Studies on the Mechanism of Action of Glucagon on Gluconeogenesis*

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SUMMARY

The mechanism by which glucagon stimulates gluconeogenesis was studied in livers perfused with 1 mM lactate, 0.1 mM pyruvate, and several different 14C-labeled substrates.

In livers perfused for 2½ min with 14C-lactate plus 14C-pyruvate, glucagon increased 14C-glucose synthesis 3-fold, increased the labeling of tissue P-pyruvate, aspartate, alanine, and glycogen, and decreased the labeling of pyruvate, oxalacetate, malate, citrate, α-ketoglutarate, and glutamate. The hormone also decreased the tissue levels of pyruvate, malate, citrate, α-ketoglutarate, and glutamate and increased those of aspartate and P-pyruvate. These changes in the liver are interpreted as indicating that glucagon stimulated the conversion of oxalacetate to aspartate and P-pyruvate.

Tryptophan produced changes in livers perfused with 14C-lactate plus 14C-pyruvate which were consistent with inhibition of gluconeogenesis at P-enolpyruvate carboxykinase. It blocked completely the effects of glucagon on the labeling of glucose and P-pyruvate at 2½ or 20 min, and caused the hormone to induce accumulation of malate and aspartate. The extra malate and aspartate which accumulated under these circumstances were derived from unlabeled sources (e.g., endogenous protein) and not from pyruvate as indicated by their decreased or unchanged radioactivity.

In livers perfused with H14CO3 for 1 min, glucagon decreased the radioactivities of oxalacetate, malate, citrate, and α-ketoglutarate and increased those of aspartate, P-pyruvate, succinate, and fumarate. The specific radioactivities of intermediates were consistent with fixation of CO2 predominately into malate, aspartate, and citrate. The changes induced by glucagon indicated stimulation of aspartate and P-pyruvate formation from oxalacetate and of succinate formation from α-ketoglutarate.

Further evidence for an effect of glucagon at a site between oxalacetate and P-pyruvate was provided by perfusions with 14C-glutamate. These showed that glucagon decreased the radioactivities of aspartate, malate, oxalacetate, and citrate and increased the radioactivity of P-pyruvate at both 2½ and 8½ min, despite the fact that the specific radioactivities of P-pyruvate precursors were reduced.

Although the interpretation of these data is subject to reservation because of the existence of multiple pools of metabolic intermediates, they provide consistent support for the view that P-pyruvate synthesis from oxalacetate is a major site of action of glucagon on gluconeogenesis in the liver.

Several studies have shown that glucagon activates gluconeogenesis in the perfused liver (3–6). The effect is mediated by adenosine 3':5'-monophosphate (5) by an unknown mechanism. It appears to be exerted primarily on a reaction(s) located in the gluconeogenic pathway between pyruvate and P-pyruvate (6, 7). Although early studies suggested that it was secondary to increased lipolysis (3, 8), this view has been disputed (9, 10).

This report presents the results of a study of the effects of glucagon on the labeling of glucose and metabolic intermediates in livers perfused with 14C-labeled lactate, pyruvate, aspartate, and bicarbonate in the absence or presence of tryptophan. The data are consistent with the view that the gluconeogenic action of glucagon involves activation of P-enolpyruvate carboxykinase.

EXPERIMENTAL PROCEDURE

The sources of animals and materials, the technique of perfusion, the basic design of the experiments and the methods of analysis of medium and tissue were those described in detail in the accompanying paper (11). Animals were either fed ad libitum or fasted for 18 to 22 hours before use.

RESULTS

Effects of Glucagon on 14C-Glucose Synthesis and Radioactivity of Metabolic Intermediates in Livers Perfused with 14C-Lactate plus 14C-Pyruvate—Glucagon increased 14C-glucose synthesis 3-fold in livers perfused for 2½ min with labeled lactate plus pyruvate (Table I). It increased the labeling of tissue P-pyruvate, aspartate, alanine, and glycogen, but decreased the radioactivity of tissue pyruvate, oxalacetate, malate, citrate, α-ketoglutarate, and glutamate (Table I). The tissue level of malate was altered while that of aspartate was increased. Citrate, α-ketoglutarate, and glutamate concentrations were decreased (Table I). The specific radioactivities of intermediates in control livers

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Metabolic intermediates in livers perfused with [14C]lactate plus less than half that of malate or citrate in livers perfused with pools of this DNP.

Several pools of a particular intermediate (12) with differing specific radioactivities of aspartate was also low relative to that of cytoplasmic acids to P-pyruvate, on the conversion of glutamate and amination of pyruvate to alanine and of oxalacetate to aspartate. This is because of the possible existence of pathways. This is because of the possible existence of a site between the 4-carbon dicarboxylic acids and P-pyruvate.

The possibility that an aspartate pool of high specific radioactivity is involved in gluconeogenesis raises the possibility that an aspartate pool of high specific radioactivity is involved in gluconeogenesis must have turned over many times. As expected, tryptophan increased the radioactivities of intermediates prior to P-pyruvate in the gluconeogenic pathway and diminished by 94%. In tryptophan-blocked livers, glucagon increased aspartate but did not alter the other intermediates.

Effects of glucagon on chemical quantity, 14C content, and specific radioactivity of intermediary metabolites in livers perfused with [14C]lactate and [14C]pyruvate

Livers from fed rats were perfused for 1 hour with recirculating medium containing no added substrate. At 1 hour, the perfusion was changed to a flow through system with medium containing 1 mM L-[U-14C]lactate (45,000 cpm per ml) plus 0.1 mM [2-14C]pyruvate (4,500 cpm per ml). Livers were frozen after 4 min. The concentration of glucagon was 5 x 10^{-9} M. Values are means ± standard errors of the mean (S.E.M.) for six observations. [14C]Malate and [14C]glutamine were determined in pooled samples. The flow rate was 7.0 ml per min.

| Chemical quantity | Radioactivity | Specific activity |
|-------------------|---------------|------------------|
|                    | Control       | Glucagon         | Control       | Glucagon         |
|                    | n mole/g      | % control        | cpm x 10^{-2} | % control        | cpm/mole        | % control |
| Pyruvate           | 100 ± 7       | 78 ± 5           | 21 ± 2        | 59 ± 7           | 21               | 75        |
| Oxalacetate        |               |                  | 7 ± 1         | 76 ± 4           | 7                | 198       |
| Malate             | 138 ± 6       | 148 ± 6          | 27 ± 1        | 87 ± 5           | 20               | 61        |
| Aspartate          | 405 ± 19      | 160 ± 5          | 28 ± 3        | 302 ± 26         | 7                | 189       |
| Citrate            | 310 ± 8       | 90 ± 2           | 37 ± 2        | 81 ± 8           | 12               | 90        |
| P-pyruvate         | 90 ± 5        | 225 ± 10         | 10 ± 1        | 150 ± 9          | 11               | 67        |
| Glucose            |               |                  | 199 ± 25      | 222 ± 44         | 9                | 95        |
| Glycogen           |               |                  | 4 ± 1         | 235 ± 22         | 7                | 94        |
| Glutamate          | 2353 ± 53     | 71 ± 3           | 214 ± 15      | 67 ± 10          | 9                | 95        |
| α-Ketoglutarate    | 274 ± 14      | 38 ± 3           | 19 ± 3        | 39 ± 4           | 7                | 94        |
| Succinate          | 34 ± 2        | 123 ± 11         | 10 ± 2        | 133 ± 12         | 10               | 115       |
| Fumarate           |               |                  | 144           | 133              | 9                | 129       |
| Alanine            | 700           | 164              | 9             | 129              |                  |           |

* p < 0.01.

† p < 0.05.

Total perfusate collected during 4 min of flow-through.

Effects of glucagon on 14C Synthesis and Radioactivity of Metabolic Intermediates in Livers Perfused with [14C]Lactate plus [14C]Pyruvate in the Absence or Presence of Tryptophan—In the preceding experiments, it was possible that activation of P-pyruvate synthesis from 4-carbon dicarboxylic acids might have obscured a simultaneous stimulatory effect of glucagon on pyruvate carboxylation. To examine this point, the effects of glucagon were examined in livers in which P-enolpyruvate carboxykinase was inhibited by addition of 2.4 mM tryptophan to the perfusion medium. In one series of experiments, the exposure to substrates and hormone was of relatively long duration, i.e. 20 min.

Table II presents the results. In the absence of tryptophan, glucagon lowered the tissue levels of pyruvate and citrate and raised those of malate and P-pyruvate. The hormone also decreased the radioactivities of pyruvate, oxalacetate, malate, and citrate and increased those of P-pyruvate and glucose. The specific radioactivities of pyruvate and malate were decreased, whereas those of aspartate, citrate, and P-pyruvate were unchanged. These changes support the view that glucagon activates gluconeogenesis at a site between the 4-carbon dicarboxylic acids and P-pyruvate.

Tryptophan treatment increased the tissue levels of pyruvate, malate, aspartate, and citrate and markedly lowered the level of P-pyruvate (Table II). In the presence of the inhibitor, glucagon increased aspartate but did not alter the other intermediates.

As expected, tryptophan increased the radioactivities of intermediates prior to P-pyruvate in the gluconeogenic pathway and lowered that of P-pyruvate (Table II). Labeling of glucose was diminished by 94%. In tryptophan-blocked livers, glucagon decreased malate and citrate radioactivities but did not significantly change the isotope content of other intermediates. The hormone also reduced the specific radioactivities of malate and citrate under these conditions. These results provide no evidence that glucagon stimulates pyruvate carboxylation. The increased levels of malate and aspartate induced by the hormone
TABLE II
Effects of glucagon in livers perfused with \[^{[4]}C\]lactate plus \[^{[14]}C\]pyruvate for 20 min in the absence or presence of tryptophan

Livers from fasted rats were perfused as described in Table I and frozen after 20 min of flow-through. Tryptophan (2.4 mM) was added 45 min prior to flow-through. Values are means ± S.E.M. for three or four observations.

| Chemical amount | Control | Glucagon |
|-----------------|---------|----------|
| Pyruvate        | 96 ± 5  | 81 ± 6   |
| Oxalacetate     | 104 ± 26| 239 ± 48 |
| Malate          | 446 ± 36| 495 ± 43 |
| Aspartate       | 210 ± 23| 137 ± 31 |
| Citrate         | 67 ± 8  | 110 ± 5  |
| P-pyruvate      | 146 ± 8 | 164 ± 9  |
| Glucose*        | 118 ± 32| 1651 ± 345|
| Without tryptophan |       |          |
| Aspartate       | 390 ± 38| 359 ± 27 |
| Glucose*        | 21 ± 3  | 23 ± 2   |
| With tryptophan |         |          |
| Pyruvate        | 146 ± 8 | 164 ± 9  |
| Oxalacetate     | 504 ± 64| 509 ± 38 |
| Malate          | 1128 ± 32| 1651 ± 345|
| Aspartate       | 390 ± 38| 359 ± 27 |
| P-pyruvate      | 21 ± 3  | 23 ± 2   |
| Glucose*        | 118 ± 32| 1651 ± 345|
| Glucose*        | 21 ± 3  | 23 ± 2   |

| Chemical amount | Control | Glucagon |
|-----------------|---------|----------|
| Radioactivity     |         |          |
| \(cpm \times \text{mole/g} \) |         |          |
| Glucose          | 25.3 ± 1.9| 12.8 ± 1.4|
| Oxalacetate      | 1.0 ± 0.1 | 1.1 ± 0.3 |
| Malate           | 33.3 ± 2.9| 25.0 ± 1.8|
| Aspartate        | 64.3 ± 7.7| 55.5 ± 4.1|
| Citrate          | 41.5 ± 4.7| 28.4 ± 1.8|
| P-pyruvate       | 11.5 ± 1.3| 18.5 ± 2.5|
| Glucose*         | 31.7 ± 5.0| 58.1 ± 3.7|
| Specific radioactivity |         |          |
| Glucose          | 26.4 ± 1.8| 16.0 ± 2.6|
| Oxalacetate      | 21.6 ± 3.0| 11.4 ± 1.9|
| Malate           | 14.4 ± 1.1| 11.5 ± 1.7|
| Aspartate        | 19.8 ± 1.0| 16.0 ± 3.0|
| Citrate          | 17.0 ± 1.6| 16.7 ± 1.8|
| P-pyruvate       | 11.5 ± 1.3| 18.5 ± 2.5|
| Glucose*         | 31.7 ± 5.0| 58.1 ± 3.7|

\(^a\) Counts per min \(\times 10^{-3}\) per ml of perfusate.

FIG. 1. Effects of tryptophan on the \(^{14}C\) content of glucose and P-pyruvate and on tissue levels of malate, aspartate, and citrate in livers perfused with \[^{[4]}C\]lactate plus \[^{[14]}C\]pyruvate in the presence or absence of glucagon. Experimental conditions were the same as in Table I. Tryptophan (2.4 mM) was added to the recirculating medium 50 min prior to flow-through. Values are means of six observations; the vertical bars indicate 2 S.E.M. C, control; G, glucagon; Try, tryptophan.

FIG. 2. Effects of glucagon on the chemical quantity, \(^{14}C\) content, and specific radioactivity of malate and aspartate in livers perfused with \[^{[4]}C\]lactate plus \[^{[14]}C\]pyruvate. Experimental conditions and numbers of observations were the same as in Fig. 1. Tryptophan (2.4 mM) was present in all experiments. C, control; G, glucagon.

are apparently due to their enhanced formation from endogenous substrates, e.g. glutamate and protein (see 11).

The possibility that an effect of glucagon on pyruvate carboxylation might have been discernible at shorter time periods was also examined in experiments in which livers were exposed to substrates and hormones for 2½ min in the absence or presence of tryptophan. The results obtained were very similar to those seen in the perfusions of longer duration. Tryptophan treatment markedly inhibited the labeling of glucose and P-pyruvate in control perfusions and abolished the increases induced by glucagon (Fig. 1). The inhibitor also increased the tissue levels of malate and aspartate and, in its presence, glucagon produced further large increases (Fig. 1). As expected, the radioactivities of malate and aspartate were increased in the tryptophan-treated livers (data not shown). In such livers, glucagon decreased malate radioactivity and did not alter aspartate radioactivity (Fig. 2). Consequently glucagon markedly decreased the specific radioactivities of malate and aspartate (Fig. 2). Thus, again, no evidence for increased pyruvate carboxylation was obtained, and the malate and aspartate which accumulated arose from unlabeled sources.

Effects of Glucagon on Labeling of Metabolic Intermediates in Livers Perfused for 1 Min with \(^{14}CO_{2}\)—A possible stimulatory effect of glucagon on pyruvate carboxylation was also tested for
in livers exposed for a short period (1 min) to H\(^{13}C\)O\(_3\), Table III shows that glucagon pretreatment for 10 min produced changes in the levels of metabolites which were consistent with increased conversion of 4-carbon dicarboxylic acids to P-pyruvate and aspartate. The labeling of intermediates in control livers was indicative of \(\frac{1}{2}\) bicarbonate fixation into a small pool of oxaloacetate which turned over extremely rapidly leading to labeling of malate, citrate, P-pyruvate, and other Krebs cycle intermediates during the 1-min time period (Table III).\(^1\) Glucagon produced marked changes in the labeling pattern. The radioactivities of \(\alpha\)-ketoglutarate, malate, oxaloacetate, citrate, and pyruvate were decreased, whereas those of succinate, fumarate, aspartate, pyruvate, and glucose were increased. These changes are similar to those observed with \(\frac{1}{2}\) malate plus \(\frac{1}{2}\) P-pyruvate (Table I) and with \(\frac{1}{2}\) glutamate (11) and are consistent with multiple effects of the hormone, namely, activation of the conversion of oxaloacetate to P-pyruvate and aspartate and of \(\alpha\)-ketoglutarate to succinate.

**Effects of Glucagon on Gluconeogenesis and Labeling of Metabolic Intermediates in Livers Perfused with \(\frac{1}{2}\) Aspartate—**Since the preceding results pointed to a possible effect of glucagon on P-enolpyruvate carboxykinase, this was tested more directly by using \(\frac{1}{2}\) aspartate, a substrate which could yield oxaloacetate in the cytosol without first participating in mitochondrial metabolism. Table IV shows the changes in livers from fasted rats perfused with lactate, pyruvate, and \(\frac{1}{2}\) aspartate and exposed to glucagon for 2\(\frac{1}{2}\) or 8\(\frac{1}{2}\) min. It is seen that the effects produced at 8\(\frac{1}{2}\) min were generally more striking than those seen at 2\(\frac{1}{2}\) min. The hormone increased the tissue concentration of P-pyruvate but had little effect on the other intermediates measured. It also reduced the radioactivities of aspartate, malate, oxaloacetate, and citrate and increased those of P-pyruvate and glucose. The specific radioactivities of malate and citrate were decreased.

Glucagon produced similar alterations in livers from fed rats perfused with \(\frac{1}{2}\) aspartate except that the increase in \(\frac{1}{2}\) glucose synthesis was more marked (4-fold) and there were no significant changes in the specific radioactivities of intermediates (data not shown). The data with this labeled amino acid are thus again consistent with an action of glucagon on P-pyruvate synthesis from oxaloacetate.

**DISCUSSION**

The present results provide several lines of evidence indicating that glucagon stimulates gluconeogenesis in part by activating P-pyruvate synthesis from oxaloacetate. First are the changes in the tissue concentrations of intermediary metabolites (Figs. 1 and 2, Tables I to IV) which are similar to those found in previous studies (6, 7, 11). These indicate a site of interaction between the 4-carbon dicarboxylic acids and P-pyruvate which is discernible at early time periods and is abolished by tryptophan (Fig. 1 and Reference 11). Second are the changes in the radioactivities of malate, citrate, oxaloacetate, and P-pyruvate in livers perfused with H\(^{13}C\)O\(_3\), \(\frac{1}{2}\) lactate plus \(\frac{1}{2}\) P-pyruvate, and \(\frac{1}{2}\) aspartate. These again indicate activation of the conversion of oxaloacetate to P-pyruvate (see Footnote 1). Third is the stimulatory effect of glucagon on P\(^{13}\)Cpyruvate and \(\frac{1}{2}\) glucose synthesis from \(\frac{1}{2}\) aspartate, a substrate which apparently yields \(\frac{1}{2}\) oxaloacetate directly in the cytosol.

Glucagon stimulation of gluconeogenesis from pyruvate or its precursors must involve increased pyruvate carboxylation.

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**Table III**

**Effect of glucagon on tissue levels and \(^{14}C\) content of intermediary metabolites in livers perfused with \(^{14}C\) bicarbonate**

| Chemical | Control | Glucagon | Control | Glucagon |
|----------|---------|----------|---------|----------|
|          | \(\mu\)mol/g | % control | \(\mu\)mol/g | \(\mu\)mol/g | % control |
| \(\alpha\)-Ketoglutarate | 378 | 31 | 15.7 | 13.9 | 42 | 41 |
| Succinate | 67 | 64 | 5.7 | 5.7 | 171 | 135 |
| Fumarate | 172 | 84 | 5.7 | 5.7 | 171 | 135 |
| Malate | 89 | 71 | 5.5 | 5.5 | 171 | 135 |
| Oxaloacetate | 2.3 | 2.5 | 46 | 46 |
| Aspartate | 588 | 398 | 158 | 127 | 83 | 83 |
| Citrate | 390 | 330 | 72 | 60 | 53 | 53 |
| P-pyruvate | 178 | 150 | 39.5 | 32.6 | 169 | 174 |
| Pyruvate | 136 | 81 | 3.7 | 3.6 | 46 | 46 |

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**Table IV**

**Effect of glucagon on tissue levels, \(^{14}C\) content, and specific radioactivity of gluconeogenic intermediates in livers from fasted rats perfused with \(^{14}C\) aspartate**

| Experimental series | Chemical amount | Radioactivity | Specific radioactivity |
|---------------------|-----------------|---------------|-----------------------|
|                     | Control | Glucagon | Control | Glucagon | Control | Glucagon |
|                     | \(\mu\)mol/g | % control | \(\mu\)mol/g | \(\mu\)mol/g | % control | \(\mu\)mol/g | \(\mu\)mol/g | % control | \(\mu\)mol/g | \(\mu\)mol/g | % control | \(\mu\)mol/g | \(\mu\)mol/g | % control |
| A (exposed to glucagon for 2\(\frac{1}{2}\) min) | Aspartate | 542 | 108 | 454 | 83 | 84 | 82 |
|                     | Malate | 75 | 60 | 75 | 60 | 87 | 87 | 86 |
|                     | Oxaloacetate | 3 | 4 | 3 | 4 | 3 | 4 |
|                     | Citrate | 488 | 80 | 114 | 56 | 29 | 70 |
|                     | P-pyruvate | 84 | 148 | 26 | 153 | 31 | 110 |
|                     | Glucose | 9000 | 128 | 2023 | 144 | 22 | 112 |
| B (exposed to glucagon for 8\(\frac{1}{2}\) min) | Aspartate | 508 | 90 | 608 | 88 | 102 | 92 |
|                     | Malate | 81 | 60 | 90 | 70 | 84 | 80 |
|                     | Oxaloacetate | 2 | 4 | 2 | 4 | 2 | 4 |
|                     | Citrate | 449 | 88 | 109 | 65 | 24 | 75 |
|                     | P-pyruvate | 122 | 177 | 53 | 159 | 43 | 115 |
|                     | Glucose | 8885 | 133 | 244 | 160 | 30 | 139 |
Since the tissue pyruvate concentration is decreased by the hormone (Table III and References 6 and 7), the stimulation must involve a mechanism such as activation of pyruvate carboxylase (6) or facilitation of mitochondrial pyruvate uptake (13). However, efforts to demonstrate effects of glucagon on pyruvate carboxylation were unsuccessful in the present study. When P-enolpyruvate carboxykinase was blocked by treatment with tryptophan, no effect of glucagon to increase the conversion of [14C]pyruvate to oxalacetate, aspartate, or malate was discerned at early or late time periods. There was also no indication that the hormone increased the total radioactivity in oxalacetate, malate, aspartate, and citrate in livers perfused with H14CO3 for 1 min.

It could be argued that the negative results in the tryptophan-blocked livers were due to the fact that the flow of substrate beyond oxalacetate was impeded. The increased levels of malate, aspartate, and citrate could conceivably have inhibited pyruvate carboxylation. However, studies with purified pyruvate carboxylase do not indicate that product inhibition is an important control mechanism for this enzyme (14, 15). Another explanation could be that stimulation of carboxylation is dependent on changes in the gluconeogenic pathway at or beyond P-enolpyruvate carboxykinase or on other changes resulting from increased flux through the gluconeogenic pathway. Many schemes can be developed which link pyruvate carboxylation with other gluconeogenic reactions, e.g. coupling pyruvate entry with movement of other metabolites across the mitochondrial membrane or linking changes in adenine nucleotides or CoA esters with alterations in pyruvate carboxylase activity (14–17).

As noted in our studies of glutamate metabolism in the perfused liver, the present findings indicate an effect of glucagon to increase the transamination of oxalacetate to aspartate and of pyruvate to alanine. The explanation for these changes is uncertain, but they could be secondary to the enhancement of α-ketoglutarate oxidation by glucagon (11). This increases the transamination of glutamate to α-ketoglutarate (11), and it is likely that oxalacetate and pyruvate serve as acceptors of amino groups.

Gluconon stimulation of gluconeogenesis from a physiological mixture of lactate and pyruvate, as in the present study, would require an increase in the mitochondrial efflux of aspartate rather than malate, since there is minimal need for hydrogen transfer into the cytosol. An increase in the transamination of glutamate relative to its oxidation would therefore be more consistent with the enhanced gluconeogenesis. Since GTP is an inhibitor of glutamate dehydrogenase (18), its increased production in the succinyl thiokinase reaction could be a factor in the apparent enhancement of glutamate transamination by glucagon.

Stimulation of P-pyruvate synthesis from oxalacetate was suggested as a mechanism for the gluconeogenic action of glucagon in 1965 (20). The effect could be due to an activation of P-enolpyruvate carboxykinase or to a stimulation of the efflux of aspartate, malate, or oxalacetate from the mitochondrion. Regarding the first mechanism, it has not been possible to observe any rapid increase in the activity of P-enolpyruvate carboxykinase in livers perfused with glucagon (21). In addition, no changes in the enzyme have been observed in cell-free extracts incubated with adenosine 3':5'-monophosphate under a wide variety of conditions (22). Thus, if activation of P-enolpyruvate carboxykinase occurs, it would appear to involve a mechanism different from phosphorylase activation.

It has been suggested that the efflux of dicarboxylic acids from mitochondria may be rate-limiting for gluconeogenesis in liver (2, 23). This would not be inconsistent with measurements of metabolite levels in livers perfused with concentrations of lactate which were saturating or supersaturating for gluconeogenesis (7). Facilitation at this site, if limiting, would lead to decreased overall levels of 4-carbon dicarboxylic acids since these would be rapidly metabolized in the cytosol. However, in the present study glucagon had little effect on the tissue concentration of malate and increased the level of aspartate. Furthermore, glucagon increased [14C]glucose formation from [14C]aspartate, a substrate which can yield oxalacetate directly in the cytosol.

Attention has been focused on the region of the gluconeogenic pathway between pyruvate and P-pyruvate in the present report since earlier studies indicated that this was the principal site at which glucagon stimulated gluconeogenesis from physiological substrates (5, 24). These studies showed, for example, that maximal glucose production from substrates entering the pathway at 2-P-glycerate or above (e.g. fructose and dihydroxyacetone) was much greater than that from lactate, pyruvate, or amino acids and was not increased by glucagon (5, 24). More recently, however, Veneziale has found that glucagon can produce a large stimulation of glucose production from fructose in the perfused liver if substratting concentrations of the hexose are employed, and that the stimulation is not diminished by prior perfusion with quinolinate (25). Although the possibility remains that the quinolinate may not have completely blocked gluconeogenesis from lactate derived from fructose (see Footnote 6), these data suggest that glucagon may exert an additional effect on a reaction located at the upper end of the gluconeogenic pathway or involved in the metabolism of fructose prior to entry into the gluconeogenic pathway (see Footnote 3 in Reference 7). The former location seems more likely since many studies of the effects of glucagon on the perfused liver have shown metabolite changes consistent with activation of the conversion of triose-P to glucose-6-P (6, 7, 26). The mechanism(s) by which glucagon affects this portion of the gluconeogenic pathway and the possible physiological importance of such an effect(s) remain to be clarified.

1 M. Ui and T. H. Claus, unpublished observations.
2 Veneziale has also raised the intriguing possibility that pyruvate may be converted to 2-P-glyceraldehyde in the liver by an unknown pathway which is not sensitive to inhibition by quinolinate (27, 28). This conclusion was based on the findings that quinolinate inhibited [14C]glucose synthesis by only 40% in livers perfused with 2 mM [14C]pyruvate or H14CO3 plus 2 mM pyruvate, and that the specific radioactivities of P-pyruvate and 3-P-glycerate in such livers were always much higher than that of aspartate and often higher than that of malate. These results differ substantially from those found in the present study which used 1 mM lactate plus 0.1 mM pyruvate as substrates and tryptophan rather than quinolinate to block P-enolpyruvate carboxykinase. As shown in Fig. 1, treatment of livers with tryptophan reduced [14C]glucose synthesis from [U-14C]lactate plus [2-14C]pyruvate by 94%. Furthermore, in livers perfused with these two labeled substrates
As discussed in greater detail in the accompanying paper, the effect of glucagon on the conversion of α-ketoglutarate to succinate could stimulate GTP production thereby increasing the activity of P-enolpyruvate carboxykinase. However, as noted before, the two enzymes are separated in rat liver by the mitochondrial membranes. GTP apparently does not cross the inner mitochondrial membrane as such but reacts with ADP to yield ATP, which leaves the mitochondrion and can react with GDP in the cytosol to yield GTP. Thus, unless these reactions are compartmentalized, the linkage between succinyl thiokinase and P-enolpyruvate carboxykinase would be lost. Another problem arising from linkage of these enzymes relates to the fact that the coupling would require two turns of the cycle per glucose molecule synthesized. This is not consistent with the relatively small increase in O2 consumption produced by glucagon in livers perfused with lactate (29) and would, in any case, generate 4 times as much ATP as would be needed to support the increased gluconeogenesis.

It thus appears that any relationships between the effects of glucagon on the Krebs cycle and on gluconeogenesis are unlikely to be stoichiometric. The possibility remains that the alterations in the levels of metabolites or coenzymes resulting from the effect of glucagon on the Krebs cycle affect gluconeogenesis because some of these compounds are allosteric effectors of gluconeogenic enzymes.

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