in the 80–110 plates of cultured cells (3.5 × 106 cells/plate) routinely obtained from a single rat liver.

Evidence of sustained viability of hepatocytes in cultures has been provided by reports from this laboratory and elsewhere (4). All cultures used in these experiments were >90% viable, judged by the criterion of trypan blue exclusion.
Experimental Incubation—Experiments were performed on 48-h cultured cells. The culture medium was replaced by Hanks' salts solution, containing, in addition, 25 mM tricine, 5 mM glucose, and 1 mM lactate, pH 7.4. Media were supplemented with 25,000 dpm of one of the following tracers: [U-14C]lactate, [1-14C]lactate, or [1,2-14C]lactate. Tracer ethanol was removed by placing the tracer on a Dowex 1 column eluted with 5 M HCl. Purified tracer was neutralized before incorporation into culture medium.

In order to assess CO2 production from cultures, a 4.7-cm diameter dish of Whatman No. 3 filter paper impregnated with 10% KOH was attached by fluid adherence to the cover of the culture dish. The culture dish was then sealed with petrolatum to prevent escape of CO2. Under these conditions it was shown that after acidification of NaH14CO3 more than 95% of CO2 released was captured by the filter paper within 10 min of its generation. Filters were removed and placed in Budget-Solve (Research Products International Corp., Mount Prospect, IL) scintillation fluid at the end of the 2-h incubation period. Prior to counting, filters were equilibrated in scintillation fluid for at least 4 h to permit complete dissociation of radioactivity from the filters. Because the method above used for CO2 capture worked well, all atmospheres were treated in addition except for the filters designed to determine the effect of atmospheric CO2 on gluconeogenesis. In these experiments 10% KOH was injected onto the filter paper after 90 min, and CO2 was collected for the next 30 min. The puncture site of the culture plate top (KOH was injected through a heated 21-gauge needle) was sealed with petrolatum to prevent CO2 escape. The possibility of CO2 escape from the sealed plates was assessed by placing the smaller culture plates in a larger Petri dish with a KOH trap outside the inner plate. A heavy seal of petrolatum prevented leakage, whereas silicone was less effective in that regard.

At completion of the 2-h incubation period, media were removed from the plate, placed on ice, and labeled glucose formed and released into the media was measured. Aliquots (0.1 ml) of media were placed on 2-cm Dowex 1 (chloride) columns. Glucose was eluted in the initial 1.5 ml neutral fraction. Following an additional 1 ml water wash, lactate (and pyruvate) were eluted with 1.5 ml of 0.5 N HCl. Eluates were collected in 22-ml polyethylene counting vials. Initial studies suggested that only a small component of the neutral fraction consisted of amino acids. Chromatography of media from later studies over sequential Dowex 1 (chloride form) and Dowex 50 (hydrogen form) columns revealed that approximately 15% of the radioactivity in the neutral fraction was from amino acids (Table I). Incorporation of radioactivity into amino acids was assessed by elution from the Dowex 50 column with 1.5 ml of 1 M NH4OH. The experiments shown in Table I and Fig. 5 examining the effect of atmospheric CO2 on gluconeogenesis used the dual column method for radioactive glucose assessment. Phosphorylation of the neutral fraction with glucokinase and ATP (5) confirmed that over 95% of the neutral fraction radioactivity obtained from the dual columns was indeed glucose. The presence of glucose in the media undoubtedly increased the lactate/pyruvate pool as 3% of [1- or 6-14C]glucose became incorporated into the media lactate/pyruvate fraction under conditions employed for these experiments. Despite this consideration, the presence or absence of glucose in the media had no appreciable effect on the rate of incorporation of lactate label into glucose, CO2, or amino acids (Table I). The contribution of hepatocyte glycogen to the lactate/pyruvate pool cannot be estimated. When glucose is present in the media, glycogenolysis may be inhibited thus possibly accounting for the failure to demonstrate an effect of glycolysis of media glucose in this system.

Since carbon 1 of lactate is lost primarily in the pyruvate dehydrogenase reaction in conversion to acetyl-CoA or as a result of fumarase equilibration after conversion to oxaloacetate by pyruvate carboxylase, our major focus was the pathways by which carbons 2 and 3 of lactate reached glucose. In order to determine the quantity of carbons 2 and 3 of lactate appearing in CO2 or glucose, the amount of CO2 or glucose formed from carbon 1 of lactate in cultures containing only [1-14C]lactate was subtracted from that formed from all three lactate carbons in cultures containing [U-14C]lactate. Since processing of carbons of lactate rather than the entire lactate molecule was being examined, nanoatoms of lactate carbon converted to glucose or CO2 calculated from [U-14C]lactate was multiplied by 3 (to obtain nanoatoms of all three carbons) before subtracting the contribution from carbon 1. The determination of 14CO2 and [1-14C]glucose from [1,2-14C]acetate served as a measure of processing of lactate carbons 2 and 3 which reached the TCA cycle. Lactate/pyruvate can reach the TCA cycle via either the pyruvate dehydrogenase or pyruvate carboxylase pathway. Clearly, [1,2-14C]acetate processing will reflect the fate of lactate carbons 2 and 3 reaching the TCA cycle via the pyruvate dehydrogenase pathway. Although less obvious, the same is true for carbons 2 and 3 of lactate entering the TCA cycle at oxaloacetate via the pyruvate carboxylase. These carbons appear as carbons 2 and 3 of oxaloacetate which after one turn of the TCA cycle and since [U-14C]lactate decarboxylated by pyruvate carboxylase was measured, nanoatoms of lactate carbon converted to glucose or CO2 calculated from [U-14C]lactate was multiplied by 3 (to obtain nanoatoms of all three carbons) before subtracting the contribution from carbon 1. The determination of 14CO2 and [1-14C]glucose from [1,2-14C]acetate served as a measure of processing of lactate carbons 2 and 3 which reached the TCA cycle. Lactate/pyruvate can reach the TCA cycle via either the pyruvate dehydrogenase or pyruvate carboxylase pathway. Clearly, [1,2-14C]acetate processing will reflect the fate of lactate carbons 2 and 3 reaching the TCA cycle via the pyruvate dehydrogenase pathway. Although less obvious, the same is true for carbons 2 and 3 of lactate entering the TCA cycle at oxaloacetate via the pyruvate carboxylase. These carbons appear as carbons 2 and 3 of oxaloacetate which after one turn of the TCA cycle will distribute equally between all four carbons of oxaloacetate, the same labeling pattern of oxaloacetate produced by 1,2-acetate. Since virtually all CO2 from carbons 2 and 3 of lactate is produced in the TCA cycle and since [U-14C]lactate decarboxylated by pyruvate dehydrogenase and 2,3-lactate entering the TCA cycle at oxaloacetate after carboxylation by pyruvate carboxylase would behave as [1,2-14C]acetate, it was possible by simple proportionality to determine the amount of carbons 2 and 3 of lactate proceeding to glucose via the TCA cycle. Any 1-14CO2 produced from the TCA cycle (via oxaloacetate entry)
TABLE 1

Effect of media glucose on lactate carbon conversion to CO₂, glucose, and amino acids in cultured hepatocytes

Cultured hepatocytes were incubated for 2 h in the presence of 25 mM tricine, pH 7.4, 1 mM lactate, and varying concentrations of glucose (0, 2.5, or 5.0 mM). Incubations were supplemented with either [U-14C]lactate or [1-14C]lactate, and the percent of radioactivity converted to CO₂ glucose and amino acids was determined (n = 3). Results are expressed as mean ± S.E.

<table>
<thead>
<tr>
<th>Media glucose</th>
<th>Labeled lactate carbon converted to CO₂</th>
<th>Labeled lactate carbon converted to glucose</th>
<th>Labeled lactate carbon converted to amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>2.5</td>
<td>9.5 ± 0.4</td>
<td>19.3 ± 3.2</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>5.0</td>
<td>10.2 ± 0.1</td>
<td>21.2 ± 0.4</td>
<td>10.2 ± 0.2</td>
</tr>
<tr>
<td>10.2 ± 0.7</td>
<td>23.3 ± 1.2</td>
<td></td>
<td>9.6 ± 0.4</td>
</tr>
</tbody>
</table>

Thus far the model has not taken into account glycogen or lipid formation, or recycling through pyruvate kinase. The incorporation of label into glycogen and lipids was found to be minimal, each accounting for less than 1% of total lactate conversion to product during the 2-h incubation. It should be obvious from Fig. 1 that a variable number of carbon atoms made their way back to pyruvate. These recycled carbons, regardless of the initial pathway traversed, would have the opportunity to use the same or another pathway. Based on a 26% recycling rate (shown later), and a 70/30 partition of pyruvate into the direct pyruvate carboxylase and TCA pathways, respectively (shown below), the net result should be a depression of CO₂ production from all lactate carbons. However, conversion of lactate carbon to glucose might not be as severely impaired because of stimulation of pyruvate carboxylase by acetate derived acetyl-CoA. The experiment depicted in Fig. 2 emphasizing 1.7 mM media ethanol gave the predicted result, a greater decrease in lactate decarboxylation than in lactate carbon conversion to glucose in the presence of ethanol (Fig. 2A). The data also indicate, as predicted, that more lactate carbon (carbons 2 and 3) reacted glucose via the direct pyruvate carboxylase pathway than by the TCA pathway in the presence of ethanol (Fig. 2B). The processing of carbons 2 and 3 of lactate through the TCA cycle to glucose was not as extensive as was predicted.
First Branch Point in Gluconeogenesis

Effect of ethanol (1.7 mM) on lactate carbon flux in cultured hepatocytes. Panel A, conversion of lactate carbons 1 ([1-14C]lactate-derived), 1, 2, and 3 ([U-14C]lactate-derived), and 2 and 3 (calculated, see model calculation) into CO2 (upper panel) and glucose (lower panel). Panel B, conversion of carbons 2 and 3 of lactate (calculated) to glucose via TCA cycle versus direct pyruvate carboxylase (PC) pathway of gluconeogenesis. Results are expressed as mean ± S.E., n = 5, separate experiments.

Fig. 2. Effect of ethanol (1.7 mM) on lactate carbon flux in cultured hepatocytes. Panel A, conversion of lactate carbons 1 ([1-14C]lactate-derived), 1, 2, and 3 ([U-14C]lactate-derived), and 2 and 3 (calculated, see model calculation) into CO2 (upper panel) and glucose (lower panel). Panel B, conversion of carbons 2 and 3 of lactate (calculated) to glucose via TCA cycle versus direct pyruvate carboxylase (PC) pathway of gluconeogenesis. Results are expressed as mean ± S.E., n = 5, separate experiments.

TABLE II
Data used for calculation of contribution of pyruvate carboxylase and TCA pathways in first branch point in gluconeogenesis by cultured periportal hepatocytes

See "Methods" for experimental detail (seven observations). Data are expressed as nanoatoms (natoms) formed per culture plate (3 x 10^6 cells) over 2 h. The number of nanoatoms formed may be slightly underestimated reflecting underestimation of the pyruvate/lactate pool which is undoubtedly increased to some extent by 3-carbon intermediates formed from glucose in the media or glycogen in the cell. Over the same time period, 3% of 1- or 6-14C-labeled glucose appears in the pyruvate/lactate fraction in the media.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glucagon (10^{-8} M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate carbon 1 to CO2</td>
<td>158 ± 7 natoms</td>
<td>173 ± 14 natoms</td>
</tr>
<tr>
<td>Lactate carbons 1, 2, 3 to CO2</td>
<td>231 ± 15 natoms</td>
<td>238 ± 10 natoms</td>
</tr>
<tr>
<td>Lactate carbons 2, 3 to CO2 (calculated)</td>
<td>73 ± 11 natoms</td>
<td>64 ± 11 natoms</td>
</tr>
<tr>
<td>Lactate carbon 1 into glucose [1-14C]glucose</td>
<td>44 ± 5 natoms</td>
<td>80 ± 6 natoms</td>
</tr>
<tr>
<td>Lactate carbons 1, 2, 3 into glucose [U-14C]lactate</td>
<td>207 ± 17 natoms</td>
<td>343 ± 28 natoms</td>
</tr>
<tr>
<td>Lactate carbons 2, 3 into glucose (calculated)</td>
<td>163 ± 13 natoms</td>
<td>264 ± 20 natoms</td>
</tr>
<tr>
<td>CO2 production from [1,2-14C]acetate (% of label)</td>
<td>8.0 ± 0.4%</td>
<td>6.6 ± 0.6%</td>
</tr>
<tr>
<td>[1,2-14C]acetate into glucose (% of label)</td>
<td>6.4 ± 0.8%</td>
<td>7.2 ± 0.9%</td>
</tr>
<tr>
<td>Carbons 2, 3 lactate to glucose via TCA pathway (calculated)</td>
<td>53 ± 8 natoms</td>
<td>71 ± 13 natoms</td>
</tr>
<tr>
<td>Carbons 2, 3 lactate to glucose via PC pathway (calculated)</td>
<td>110 ± 13 natoms</td>
<td>194 ± 24 natoms</td>
</tr>
</tbody>
</table>

Inhibited to approximately 60% of normal (p = 0.05), whereas carbons converted to glucose via the direct PC pathway were, if anything, slightly increased. Some of the carbon flux thru the TCA cycle observed with ethanol may have resulted from entry into the cycle at oxaloacetate.

Effect of Glucagon on Lactate Carbon Conversion to Glucose—As shown in Fig. 3 and Table II, glucagon stimulates incorporation of all three carbon atoms of lactate into glucose. Although carbons 2 and 3 were not monitored individually, the 60% increase in incorporation of carbon 2 plus carbon 3 likely represents an increase in both with perhaps slightly less carbon 2 incorporation because of greater loss of this carbon as CO2 during subsequent turns in the TCA cycle. Perivenous cells (see "Methods") also were responsive to glucagon (lower panel, Fig. 3) although stimulation of lactate carbon to glucose appeared marginally less in these cells than in periportal cells.

Fig. 3. Effect of glucagon (10^{-8} M) on lactate carbon conversion to glucose in glucagon-cultured (periportal) (n = 7) (upper panel) and insulin-cultured (perivenous) (n = 5) (lower panel) cultured hepatocytes. Lactate carbons 1 ([1-14C]lactate-derived), 1, 2, and 3 ([U-14C]lactate-derived), and 2 and 3 (calculated, see model calculations) are shown. Results are expressed as mean ± S.E., n = 5, separate experiments.

Effect of Glucagon on the Direct Pyruvate Carboxylase Pathway and the TCA Pathway during Gluconeogenesis—From data in Table I, the amount of carbon 2 plus carbon 3 of lactate converted to glucose by either the TCA or the direct pyruvate carboxylase pathway was determined (Fig. 4). Although TCA pathway flux was slightly increased in perivenous cells exposed to glucagon (p = 0.08), a significant increase was observed in direct PC pathway flux only (p < 0.001). A

Fig. 4. Effect of glucagon (10^{-8} M) on conversion of lactate carbons 2 and 3 to glucose (total) and via TCA cycle and direct pyruvate carboxylase (PC) pathways of gluconeogenesis in cultured hepatocytes. Lactate carbon flux was determined in both glucagon-cultured (periportal) (upper panel, n = 7) and insulin-cultured (perivenous) (lower panel, n = 5) hepatocytes. Results are expressed as mean ± S.E.
similar pattern was observed in perivenous cells (Fig. 5, bottom) although the stimulatory effect of glucagon was not as great and reached significance only when total lactate carbon 2 and 3 flux to glucose (pyruvate carboxylase + TCA) was considered (p < 0.02).

Effect of Glucagon on Pyruvate Kinase Activity—One of the postulated mechanisms of glucagon's effect on gluconeogenesis is to decrease pyruvate kinase. The effect that pyruvate kinase activity might have on carbon flux through the two pathways is discussed in the section on description of the model. Assessment of pyruvate kinase activity by modification of the method of Rognstad (8) revealed pyruvate kinase activity to be somewhat modest in these cultured cells and surprisingly not affected by glucagon (Table III). PK flux was approximately 26% of the gluconeogenesis rate, a figure similar to the 25% rate reported for perfused fasted liver (9) and between the 10 and 50% figure noted by Rognstad and Katz (10) with fasted and fed hepatocytes, respectively.

Effect of Atmospheric CO₂ on Lactate Carbon Conversion to CO₂ and Glucose—In the previously described studies, cultured cells were incubated in an essentially CO₂-free environment since the KOH-impregnated trap was present in the sealed culture dishes throughout the experiment. In order to examine the effect of atmospheric CO₂ on lactate carbon conversion to CO₂ and glucose, paired plates of cultured hepatocytes were incubated in culture dishes in which KOH was present on the filter either throughout the experiment (CO₂-deplete) or for only the last 30 min (CO₂-replete) (see "Methods" for details). As shown in Fig. 5, panel A, the conversion of lactate carbon to CO₂ was enhanced somewhat in the presence of atmospheric CO₂ both with and without added glucagon. However, lactate carbon conversion to glucose increased almost 2-fold when the hepatocytes were incubated in the presence of CO₂ compared to that in the absence of CO₂ (p < 0.01) (Fig. 5, panel A). When lactate carbon flux through the first branch point in gluconeogenesis is examined, it is apparent that the increased lactate carbon conversion to glucose when atmospheric CO₂ is present reflects mainly an increase in carbon flux through the direct pyruvate carboxylase pathway (Fig. 5, panel B). Under conditions of glucagon stimulation, lactate carbon conversion to glucose is stimulated via both the TCA and pyruvate carboxylase pathways with quantitatively more lactate carbon traversing the pyruvate carboxylase pathway in the presence than in the absence of atmospheric CO₂.

DISCUSSION

We have presented a simple model for determining carbon flux from lactate to glucose via the TCA cycle whether lactate reaches the cycle through pyruvate dehydrogenase or pyruvate carboxylase. The remaining lactate carbon flux to glucose occurs as a result of pyruvate carboxylase and direct conversion of oxaloacetate to P-enolpyruvate. Neither measurement of specific activity of the product nor assessment of the location of radioactivity in the glucose molecule is required for this method. Although the model cannot be used to quantify gluconeogenesis precisely, it allows calculation of the route by which lactate carbons reach glucose. This calculation depends on a simple proportionality equation in which the ratio of lactate carbons 2 and 3 incorporated into glucose divided by carbons 2 and 3 of lactate oxidized to CO₂ equals the ratio of percent labeled 1,2-acetate incorporated into glucose divided by percent labeled acetate oxidized to CO₂.

For the proposed model to be valid two assumptions must be made. First, virtually all CO₂ generated from carbons 2 and 3 of lactate must occur as a result of decarboxylations in the TCA cycle. The second assumption is that processing of acetate carbons 1 and 2 entering the TCA cycle must mimic that of 1,2-acetyl-CoA formed by decarboxylation of pyruvate and that of pyruvate carbons 2 and 3 entering the TCA cycle at oxaloacetate. These assumptions have been made previously by others (11, 12). As stated earlier, the labeling pattern of oxaloacetate at the end of the first TCA cycle is identical from [1,2-14C]acetate, [1,2-14C]acetate-CoA after decarboxylation of [U-14C]acetate and [2,3-14C]pyruvate entering the TCA cycle at oxaloacetate after pyruvate carboxylation. Thus the entry site (oxaloacetate or acetyl-CoA) of lactate carbon into the cycle does not affect the calculations used to assess the amount of lactate carbons 2 and 3 incorporated into glucose via the TCA cycle.

Our model should not be confused with Kelleher's method.

TABLE III
Pyruvate kinase activity in cultured hepatocytes (± glucagon)

<table>
<thead>
<tr>
<th>No. of observations</th>
<th>Pyruvate kinase activity*</th>
<th>Alanine partition factor*</th>
<th>Adjusted pyruvate kinase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>0.24 ± 0.01</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td>Glucagon (10⁻⁷ M)</td>
<td>4</td>
<td>0.27 ± 0.04</td>
<td>1.14 ± 0.10</td>
</tr>
</tbody>
</table>

* Cells incubated with NaH¹⁴CO₃ for 30 min: [lactate + pyruvate]/[lactate + pyruvate + glucose].

* Cells incubated with [¹⁴C]l-alanine for 10 min: [lactate + pyruvate + glucose + CO₂]/[lactate + pyruvate].

* Pyruvate kinase activity when [¹⁴C]lactate (pyruvate) used in pyruvate kinase activity* above multiplied by partition factor.

Fig. 5. Effect of atmospheric CO₂ on lactate carbon conversion to CO₂ and glucose in cultured hepatocytes (paired experiments, n = 5). Incubations were performed with continuous CO₂ removal (□ CO₂) or in the presence of CO₂ (□ + CO₂). Panel A, lactate carbon conversion to CO₂ (upper panel) and glucose (lower panel) from either lactate carbons 1, 2, and 3 ([U-¹⁴C]lactate-derived) or lactate carbons 1 ([¹⁴C]lactate-derived) in absence (control) or presence of glucagon (10⁻⁸ M). Panel B, conversion of lactate carbons 2 and 3 to glucose via TCA cycle and direct pyruvate carboxylase (PC) pathway in absence or presence of glucagon (10⁻⁸ M). Results are expressed as mean ± S.E.
which assesses the relative magnitude of pyruvate entry into the TCA cycle via either pyruvate dehydrogenase or pyruvate carboxylase. This determination is dependent on the degree of randomization which occurs during equilibration at the fumarase step. Since our model assesses only carbon flux through the TCA and direct pyruvate carboxylase pathways without defining the site of entry of pyruvate into the cycle, maximal information could be obtained by the use of both methods. It is widely appreciated that isotopic carbon exchange in the TCA cycle leads to a variable underestimation of gluconeogenesis (2, 11, 14, 15). Therefore a method assessing quantitatively the relative amount of lactate carbon incorporated into glucose as a result of traffic through the TCA cycle where isotopic exchange occurs is important. The contribution of the pyruvate carboxylase and TCA pathways to gluconeogenesis from lactate has been examined under control conditions and in the presence of a metabolic inhibitor. Pyruvate, CO₂ production from lactate was dramatically reduced whereas the production of lactate carbon conversion to CO₂. These findings, predictably the model.

Expected to increase labeled carbon incorporation into glucose via the TCA cycle route was anticipated. Diminished TCA cycle traffic with reduced carbon exchange would be expected to increase labeled carbon incorporation into glucose and thus might explain why ethanol inhibition of lactate carbon incorporation into glucose was less than the inhibition of lactate carbon conversion to CO₂. These findings, predictable from present knowledge about alcohol metabolism, serve as partial validation of the model.

The effect of glucagon on the two arms of the first branch point in gluconeogenesis was examined using this model. Glucagon increased incorporation of carbons 2 and 3 of lactate into glucose via both the TCA and direct pyruvate carboxylase pathways. Quantitatively the effect of glucagon's action in promoting gluconeogenesis have been proposed. These include an increase in P-enolpyruvate carboxykinase (16), an increase in pyruvate carboxylase (17), and a decrease in pyruvate kinase (18, 19). Surprisingly, an effect of glucagon on pyruvate kinase activity in cultured hepatocytes could not be demonstrated. The greater increase in lactate carbon incorporation into glucose via the direct pyruvate carboxylase pathway compared to the TCA pathway suggests that a major effect of glucagon is on pyruvate carboxylase enzyme activity. The fact that the activities of both pathways are increased suggests in addition a possible pull-through effect exerted at either P-enolpyruvate carboxykinase or higher (i.e. modulation of fructose-2,6-diphosphatase). However, it is also possible that the glucagon-induced increase in lactate carbon incorporation into glucose via the TCA cycle could be related to increased pyruvate carboxylation with a portion of the oxaloacetate so formed traversing the TCA cycle.

Two additional studies have been performed using the model. The first was to examine the effect of atmospheric CO₂ on lactate carbon flux through the two pathways. Since atmospheric CO₂ serves as a carbon source for the pyruvate carboxylase reaction, it should not be surprising that atmospheric CO₂ enhances carbon flux to glucose via the direct pyruvate carboxylase pathway under control and glucagon-stimulated conditions. Despite similar qualitative results in the presence and absence of CO₂, subsequent studies should be performed under conditions where atmospheric CO₂ is preserved through maintenance of CO₂ in the sealed cultures makes experiments technically more demanding. The second observation relates to the comparison of lactate carbon flux in periporal and perivenous cells. Jungermann and Katz (20) have popularized the concept of liver cell heterogeneity. In their hands periporal cells are more responsive than perivenous cells to glucagon. Since it is difficult to obtain the two cell populations by cell sorting after liver digestion, Probst and co-workers (3) proposed a method for inducing periporal and perivenous cell behavior in culture by differential exposure to glucagon and insulin, respectively (see “Methods”). Our results failed to demonstrate a qualitative difference in the pathways of gluconeogenesis in the two cell types, although quantitatively the perivenous cells responded slightly less to glucagon than did the periporal cells.

In summary, a method is described for determining carbon flux through either the classical (pyruvate carboxylase) or the TCA arm of the first branch point in gluconeogenesis. The number of lactate carbon atoms reaching glucose via the TCA cycle will, of course, be an underestimation depending on the degree of dilution and exchange taking place in the TCA cycle. Nevertheless, the method provides a means for assessing the effect of a variety of factors (hormones, cofactors, metabolites, etc.) on lactate carbon incorporation into glucose via the two arms of the pathway. Further studies to examine the magnitude of dilution and exchange occurring in the TCA cycle as well as the route of entry into the TCA cycle should expand the usefulness of this model.

REFERENCES