The Development and Application of a Radioimmunoassay for Rat Phosphoenolpyruvate Carboxykinase*

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We employed a newly developed radioimmunoassay to quantitate P-enolpyruvate carboxykinase protein directly in liver and kidney of intact rats. The radioimmunoassay was dependent upon the presence of sodium dodecyl sulfate in the competitive binding reaction mixture. In conjunction with assayable activity measurements, the radioimmunoassay results also made it possible to assess the average specific activity of the enzyme. In the fed state, liver P-enolpyruvate carboxykinase was 0.89 μM (micromoles/kg of tissue) and the kidney enzyme was 1.90 μM. Following a 48-h fast, the enzyme in liver increased to 2.83 μM and that in kidney to 6.83 μM. Although liver enzyme concentration increased 3-fold, total liver enzymic activity was increased only about 30%. The discrepancy was due to the decrease in liver weight which occurred during fasting. The kidneys, which do not lose weight during fasting, had a 65% elevation of total organ activity. Total organ enzyme activity was predominantly dependent on changes in enzyme mass. In the fed state, the kidney/liver enzyme mass ratio was 0.44, in the fasted state it was 0.68. Variation in enzyme concentration, enzyme half-life, and response to tryptophan and chronic triamcinolone administration all gave evidence for organ-specific regulation.

P-enolpyruvate carboxykinase is generally believed to catalyze the rate-limiting reaction of gluconeogenesis from three carbon precursors. Augmented enzyme activity contributes to the maintenance of plasma glucose in starvation, in multiple hormonal and nutritional states, and in diabetes mellitus (1-4). Liver and kidney P-enolpyruvate carboxykinase activities vary over a wide range in response to regulatory influences. Others have employed immunoprecipitation and immunoinvolvement techniques and in vitro mRNA translation systems to conclude that P-enolpyruvate carboxykinase activity is regulated by changes in enzyme mass (for reviews, see Refs. 5-7). Exactly how P-enolpyruvate carboxykinase activity is changed has not until now been documented by direct measurement of enzyme protein concentration. In particular, it is crucial to establish the magnitude of concentration change which can occur intracellularly, the contribution of that change to overall activity, and even whether change in the specific activity of the enzyme might also occur.

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MATERIALS AND METHODS

Animals
Male Sprague-Dawley rats were maintained on a standard Purina diet for at least 2 weeks before all experiments. Lighting was on from 8 a.m. to 8 p.m. and off between 8 p.m. and 8 a.m. Water was provided at all times. When animals were fasted, food was removed 48 to 52 h before they were killed.

Triamcinolone diacetate (40 mg/ml in a long acting suspension) was injected into leg muscle. Tryptophan, suspended to 50 mg/ml in 0.9% NaCl solution, was sonicated with an immersed probe (Biosonik III) and further sonicated in the water bath of a Bransonic 12 sonicator just prior to intraperitoneal injection. Animals were stunned and killed by decapitation. See the tables for further details.

Cytosol Preparations
Livers and kidneys were excised and placed in 0.9% NaCl solution at 4 °C. Organs were suspended in four volumes of buffer (10 mM triethanolamine, pH 7.5, 0.25 mM sucrose, 1 mM EDTA, 1 mM dithiothreitol) and dispersed with a polytron (Brinkmann Instruments) operated 10 to 15 s at maximum speed at 4 °C. Both kidneys from each animal were homogenized together. The homogenate was centrifuged at 4 °C at 110,000 × g for 1 h to yield a cytosolic fraction that was used for enzyme assays and RIAs.

Storage at −20 °C for up to 1 month did not result in a decrease in enzyme activity.

Enzyme Purification
P-enolpyruvate carboxykinase was purified according to Colombo et al. (8). Enzyme, further purified by affinity chromatography on GTP-Sepharose, exhibited maximal specific activity. If stored for prolonged periods of time at 4 °C the gradual appearance of a less active form of the enzyme was noted.

Enzyme activity was monitored by the decrease in A254 in a Gilford spectrophotometer. NaHCO3 was added to 50 mM, and the new rate of NADH utilization due to P-enolpyruvate carboxykinase was monitored. The OAA produced by P-enolpyruvate carboxykinase was quantitatively reduced with NADH by malate dehydrogenase in the coupled enzyme reaction. The rate of utilization of NADH over background was employed to calculate P-enolpyruvate carboxykinase activity (1 unit = 1 μmol of OAA formed/min at 25 °C).

Enzyme Activity Measurements
P-enolpyruvate to OAA—25 to 100 μl of liver or kidney high speed supernatant were assayed at 25 °C in a final volume of 2.0 ml containing 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.2, 1 mM DTP, 2 mM MnCl2, 1 mM dithiothreitol, 0.25 mM NADH, 2 mM P-enolpyruvate, and 10 μg of malate dehydrogenase. Background utilization of NADH was monitored by the decrease in A340 in a Gilford spectrophotometer. NaHCO3 was added to 50 mM, and the new rate of NADH utilization due to P-enolpyruvate carboxykinase was monitored. The OAA produced by P-enolpyruvate carboxykinase was quantitatively reduced with NADH by malate dehydrogenase in the coupled enzyme reaction. The rate of utilization of NADH over background was employed to calculate P-enolpyruvate carboxykinase activity (1 unit = 1 μmol of OAA formed/min at 25 °C).

OAA to P-enolpyruvate—25 to 200 μl of high speed supernatant was assayed in a 1-ml final volume containing 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.5, 2 mM ITP, 3 mM MgCl2, and 1.5 mM OAA. The reaction was begun by the addition of OAA. P-enolpyruvate formation was allowed to proceed for 5 to 10 min.
Regulation of P-enolpyruvate Carboxykinase in Liver and Kidney

At 25°C, 10-15 mg of KBH₄ were added to each assay mixture to terminate the reaction. Tubes were chilled on ice, and after 2-4 min 0.4 ml of 6% (w/v) perchloric acid was added. After 2-4 min on ice, 5 μl of 0.05% methyl orange was added and the solution neutralized with 30% KOH. Precipitates were removed by centrifugation at 2000 rpm for 10 min at room temperature in a Beckman TJ-6 centrifuge. P-enolpyruvate in the supernatant fraction was quantitated in a reaction coupled to NADH oxidation.

**125I Labeling of P-enolpyruvate Carboxykinase**

10 μg of P-enolpyruvate carboxykinase in 50 μl of buffer composed of 10 mM N-(tris(hydroxymethyl)methyl-2-amino)ethanesulfonic acid, pH 7.2, 0.10 mM EDTA, 1 mM GSH, 0.12 mM KCl, and 10% (v/v) glycerol, was labeled by the chloramine-T procedure (9, 10). Approximately 1 to 5 × 10⁶ atoms were introduced for every 10 enzyme molecules. It should be noted that labeling P-enolpyruvate carboxykinase with ²²⁵T-TagIT (3-(4-hydroxphenyl) propionic acid N-hydroxysuccinimide ester) as employed previously in this laboratory for other proteins (11-17) always yielded multiple enzyme fragments of P-enolpyruvate carboxykinase that were no longer precipitable with antibody.

P-enolpyruvate carboxykinase contains 13 -SH groups that are labile to oxidation (8). After labeling, the enzyme was diluted with a stabilizing buffer consisting of 50 mM Na(H)PO₄, pH 7.5, 0.1 mM EDTA, 0.1 mM β-mercaptoethanol, 0.1 mM KCl, 10% (v/v) glycerol, and 0.02% NaN₃. The enzyme was chromatographed through a P-10 column equilibrated with stabilizing buffer. ²²⁵I-P-enolpyruvate carboxykinase, free from soluble ²²⁵I and labeling reagents, was then passed through a G-150 column equilibrated with stabilizing buffer at 4°C. The enzyme freed from fragments and aggregates was collected and stored at 4°C. Radiolabeled enzyme could be used in RIAs for 4 to 6 weeks.

**Antibody Preparation and Characterization**

γ-Globulin against rat liver P-enolpyruvate carboxykinase was raised in goats by procedures previously described (11-17). The antiserum was demonstrated to be specific for P-enolpyruvate carboxykinase by Ouchterlony double diffusion, and by immunoelectrophoresis. The antiserum was tested for its ability to precipitate standard P-enolpyruvate carboxykinase as well as P-enolpyruvate carboxykinase in tissue extracts (16, 17).

**RIAs: The Generation of Standard Competitive Binding Curves**

Assays were conducted in a final volume of 1 ml containing 50 mM K(H)PO₄, pH 7.5, 1% ovalbumin, 0.1% SDS, 0.02% NaN₃. The assay tubes contained increasing quantities (0 to 80 ng) of standard P-enolpyruvate carboxykinase, a constant amount of antiserum, and a constant amount of ²²⁵I-P-enolpyruvate carboxykinase. All reaction mixtures were made in duplicate or triplicate and maintained at 4°C for 24 h. The amount of ²²⁵I-P-enolpyruvate carboxykinase in 2 ng containing 20,000 cpm. The amount of antiserum was 50 μl of a 1:2,000 dilution of antiserum. This amount of antiserum was sufficient to precipitate 40 to 50% of the labeled enzyme.

After the first stage incubation, we added 25 μl of a 1:50 dilution of goat normal serum and 25 μl of rabbit anti-goat serum to each reaction mixture, which was then incubated at 4°C for 24 h. We then counted the total radioactivity and pellet radioactivity after centrifugation and aspiration of the supernatants. The data were plotted on log-logit paper and analyzed by means of a computer program described previously (16, 17).

**Unknown Cytosol Samples**

These were assayed as described above, except that in place of standard enzyme we used varying volumes of 1:10 and 1:100 dilutions of high speed liver and kidney supernatant fractions. The expression of results is described in the legends of appropriate tables and figures.

**RESULTS**

**Inhibition of Enzyme Activity by Antiserum.—**Antibody to liver P-enolpyruvate carboxykinase was shown to inhibit the activity of the purified enzyme and of the enzyme in liver and kidney extracts. Conditions of these experiments were set up so that 100 μl of antiserum removed (precipitated) all the enzyme activity in 100 μl of kidney or liver extracts.

**Immunoelectrophoretic and Double Diffusion Studies—**

Immunoelectrophoretic analyses showed that purified P-enolpyruvate carboxykinase and the enzyme in liver/kidney cytosol have identical electrophoretic mobilities. The identity of these enzymes was further confirmed by their formation of continuous precipitin lines in agar diffusion analyses.

**Behavior of ²²⁵I-P-enolpyruvate Carboxykinase Labeled by the Chloramine-T Method—**Chromatography on Sephadex G-150 gave a single sharp peak which corresponded to the elution position of standard enzyme (Fig. 1). Electrophoresis

**FIG. 1. Sephadex G-150 chromatography of ²²⁵I-P-enolpyruvate carboxykinase.** Enzyme had been prechromatographed on a Bio-Gel P-10 column. Fractions 45-55 (1.0 ml each) were combined for use in the RIA. 

**FIG. 2. Electrophoresis of ²²⁵I-P-enolpyruvate carboxykinase.** 50 μl of purified labeled enzyme (approximately 50,000 cpm containing 3-5 ng of enzyme) in 8% urea and 1% SDS and was heated for 5 min in a boiling water bath. Electrophoresis was in 5.6% polyacrylamide-SDS gels. The gels were frozen, sectioned, and then counted in a Beckman γ-counter at 50% efficiency. PEPCK, P-enolpyruvate carboxykinase.

**FIG. 3. SDS gel electrophoresis of ²²⁵I-P-enolpyruvate carboxykinase.**

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*Note: The above text is a transcription of the image and may contain some formatting or layout issues due to the nature of the document.*
of $^{125}$I-P-enolpyruvate carboxykinase on an SDS-polyacrylamide gel gave a single peak also corresponding to unlabeled enzyme (Fig. 2). The use of lactoperoxidase and Bolton-Hunter procedures was totally unsatisfactory for labeling the enzyme. The former was an ineffective labeling procedure in our hands, and the latter produced fragmented labeled products.

**RIA Development**—We found that antibody reactivity was much greater with $^{125}$I-P-enolpyruvate carboxykinase than with unlabeled P-enolpyruvate carboxykinase. However, in the absence of SDS, antibody reactivity was comparable for both antigens. Fig. 3 shows that without SDS (Fig. 3A), standard enzyme could not compete with $^{125}$I-P-enolpyruvate carboxykinase for antibody. The presence of only 0.1% SDS made unlabeled antigen highly competitive. We showed that stored P-enolpyruvate carboxykinase preparations, which had spontaneously fragmented into two polypeptides of 50,000 and 22,000 daltons, competed moderately well (Fig. 3B) in the absence of SDS. In separate experiments we varied the procedure by adding standard P-enolpyruvate carboxykinase in the RIA 24 h before addition of $^{125}$I-P-enolpyruvate carboxy-

**Competitive Binding Assays**

![Graph](https://via.placeholder.com/150)

*Fig. 3. Competitive binding assays.* A, standard (native) enzyme competes simultaneously with $^{125}$I-P-enolpyruvate carboxykinase for antibody binding only if SDS is present. B, partially degraded enzyme (two fragments) competes simultaneously with $^{125}$I-P-enolpyruvate carboxykinase for antibody binding even in the absence of SDS. C, standard enzyme when preincubated for 24 h with antiserum before the addition of $^{125}$I-enzyme leads to the generation of a standard competitive curve. Scattering of the data points occurs. PEPCK, P-enolpyruvate carboxykinase.

**Effect of SDS on Antigen-antibody Reaction**

![Graph](https://via.placeholder.com/150)

*Fig. 4. Effect of SDS on antigen-antibody reaction.* A fixed amount of $^{125}$I-P-enolpyruvate carboxykinase (50 μl containing 20,000 cpm) was reacted with increasing volumes of antiserum. Final reaction volumes were 1.0 ml in egg albumin containing buffer. Incubation time for the first step was 48 h at 4 °C, after which second stage antibody was added and incubation continued overnight. After centrifugation, supernatants were aspirated and the counts/min in the antigen-antibody complexes determined. PEPCK, P-enolpyruvate carboxykinase.

**Effects of SDS Concentration on Antigen-Antibody Reaction**

The ability of antibody to precipitate $^{125}$I-P-enolpyruvate carboxykinase was tested at different concentrations of SDS (Fig. 4). 0.1% SDS (w/v) had virtually no adverse effect and was included in all assays.

**Effect of Cytosol on Standard Competitive Binding**

![Graph](https://via.placeholder.com/150)

*Fig. 5. Effect of liver extract on the standard competitive binding curve.* Control tubes contained no tissue extract. To assess the effect of added liver extract on the extent of competition, of unlabeled enzyme for $^{125}$I-enzyme antibody binding sites, we added 75 μl of a 1:100 dilution of liver cytosol per reaction mixture. The effect was to shift the competition curve to the left as expected due to the addition of liver P-enolpyruvate carboxykinase (PEPCK) to standard enzyme. The addition of tissue extracts which lack P-enolpyruvate carboxykinase did not shift the position of the standard curve, demonstrating that cytosol alone caused no interference.
Fed and fasted Sprague-Dawley rats were used. Enzyme protein concentration (milligrams/1000 g of tissue, wet weight) was determined by RIA. Assayable activity measurements reflect the rates of conversion of OAA to P-enolpyruvate. Units/mg of P-enolpyruvate carboxykinase represents the enzyme activity specific based on a molecular weight of 72,000. Total liver enzyme (nanomoles/liver) and total liver activity (units/liver) were obtained by multiplying liver weight by the concentration and units/g of liver, respectively. Data are presented as mean ± S.E. (n). Statistical analysis was based on comparison with fed rats by using Student’s t test. Fed animals (first row data) and their livers weighed 242 ± 14 g (7) and 11.20 ± 0.80 g (8), respectively. 48-h fasted animals and their livers weighed 185 ± 6 g (12) and 6.5 ± 0.20 g (12), respectively. Five rats were fed Purina Chow ad libitum and injected intramuscularly with 4 mg of triamcinolone three times weekly for 2 weeks (third row data). They were killed on the 3rd day after the last injection. Animal weights, which remained unchanged throughout the 2-week period, were 124 ± 6 g at 3 days of the fasted state. Livers weighed 6.90 ± 0.33 g.

### Table I
Liver P-enolpyruvate carboxykinase

<table>
<thead>
<tr>
<th>Condition</th>
<th>Concentration</th>
<th>Enzyme</th>
<th>Units/g liver</th>
<th>Units/liver</th>
<th>Units/nmol enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>0.89 ± 0.12</td>
<td>9.56 ± 1.31</td>
<td>0.32 ± 0.05</td>
<td>3.81 ± 0.55</td>
<td>0.37 ± 0.03</td>
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<td></td>
<td>(7)</td>
<td>(7)</td>
<td>(6)</td>
<td>(6)</td>
<td>(5)</td>
</tr>
<tr>
<td>Fasted</td>
<td>2.83 ± 0.28</td>
<td>18.95 ± 2.60</td>
<td>0.78 ± 0.05</td>
<td>4.87 ± 0.27</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
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<tr>
<td><em>p = 0.0001</em></td>
<td><em>p = 0.0179</em></td>
<td><em>p = 0.0001</em></td>
<td><em>NS</em></td>
<td><em>p = 0.0479</em></td>
<td></td>
</tr>
<tr>
<td>Fed plus hypercortisonism</td>
<td>2.76 ± 0.15</td>
<td>18.96 ± 1.01</td>
<td>0.88 ± 0.03</td>
<td>6.98 ± 0.31</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
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</tr>
<tr>
<td><em>p &lt; 0.0001</em></td>
<td><em>p &lt; 0.0004</em></td>
<td><em>p &lt; 0.0001</em></td>
<td><em>p &lt; 0.0080</em></td>
<td><em>NS</em></td>
<td></td>
</tr>
</tbody>
</table>

*The mean nanomoles/liver for these six animals was 16.98.

### Table II
Kidney P-enolpyruvate carboxykinase

<table>
<thead>
<tr>
<th>Condition</th>
<th>Concentration</th>
<th>Enzyme</th>
<th>Units/g kidney</th>
<th>Units/kidney pair</th>
<th>Units/nmol enzyme</th>
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</thead>
<tbody>
<tr>
<td>Fed</td>
<td>1.90 ± 0.26</td>
<td>4.16 ± 0.62</td>
<td>0.77 ± 0.05</td>
<td>1.75 ± 0.14</td>
<td>0.48 ± 0.07</td>
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<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>Fasted</td>
<td>6.83 ± 0.73</td>
<td>12.87 ± 1.52</td>
<td>1.65 ± 0.05</td>
<td>2.90 ± 0.05*</td>
<td>0.30 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p = 0.0001</em></td>
<td><em>p = 0.0001</em></td>
<td><em>p &lt; 0.0001</em></td>
<td><em>p &lt; 0.0001</em></td>
<td><em>p = 0.0496</em></td>
<td></td>
</tr>
<tr>
<td>Fed plus hypercortisonism</td>
<td>2.20 ± 0.21</td>
<td>3.61 ± 0.22</td>
<td>0.87 ± 0.45</td>
<td>1.56 ± 0.07</td>
<td>0.40 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
<td>(5)</td>
</tr>
<tr>
<td><em>NS</em></td>
<td><em>NS</em></td>
<td><em>NS</em></td>
<td><em>NS</em></td>
<td><em>NS</em></td>
<td></td>
</tr>
</tbody>
</table>

*The mean nanomoles/kidney pair for these six animals was 10.70.

*NS, not significant.

### Curve
Liver cytosol appropriately displaced the standard curve to the left consistent with the presence of endogenous enzyme (Fig. 5). We found the same result when kidney extract was used. Addition of cytosol lacking P-enolpyruvate carboxykinase did not interfere with the quantitation of standard enzyme. Thus, no cytosolic substances appear to interfere under the conditions of the RIA. We routinely assayed a standard liver extract with every batch of assays conducted. Only RIAs which correctly measured the standard were acceptable.

**Application of RIA**—In the liver of fed rats, P-enolpyruvate carboxykinase concentration was 0.89 μM (Table I), which increased to 2.83 μM after a 48-h fast. Liver enzyme activity per g of tissue increased greatly in the fasted state. Total liver P-enolpyruvate carboxykinase activity increased nearly 30%, but because of some scatter of the data the p value was greater than 0.05.

In kidney, P-enolpyruvate carboxykinase concentration increased more than 3-fold (Table II). There was no loss of kidney weight, and the total activity of paired kidneys was significantly elevated during fasting. P-enolpyruvate carboxykinase specific activity was higher in the direction P-enolpyruvate to OAA than in the direction OAA to P-enolpyruvate (compare Tables I-III). Based on assays in either direction, fasting tended to decrease the specific activity slightly in both liver and kidney (Tables I-III). Although consistently observed, the decrease was small and probably not significant.

**Effects of Triamcinolone**—Hyperglucocorticoidism was brought about by repeated injections of triamcinolone over a 2-week period. Liver P-enolpyruvate carboxykinase concentration and activity increased (Table I). The same multiple injection program did not give that response in kidney (Table II), although a single injection may have an effect (6).

**Effects of Tryptophan**—Following tryptophan administra-

### Table III
Activity in reverse direction

<table>
<thead>
<tr>
<th>Condition</th>
<th>Units/g liver</th>
<th>Units/liver</th>
<th>Units/nmol enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>0.76 ± 0.07</td>
<td>9.08 ± 1.06</td>
<td>1.01 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(6)</td>
<td>(5)</td>
</tr>
<tr>
<td>Fasted</td>
<td>2.06 ± 0.07</td>
<td>13.58 ± 0.88</td>
<td>0.79 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>(12)</td>
<td>(12)</td>
<td>(12)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>p &lt; 0.0001</em></td>
<td><em>p = 0.0071</em></td>
<td><em>NS</em></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>1.78 ± 0.12</td>
<td>3.88 ± 0.35</td>
<td>1.07 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>Fasted</td>
<td>5.21 ± 0.21</td>
<td>9.77 ± 0.57</td>
<td>0.84 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>p &lt; 0.0001</em></td>
<td><em>p &lt; 0.0001</em></td>
<td><em>NS</em></td>
<td></td>
</tr>
</tbody>
</table>

*NS, not significant.
Regulation of P-enolpyruvate Carboxykinase in Liver and Kidney

Six rats were injected intraperitoneally with a tryptophan suspension (50 mg/ml of 0.9% NaCl solution, 75 mg injected/100 g of body weight) at 46 and 49 h during the course of a 52-h fast. The rats were killed at 52 h. For details and controls see Tables I-III. Animals weighed 202 ± 8 g. Liver weights were 7.16 ± 0.50 g, and kidneys 2.03 ± 0.10 g.

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>Enzyme Specific Activity</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.38 ± 0.13</td>
<td>24.11 ± 2.07</td>
<td>NS</td>
</tr>
<tr>
<td>6.73 ± 0.09</td>
<td>13.82 ± 1.61</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.94 ± 0.27</td>
<td>10.12 ± 1.02</td>
<td>NS</td>
</tr>
<tr>
<td>6.73 ± 0.49</td>
<td>13.82 ± 1.61</td>
<td>+62</td>
</tr>
</tbody>
</table>

Indicates activity measured in forward direction. See Table I for liver controls and Table II for kidney controls.

Fig. 6. Enzyme response to refeeding. Twenty-two rats were fasted for 48 h; 10 were then killed to provide zero time data. The remaining 12 were provided with Purina Chow. At 4, 6, 8, and 12 h of refeeding, three animals per time point were killed: liver and kidney cytosols were prepared and assayed for P-enolpyruvate carboxykinase (PEPCK) protein concentration by RIA. Eight rats were fed ad libitum for 3 weeks and were then killed to provide fed control P-enolpyruvate carboxykinase concentration values. The values (hours, micromolar P-enolpyruvate carboxykinase) for kidney were: 0, 6.83; 4, 1.98; 6, 1.84; 8, 1.43; and 12, 0.99. The control value of 0.89 μM P-enolpyruvate carboxykinase for liver of fed rats (Table I) was subtracted at each time point and the resulting coordinates plotted. The plots indicate first order decay of enzyme concentration with time. The values (hours, micromolar P-enolpyruvate carboxykinase) for liver (three animals killed per time point) were: 0, 2.83; 4, 1.98; 6, 1.84; 8, 1.43; and 12, 0.99. The control value of 0.89 μM P-enolpyruvate carboxykinase for liver of fed rats (Table I) was subtracted at each time point and the resulting coordinates plotted. The plots indicate first order decay of enzyme concentration with time.
ties of extracts of muscle which does not contain the enzyme to RIA reaction mixtures containing liver or kidney extracts, we could still measure the enzyme correctly. Even when undiluted muscle supernatant was present, the RIA measured the kidney or liver enzyme with normal precision and accuracy. Furthermore, the addition of kidney extract to the assay for liver enzyme, and vice versa, failed to interfere. Documentation for the validity of the RIA rests also on the fact that enzyme protein concentration followed activity measurements pari passu. Documentation convincingly rests on agreement between the specific activity of the enzyme in tissue extracts (based on RIA and assayable activity measurements) and the specific activity of homogeneously purified enzyme which is free of putative interfering factors.

The measurement of enzyme mass by RIA in conjunction with standard activity measurements allowed us to calculate units/nmol of P-enolpyruvate carboxykinase, which we found to be virtually identical with that reported for homogeneously purified enzyme (8, 19–21). 16–18 units/mg based on activity in the backward direction has been reported. We found values of 1.0–1.3 units/nmol (Tables III and IV), which converts to 14–18 units/mg. Assays in the forward direction were conducted in the absence of divalent transition metal salts and are, therefore, lower. Furthermore, our measurements show that the enzyme is capable of producing more than adequate amounts of P-enolpyruvate to meet the animals’ requirement for gluconeogenesis. Extrapolating to a human liver weight of 1500 g and paired kidneys of 340 g, one will conclude that the fasting activity data (0.78 and 1.65 units/mg, respectively) would indicate glucose production of 152 g + 73 g/24 h. Even at 25 °C, the rates so measured would more than meet the requirements of the central nervous system and red blood cell for glucose.

Much evidence exists that P-enolpyruvate carboxykinase concentration is able to be modulated to account for the observed changes in activity. Alterations in the rate of P-enolpyruvate carboxykinase synthesis occur (2). Others have concluded that large fluxes in the rate of synthesis account for the changes in P-enolpyruvate carboxykinase concentration. Other regulatory mechanisms, such as alternative splicing, may also play a role in modulating P-enolpyruvate carboxykinase activity.

REFERENCES


Regulation of P-enolpyruvate Carboxykinase in Liver and Kidney