The Regulation of Phosphoenolpyruvate Carboxykinase (GTP) Synthesis in Rat Kidney Cortex

THE ROLE OF ACID-BASE BALANCE AND GLUCOCORTICOIDS*

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The effects of metabolic acidosis and of hormones on the activity, synthesis, and degradation of renal cytosolic P-enolpyruvate carboxykinase (GTP) (EC 4.1.1.32) were studied in the rat using isotopic-immunochemical procedures. At normal acid-base balance, the synthesis of the enzyme accounted for between 2 and 3.5% of the synthesis of all soluble protein in the kidney cortex. P-enolpyruvate carboxykinase synthesis was selectively stimulated in acute metabolic acidosis, so that the relative rate of synthesis of the enzyme was increased to 7% 13 hours after oral administration of ammonium chloride. The stimulation of P-enolpyruvate carboxykinase synthesis preceded any increase in the assayable activity of the enzyme. The administration of sodium bicarbonate to acutely acidic rats returned the rate of enzyme synthesis to normal in 8 hours. The effect of acidosis on both the synthesis and the activity of P-enolpyruvate carboxykinase was prevented by actinomycin D, cordycepin, and cycloheximide. The degradation in vivo of pulse-labeled P-enolpyruvate carboxykinase was not affected by acidosis. Thus, the stimulation of P-enolpyruvate carboxykinase synthesis is the major mechanism for the increase in the level of the enzyme observed in metabolic acidosis.

The administration of the glucocorticoid triamcinolone resulted in an increase in the relative rate of P-enolpyruvate carboxykinase synthesis and a commensurate increase in the activity of the enzyme in the renal cortex. Both changes were abolished by actinomycin D. Fasting was characterized by a high enzyme activity and a rapid rate of enzyme synthesis in the kidney cortex. This high rate of synthesis was reduced after the administration of sodium bicarbonate, but not after glucose feeding. Moreover, the injection of insulin to diabetic rats did not repress P-enolpyruvate carboxykinase synthesis in the renal cortex. Theophylline plus N6,02'-dibutyryl adenosine 3':5'-monophosphate stimulated P-enolpyruvate carboxykinase synthesis in the kidney of intact rats. However, the latter effect was probably due to glucocorticoid secretion, since it did not occur in adrenalectomized animals. The administration of parathyroid extracts did not result in the induction of the enzyme. Thus, the hormonal regulation of cytosolic P-enolpyruvate carboxykinase synthesis in the kidney differs markedly from that in the liver.

The regulation of renal gluconeogenesis has received increasing attention since Goodman et al. (1) reported in 1966 that the process is stimulated by metabolic acidosis. This initial observation suggested a functional link between the increased gluconeogenic flux and the enhanced level of ammonia production which provides a buffer for the excretion of excess protons by the acidic kidney (2). Since then, evidence has been presented that the stimulation of renal gluconeogenesis characteristic of acidosis is primarily due to an increase in the activity of cytosolic P-enolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (3, 4) and interest has focused on the control of the level of this enzyme in the kidney cortex. It is known that, in addition to metabolic acidosis induced by ammonium chloride feeding, glucocorticoid administration (5), starvation (5), and diabetes (6) also increase the activity of the renal enzyme. To date, however, only limited and largely indirect information has been available on the mechanism underlying the observed changes in P-enolpyruvate carboxykinase activity.

Flores and Alleyne (7) reported that both actinomycin D and ethionine were ineffective in blocking the increase in renal P-enolpyruvate carboxykinase activity during acidosis, and they suggested that de novo enzyme synthesis was not involved in this condition. In a more detailed study, Longshaw and Pogson (8) found an increased level of enzyme protein in the kidney after treatment with acid, but were also unable to
inhibit significantly the increase in enzyme activity with actinomycin D. These authors proposed that the accumulation of P-enolpyruvate carboxykinase without stimulation of enzyme synthesis could be due to a prolongation in the half-life of the enzyme or to the conversion of a precursor into active enzyme (9). They reported, moreover, that the apparent molecular weight of the enzyme in acidic kidney was higher than in normal tissue (8). In contrast with the observations in acidosis, the increase in renal P-enolpyruvate carboxykinase activity caused by glucocorticoids is readily suppressed by actinomycin D (7, 8), which suggests a requirement for new protein synthesis. Also, extensive immunochemical studies of the regulation of the enzyme in the liver have indicated that most physiologically induced increases in hepatic P-enolpyruvate carboxykinase activity are accounted for by changes in the synthesis rate of the enzyme (10–12).

The experiments to be described are an attempt to delineate more clearly the mechanisms which control the level of cytosolic P-enolpyruvate carboxykinase activity in the rat kidney. Using specific antibodies against the enzyme, we have studied the effects of acid base alterations and glucocorticoid treatment on the synthesis and degradation of the enzyme. The role of insulin and cyclic AMP in the regulation of the renal enzyme has also been examined.

**Materials and Methods**

Biochemicals and Drugs—NADH, IDP, potassium P-enolpyruvate, and malate dehydrogenase were purchased from Boehringer Mannheim Corp. (New York, N. Y.); triacminolone acetonide from E. R. Squibb and Sons, Inc. (New York, N. Y.); actinomycin D, cordycepin, cycloheximide, l-tyrosine, theophylline, 0.02% (w/v) adenosine cyclic 3':5'-monophosphoric acid, and parathyroid trichloroacetic acid powder (100 to 300 U.S. units/kg) from Sigma Chemical Co. (St. Louis, Mo.). Alloxan was from Eastman Kodak Co. (Rochester, N. Y.). Crystalline porcine insulin was a generous gift from Dr. Walter Shaw of the Eli Lilly Laboratories (Indianapolis, Ind.). Sodium 14C bicarbonate (2 to 10 mCi/mmol) and L-[4,5-3H]leucine (30 to 50 Ci/mmol) were purchased from New England Nuclear (Boston, Mass.). Triton X 105 was kindly donated by Rohm and Haas (Philadelphia, Pa.). NCS tissue solubilizer was purchased from Amersham/Searle (Arlington Heights, III.).

**Animals**—Male Sprague-Dawley rats (from Charles River Breeding Laboratories, Wilmington, Mass.) weighing between 180 and 240 g (7 to 8 weeks old) were used throughout these experiments. The animals were housed in individual cages in 100-cm (2.00 p.m.) and temperature (20°C) regulated quarters, and they had permanent access to water and commercial laboratory chow (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.) unless otherwise specified. Thyroparathyroidectomized and hypophysectomized rats were purchased from Charles River. The former were maintained on 1% (w/v) calcium chloride in the latter on 5% (w/v) glucose as source of fluid. Adrenalectomy was performed under ether narcosis; after the operation the animals received 1% (w/v) sodium chloride as fluid. All experiments were commenced at 8:00 a.m.

Acute metabolic acidosis was induced by tube feeding the animals 10 m mol of ammonium chloride/kg of body weight intraperitoneally 45 min after the radioactive leucine. In some experiments, the animals were given 1% (w/v) sodium bicarbonate before the leucine “chase” to block protein synthesis. The animals were killed 9 hours later, and the amount of radioactive enzyme remaining was determined by the immunochemical procedures outlined above.

**Specificity of Antibody**—In order to check the specificity of the antibody, antigen-antibody precipitates were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. In this procedure, the antigen-antibody complex was suspended in 0.02% (w/v) sodium dodecyl sulfate polyacrylamide gel electrophoresis. In this procedure, the antigen-antibody complex was suspended in 0.02% (w/v) sodium dodecyl sulfate and electrophoresed. As shown in Fig. 1, the absorbance scan at 280 mm of the heavy and light chains of the precipitate was then suspended in 10 mM pH 7.0, sodium phosphate buffer, and 250 milliunit equivalents of antibody to the supernatant of the first immune precipitation was performed by adding 250 milliunits of unlabeled partially purified hepatic P-enolpyruvate carboxykinase to 180 milliunits of enzyme. In some experiments, the antigen-antibody mixture was incubated with 1% (w/v) sodium bicarbonate before the leucine “chase” to block protein synthesis. The animals were killed 9 hours later, and the amount of radioactive enzyme remaining was determined by the immunochemical procedures outlined above.

**Acid-Base Balance**—Blood pH and P,CO2 were determined anaerobically at 37°C with a glass and a CO2-sensitive electrode, respectively. Total carbon dioxide concentration in the blood was calculated from the Henderson-Hasselbalch equation. For those experiments, rats were anesthetized by intraperitoneal injection of 60 mg of pentobarbital/kg of body weight intraperitoneally 45 min after the radioactive leucine. In some experiments, the animals were given 1% (w/v) sodium bicarbonate before the leucine “chase” to block protein synthesis. The animals were killed 9 hours later, and the amount of radioactive enzyme remaining was determined by the immunochemical procedures outlined above.

**RESULTS**

**Specificity of Antibody**—Antibody prepared against P-enolpyruvate carboxykinase purified from the cytosol of rat liver has been shown previously to cross-react with the renal enzyme (8). To test the specificity of the reaction with kidney enzyme, an immune precipitate made from the renal cortex cytosol of an acidic rat which had been injected with tritiated leucine was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. As shown in Fig. 1, the absorbance scan at 280 nm indicates three peaks which correspond to the enzyme (Fractions 10 to 18) and the heavy and light chains of the
FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the antigen-antibody complex obtained from the renal cortex cytosol of an acidotic rat. The enzyme was precipitated using antibody developed in the goat against rat liver cytosolic P-enolpyruvate carboxykinase. The method for the dissociation of the immune precipitate is described under "Materials and Methods." Electrophoresis (3 ma/gel) was carried out for 16 hours in 10% (w/v) polyacrylamide gels prepared as described by Hopgood et al. (11). ---, absorbance at 280 nm; O-0, radioactivity in counts per min in the first precipitate; O--O, radioactivity in the second precipitate obtained with purified hepatic enzyme. The shaded area is the mean background in counts per min ± 2 S.D. The top of the gels is at left. Immunoglobulin (11). Radioactivity is located exclusively in the fractions corresponding to the enzyme. The electrophoresis of a second immune precipitate obtained after addition of purified liver enzyme to the supernatant of the first antigen-antibody reaction gave an overlapping protein pattern, but with no appreciable radioactivity present in the gel (Fig. 1). Although not shown, an identical electrophoretic pattern was found for the enzyme for the kidney cortex of normal rats. From these observations, it is concluded that the precipitation of P-enolpyruvate carboxykinase from kidney cortex cytosol by the antibody is specific.

Time Course of P-enolpyruvate Carboxykinase Induction in Acidosis—The time course of the change in the relative rate of synthesis (Panel A) and activity (Panel B) of renal cortex P-enolpyruvate carboxykinase in rats made acutely acidic by a single oral administration of ammonium chloride is illustrated in Fig. 2. The rate of synthesis, expressed as the fractional incorporation of labeled leucine in enzyme relative to the incorporation in total soluble protein (relative rate of synthesis), increases progressively from a preinduced level of 2% to approximately 7% 13 hours after the acid load. No differences in the incorporation of L-leucine in total cytosol protein between groups of animals killed at various times were noted, except for a marginally significant increase of approximately 30% at 13 hours after ammonium chloride administration. As shown in panel B of Fig. 2, the first detectable increase in enzyme activity lags behind the change in enzyme synthesis rate. Also, the magnitude of the activity increase appears to be accountable for by the increase in enzyme formation. In control animals given sodium chloride, there is an increase in the rate of synthesis to 3.5% by 13 hours, possibly related to the circadian rhythm of corticosterone secretion (16), as the renal enzyme synthesis is stimulated by glucocorticoids (see below).

The induction of enzyme by acid can be slowly reversed when an amount of sodium bicarbonate sufficient to elevate rapidly the blood pH (9) is fed to acutely acidic rats (Fig. 2). However, the restoration of normal pH does not abruptly return the rate of enzyme synthesis to the preinduced level. An increase in the relative rate of P-enolpyruvate carboxykinase synthesis from 3.84 ± 0.37 (mean ± S.E.) % in controls to 6.72 ± 0.30% 9 hours after ammonium chloride administration occurs in adrenalectomized rats. The induction of the enzyme during metabolic acidosis is therefore not dependent on the presence of the adrenal glands. A normal enhancement of enzyme activity after acid treatment in adrenalectomized animals has recently been observed by others (17).

Induction of Renal P-enolpyruvate Carboxykinase by Triam...
Ammonium chloride—The effect of triamcinolone on the activity and rate of synthesis of renal P-enolpyruvate carboxykinase in the renal cortex is shown in Table I. By 8 hours after treatment, the relative rate of synthesis as well as the activity is approximately doubled. Both the effects on synthesis and on activity of the enzyme are subject to inhibition by actinomycin D at doses which have been shown to inhibit mRNA synthesis in rat tissues (18). At these doses, there is only a slight and statistically insignificant decrease in the rate of L-leucine incorporation in total protein (Table I). These data clearly demonstrate that the classical increase in renal P-enolpyruvate carboxykinase caused by glucocorticoids (5, 8) results from a selective stimulation of enzyme synthesis.

Effect of Actinomycin D and Cordycepin on Induction of P-enolpyruvate Carboxykinase in Acidosis—Two reports indicated that the increase in renal P-enolpyruvate carboxykinase activity caused by ammonium chloride feeding was not inhibited by actinomycin D. On this basis, it was concluded that the effect of acidosis did not involve new enzyme synthesis (7, 8). This earlier conclusion is at variance with the results of our experiments described above (Fig. 2). We therefore decided to re-evaluate the effect of actinomycin D on the induction after acid treatment. Table II shows that actinomycin, within the dosage range effective against glucocorticoid induction, also blocks the induction noted 6 hours after ammonium chloride administration. The effectiveness of the drug, however, is limited in duration: 11 hours after the acid load, a considerable increase in the relative rate of P-enolpyruvate carboxykinase synthesis was observed. Since actinomycin has toxic side effects, it was important to determine whether the drug interferes with the development of metabolic acidosis after oral administration of ammonium chloride. The last column of Table II, reporting the total concentration of carbon dioxide in the blood of the animals at the time of death, demonstrates that rats treated with 2 mg of actinomycin/kg of body weight displayed metabolic acidosis, although of somewhat milder degree than nontreated rats. This difference is presumably due to impaired intestinal absorption (we noted that actinomycin-treated rats had distended stomachs).

To evaluate further the apparent requirement for RNA synthesis in the induction of P-enolpyruvate carboxykinase during acidosis, additional experiments were done with the antibiotic cordycepin, a drug which blocks the adenylylation of mRNA (19). As shown in Table III, cordycepin markedly inhibited mRNA synthesis in rat tissues (18). At these doses, inhibition by actinomycin D at doses which have been shown to interfere with the development of metabolic acidosis after oral administration of ammonium chloride. The last column of Table II, reporting the total concentration of carbon dioxide in the blood of the animals at the time of death, demonstrates that rats treated with 2 mg of actinomycin/kg of body weight displayed metabolic acidosis, although of somewhat milder degree than nontreated rats. This difference is presumably due to impaired intestinal absorption (we noted that actinomycin-treated rats had distended stomachs).

Table I

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Actinomycin D</th>
<th>No. of animals</th>
<th>Radioactivity incorporated into Cytosol protein</th>
<th>% Radioactivity in enzyme</th>
<th>Enzyme activity</th>
<th>units/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>8</td>
<td>475.8 ± 46.5</td>
<td>17.13 ± 3.30</td>
<td>3.42 ± 0.31</td>
<td>6.1 ± 0.50</td>
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<tr>
<td>Triamcinolone</td>
<td>0</td>
<td>8</td>
<td>437.2 ± 55.5</td>
<td>28.71 ± 5.36</td>
<td>6.55 ± 0.34</td>
<td>11.2 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4</td>
<td>359.5 ± 54.0</td>
<td>14.69 ± 4.76</td>
<td>3.94 ± 0.84</td>
<td>7.7 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>9</td>
<td>323.5 ± 55.9</td>
<td>8.31 ± 2.91</td>
<td>2.14 ± 0.47</td>
<td>5.4 ± 0.34</td>
</tr>
<tr>
<td>None</td>
<td>2.0</td>
<td>11</td>
<td>317.5 ± 48.5</td>
<td>4.52 ± 1.07</td>
<td>1.33 ± 0.15</td>
<td>4.4 ± 0.37</td>
</tr>
</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Time</th>
<th>Actinomycin D</th>
<th>No. of animals</th>
<th>Radioactivity incorporated into Cytosol protein</th>
<th>% Radioactivity in enzyme</th>
<th>Enzyme activity</th>
<th>units/g</th>
<th>Total CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>hr</td>
<td>mg/kg body weight</td>
<td>7</td>
<td>403.3 ± 58.3</td>
<td>11.57 ± 2.04</td>
<td>9.77 ± 0.19</td>
<td>4.8 ± 0.4</td>
<td>29.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>370.7 ± 56.9</td>
<td>21.29 ± 3.93</td>
<td>5.69 ± 0.35</td>
<td>7.6 ± 0.5</td>
<td>20.7 ± 1.0</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>6</td>
<td>0</td>
<td>7</td>
<td>299.9 ± 42.4</td>
<td>9.07 ± 2.26</td>
<td>3.20 ± 0.94</td>
<td>5.4 ± 0.7</td>
<td>23.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>295.9 ± 44.2</td>
<td>8.36 ± 2.07</td>
<td>2.69 ± 0.39</td>
<td>5.0 ± 0.3</td>
<td>23.4 ± 0.7</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>493.1 ± 123.1</td>
<td>25.28 ± 7.90</td>
<td>5.03 ± 0.45</td>
<td>6.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6</td>
<td>2</td>
<td>15</td>
<td>317.9 ± 42.7</td>
<td>6.04 ± 1.11</td>
<td>1.90 ± 0.18</td>
<td>4.1 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>
inhibits the increase in rate of synthesis and in activity of P-enolpyruvate carboxykinase normally observed after ammonium acidosis, without interfering with the development of metabolic acidosis.

### Acidosis and Degradation of Renal P-enolpyruvate Carboxykinase

The degradation of renal P-enolpyruvate carboxykinase in acidotic animals was measured by immunochemical techniques in an attempt to verify the previous claim (9) that a decreased rate constant for enzyme degradation contributed to the augmentation in enzyme content in the kidney of acidotic rats. As shown in Table IV, the radioactivity in P-enolpyruvate carboxykinase in normal animals decreased from 1.96% of the radioactivity in total cytosol protein immediately after the pulse period to 1.34% over the 9-hour decay interval. Accordingly, the relative half-life of the enzyme would be about 13 hours, approximating the value reported for the hepatic enzyme (20) and far longer than the 1.4 to 3.2 hour figures presented by Longshaw et al. (9). In acidotic animals, we observed a much smaller loss of radioactivity in the enzyme over the 9 hours of the experiment. However, when cycloheximide was used to inhibit protein synthesis during the decay period, the radioactivity remaining in renal P-enolpyruvate carboxykinase at the end of the experiment was markedly reduced in acidotic rats, and no difference was then observed between the latter and control animals (Table IV). Presumably because of the high rate of enzyme synthesis, substantial reincorporation of radioactive leucine might have occurred in acidotic rats which were not treated with cycloheximide, resulting in an overestimation of the stability of the enzyme. Taken together, these data indicate that the degradation of renal P-enolpyruvate carboxykinase is apparently not affected in acute acidosis. On the other hand, the essential role of enzyme synthesis for the increase in P-enolpyruvate carboxykinase level during acidosis is underscored by the fact that no increase in enzyme activity takes place in acidotic rats treated with cycloheximide (Table IV), in agreement with a recent report by Pogson et al. (17).

### Insulin and Renal P-enolpyruvate Carboxykinase Synthesis

Kamm et al. (21) observed that sodium bicarbonate feeding prevented the increase in renal P-enolpyruvate carboxykinase activity normally seen in diabetic rats, and they suggested that metabolic acidosis, rather than insulin deficiency per se, is the cause of the increase in the enzyme level in the kidney. The experiments summarized in Fig. 3 show that, after 3 days of fasting, the high rate of P-enolpyruvate carboxykinase synthesis in the kidney cortex slowly declines during 6 hours following bicarbonate administration, while it is not affected by glucose loading. Conversely, sodium bicarbonate has no effect on the synthesis of the liver enzyme, which, on the other hand, falls rapidly after glucose administration (Fig. 3B). Data in Table V demonstrate that in alloxan diabetic rats, the injection of large amounts of insulin does not alter the rate of enzyme synthesis in the kidney cortex over a 5-hour period. On the contrary, in the same animals the relative rate of leucine incorporation in the hepatic enzyme was markedly depressed from 2.14% to 0.21% of the total leucine incorporated into cytosol protein, in agreement with previous results from our laboratory (12).

### Cyclic Adenosine 3',5'-Monophosphate and Renal P-enolpyruvate Carboxykinase

Cyclic AMP is an effective inducer of cytosolic P-enolpyruvate carboxykinase in the liver, as demonstrated by several studies in vivo (10, 22) and in vitro (23, 24). In contrast, the role of the nucleotide in the renal cortex has not been well documented, although it was reported recently that cyclic AMP plus theophylline caused an increase in renal P-enolpyruvate carboxykinase activity (25). Our data (Table VI) show that theophylline alone stimulates the synthesis of the enzyme in the rat kidney, and that dibutyryl cyclic AMP, although ineffective by itself, further enhances the rate of synthesis over that achieved with the theophylline treat-

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### Table III

**Effect of cordycepin on induction of renal P-enolpyruvate carboxykinase during acidosis**

Two intraperitoneal injections of cordycepin (each of 20 mg/kg of body weight) were administered 30 min prior and 1.5 hours after oral administration of sodium chloride or ammonium chloride (10 mmol/kg of body weight). The rats were killed 6 hours after tube feeding, and both the activity and rate of synthesis of the enzyme were determined. Values are means ± S.E. Total CO2 concentration in aortic blood was determined in five animals per group. See Table II for the effect of ammonium chloride in untreated rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>% Radioactivity in enzyme</th>
<th>Enzyme activity</th>
<th>Total CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cordycepin</td>
<td>10</td>
<td>2.33 ± 0.27</td>
<td>9.6 ± 1.0</td>
<td>26.0 ± 0.5 (5)</td>
</tr>
<tr>
<td>Cordycepin + ammonium chloride</td>
<td>11</td>
<td>3.26 ± 0.24</td>
<td>10.0 ± 0.9</td>
<td>16.7 ± 1.1 (5)</td>
</tr>
</tbody>
</table>

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### Table IV

**Effect of acidosis on degradation of renal P-enolpyruvate carboxykinase**

All animals were injected with [3H]leucine. Forty-five minutes later (0 time) some were killed and the radioactivity incorporated in various fractions was measured; the other rats were injected with unlabeled leucine and were fed with ammonium or sodium chloride. They were killed 9 hours later. To some animals, cycloheximide (1 mg/kg of body weight) was administered intraperitoneally at 0 time and again 4 hours later. Results are means ± S.E. for four animals in each group. Note Added in Proof—In an identical experiment, the control CO2 content of aortic blood was 28.3 ± 0.5 mEq, as compared to 20.4 ± 1.6 in rats tube-fed ammonium chloride and to 22.9 ± 1.3 in rats treated with cycloheximide plus ammonium chloride.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Radioactivity incorporated into</th>
<th>% Radioactivity in enzyme</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hr</td>
<td>Cytosol protein</td>
<td>Enzyme</td>
<td>units/g</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>482.7 ± 15.6</td>
<td>9.49 ± 0.78</td>
<td>1.96 ± 0.11</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>482.3 ± 26.1</td>
<td>6.31 ± 0.40</td>
<td>1.34 ± 0.09</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>9</td>
<td>507.4 ± 23.5</td>
<td>8.95 ± 0.83</td>
<td>1.74 ± 0.09</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>9</td>
<td>397.2 ± 31.3</td>
<td>5.57 ± 0.75</td>
<td>1.37 ± 0.10</td>
</tr>
<tr>
<td>Cycloheximide + ammonium chloride</td>
<td>9</td>
<td>378.7 ± 27.8</td>
<td>5.22 ± 0.42</td>
<td>1.39 ± 0.07</td>
</tr>
</tbody>
</table>
In these experiments, no increase in enzyme activity was detected. However, in similar experiments in which the animals were killed 8 hours instead of 4 hours after the start of the treatment with theophylline plus dibutyl cyclic AMP, a slight but significant increase in P-enolpyruvate carboxykinase activity was observed (data not shown).

To define better the possible participation of cyclic AMP as a second messenger in the regulation of renal P-enolpyruvate carboxykinase, we tried to duplicate the effect of exogenous nucleotide with parathyroid hormone, which is known to stimulate renal cortex adenyl cyclase (26, 27) and to increase intrarenal cyclic AMP concentration (28). As shown in Table VI, large amounts of bovine parathyroid extracts injected to thyroparathyroidectomized rats together with theophylline do not significantly stimulate the synthesis of P-enolpyruvate

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**TABLE V**

**Effect of insulin on relative rate of synthesis of renal and hepatic P-enolpyruvate carboxykinase in diabetic rats**

Rats were made diabetic as outlined under "Materials and Methods." Crystalline porcine insulin, 12 units/kg of body weight, was injected subcutaneously and the animals were killed 5 hours later. Control animals received 0.9% sodium chloride instead of insulin. Values are expressed as the mean ± S.E. for four animals.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>Radioactivity incorporated into Cytosol protein 10^13 x cpm/g</th>
<th>% Radioactivity in enzyme Enzyme activity</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cortex</td>
<td>Diabetes</td>
<td>207.4 ± 37.7</td>
<td>10.14 ± 1.79</td>
<td>4.95 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>Diabetes + insulin</td>
<td>321.7 ± 73.7</td>
<td>15.64 ± 0.47</td>
<td>4.99 ± 0.23</td>
</tr>
<tr>
<td>Liver</td>
<td>Diabetes</td>
<td>436.2 ± 111.2</td>
<td>8.54 ± 1.59</td>
<td>2.14 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>Diabetes + insulin</td>
<td>554.2 ± 133.8</td>
<td>1.45 ± 0.83</td>
<td>0.21 ± 0.12</td>
</tr>
</tbody>
</table>

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**TABLE VI**

**Effect of theophylline, dibutyl adenosine cyclic 3':5'-monophosphate and parathyroid hormone on relative rate of synthesis of renal P-enolpyruvate carboxykinase**

Intact and adrenalectomized rats were injected intraperitoneally with 20 mg of theophylline/kg of body weight at 0 and 2 hours. 10 mg of dibutyl cyclic AMP/kg of body weight were injected at 0.5, 1.5, 2.5, and 3.5 hours. Controls received 0.9% sodium chloride and all animals were killed at 4.5 hours. Thyroparathyroidectomized rats were injected intraperitoneally with 20 mg of theophylline/kg of body weight 0.5 hours prior to a subcutaneous injection of 200 U.S.P. units of bovine parathyroid extract/kg of body weight and were killed at 5.0 hours. P-enolpyruvate carboxykinase activity and incorporation of [H]leucine in the enzyme and in total cytosol protein were determined as described under "Materials and Methods." Results are means ± S.E. for four to six animals.

<table>
<thead>
<tr>
<th>Endocrine status</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Radioactivity incorporated into Cytosol protein 10^13 x cpm/g</th>
<th>% Radioactivity in enzyme Enzyme activity</th>
<th>Enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Control</td>
<td>11</td>
<td>386.9 ± 29.4</td>
<td>9.50 ± 0.69</td>
<td>2.55 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>Bt,cAMP</td>
<td>6</td>
<td>336.3 ± 48.1</td>
<td>9.67 ± 1.67</td>
<td>2.84 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Theophylline</td>
<td>7</td>
<td>417.8 ± 51.0</td>
<td>16.32 ± 2.88</td>
<td>3.45 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>Theophylline + Bt,cAMP</td>
<td>11</td>
<td>415.7 ± 32.7</td>
<td>18.51 ± 2.22</td>
<td>4.32 ± 0.26</td>
</tr>
<tr>
<td>Thyroparathyroidectomized</td>
<td>Control</td>
<td>6</td>
<td>441.9 ± 99.8</td>
<td>13.98 ± 1.05</td>
<td>3.90 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>Theophylline + PTE</td>
<td>8</td>
<td>349.2 ± 42.0</td>
<td>13.38 ± 2.46</td>
<td>3.75 ± 0.35</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>Control</td>
<td>7</td>
<td>533.0 ± 79.6</td>
<td>10.44 ± 1.99</td>
<td>1.96 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Theophylline + Bt,cAMP</td>
<td>7</td>
<td>424.4 ± 76.1</td>
<td>10.37 ± 1.70</td>
<td>2.51 ± 0.13</td>
</tr>
</tbody>
</table>
carboxykinase. The latter observation suggests that the action of injected theophylline and dibutyryl cyclic AMP might be due to an indirect, extrarenal effect. Actually, when adrenalectomized rats are given theophylline plus dibutyryl cyclic AMP, no induction of the enzyme takes place (Table VI).

**DISCUSSION**

The physiological significance of P-enolpyruvate carboxykinase induction in the kidney of acidotic animals has been emphasized in several recent reviews (29, 30). In metabolic acidosis a substantial part of the excess protons are excreted by the kidneys in the form of ammonium ions. The ammonium moiety is supplied primarily by the deamination and deamination of glutamine, which is extracted in increased amounts from the blood by the renal cell (2). The deamination of glutamine generates α-ketoglutarate, and the renal response to acidosis must involve metabolic adaptations for the removal of this intermediate. There is evidence that glutamine-derived α-ketoglutarate is mainly metabolized to glucose in the rat kidney (31) and to carbon dioxide in the dog kidney (32).

P-enolpyruvate carboxykinase is one of the enzymes specific to the gluconeogenic pathway and plays, therefore, an important role in the conversion of α-ketoglutarate to glucose. On the other hand, Pitts (33) and Goodman (29) have suggested that the oxidation of glutamine to carbon dioxide might occur by a pathway involving the conversion of glutamine-derived oxalacetate to P-enolpyruvate and its re-entry into the Krebs cycle as acetyl-coenzyme A. According to this proposal, the rate of glutamine oxidation could also be regulated by the activity of P-enolpyruvate carboxykinase.

In the present work, we have demonstrated a selective stimulation of the synthesis of P-enolpyruvate carboxykinase in the renal cortex of the rat during ammonium chloride acidosis. Three lines of evidence indicate that the stimulation of enzyme synthesis is probably the only mechanism for the increase in P-enolpyruvate carboxykinase level. First, the acceleration of enzyme synthesis occurs early enough and is apparently of sufficient magnitude to explain the change in activity. Second, treatments with actinomycin D, cordycepin, or cycloheximide, which interfere with the acid-stimulated synthesis of the enzyme, also suppress the rise in P-enolpyruvate carboxykinase activity. The latter observation negates the possibility of an alteration in the catalytic properties of preformed enzyme molecules as a mechanism for the increase in assayable activity. Third, the stability of P-enolpyruvate carboxykinase, estimated by measuring the loss of radioactivity from the enzyme pool which had been labeled with [3H]leucine, is apparently not increased during acidosis. Thus, when cycloheximide was used to prevent reincorporation of radioactive leucine during the decay period, no difference in the apparent half-life of P-enolpyruvate carboxykinase could be detected in the renal cortex of normal and acidic rats. Unfortunately, this experiment is somewhat equivocal since there is evidence that cycloheximide itself may interfere with protein degradation processes, and that the use of this inhibitor may therefore result in an overestimation of the stability of enzymes (20, 34). The data do indicate, however, that the half-life or renal P-enolpyruvate carboxykinase, in acidosis as well as in the normal state, is about 13 hours, that is in the same order as noted for the hepatic enzyme using the same procedure (20), and much longer than reported by Longshaw et al. (9).

Flores and Alleyne (7) and Longshaw and Pogson (8) reported that the increase in P-enolpyruvate carboxykinase activity after acid administration could not be blocked with actinomycin D, while the induction caused by glucocorticoids was suppressed by the drug. On the basis of these findings, they suggested that the two inductive processes were achieved by different mechanisms. The present work provides a direct demonstration that glucocorticoids stimulate the synthesis of the renal enzyme, and that the stimulation of enzyme synthesis is suppressed by actinomycin D. On the other hand, we show that actinomycin D also inhibits the induction of renal P-enolpyruvate carboxykinase caused by ammonium chloride acidosis. The failure to detect this effect of actinomycin in the studies of Flores and Alleyne (7) and of Longshaw and Pogson (8) can probably be explained by the use of low doses of the drug and by a long interval between drug administration and the measurement of enzyme activity. The effectiveness of actinomycin D in suppressing the ammonium chloride or glucocorticoid induced stimulation of P-enolpyruvate carboxykinase synthesis suggests that DNA-directed RNA synthesis is involved in the induction by both stimuli. Furthermore, the formation of new mRNA coding for the enzyme is probably required, since cordycepin inhibited the induction caused by acidosis. The slow and gradual changes in the synthesis rate of the enzyme during acidosis or after bicarbonate administration are also consistent with a regulation at the transcriptional level. The stimulation of a specific enzyme synthesis may be related to the earlier observations of Lotspeich (35) showing a transcription-dependent stimulation of protein synthesis in the kidney of the acidotic rat.

There are marked differences between the kidney and the liver in the physiological regulation of P-enolpyruvate carboxykinase levels. The hepatic enzyme is regulated by an interaction between cyclic AMP, which stimulates enzyme synthesis and insulin, which causes a rapid decrease in the synthesis rate of the enzyme (12). Recently, Kamm et al. (21) reported that the rise in enzyme activity in the renal cortex of diabetic rats was prevented not only by insulin treatment but also by the administration of bicarbonate, while in the liver only insulin was effective. These authors then suggested that in diabetes, renal P-enolpyruvate carboxykinase is regulated by the acid-base status, independently of the defect in carbohydrate metabolism. Our experiments extend these observations and demonstrate that an oral administration of glucose to fasted rats, or a single injection of insulin to diabetic animals, does not acutely decrease the high relative rate of enzyme synthesis prevailing in the renal cortex of these animals, while these treatments cause a marked and rapid decline in the rate of enzyme synthesis in the liver. In contrast, bicarbonate administration to fasted rats slowly represses P-enolpyruvate carboxykinase synthesis in kidney without affecting the relative rate of enzyme synthesis in liver. This lends support to the concept that the disturbance in acid-base balance is responsible for the induction of renal P-enolpyruvate carboxykinase in starvation (36) and diabetes (21). The regulation of the synthesis of the renal enzyme by the acid-base status, independently of variations in the level of circulating insulin, is also in keeping with a role of renal gluconeogenesis associated with ammoniagenesis and the homeostasis of acid-base balance rather than with net glucose production and the homeostasis of blood glucose.

The dissimilarity between the regulation of P-enolpyruvate carboxykinase in the renal cortex and in the liver is further illustrated by the different role of cyclic AMP in the two
tissues. In the liver the cyclic nucleotide is a major modulator of P-enolpyruvate carboxykinase synthesis (37). In contrast, we have been unable to induce the renal enzyme with dibutyryl cyclic AMP plus theophylline in adrenalectomized rats. Moreover, the administration of parathyroid hormone, which increases the renal content in cyclic AMP (38), fails to stimulate enzyme synthesis. Therefore, the rate of P-enolpyruvate carboxykinase synthesis in the kidney does not appear to be controlled by the tissue level of cyclic AMP. The increase in relative rate of enzyme synthesis which we have observed in intact rats after administration of theophylline and dibutyryl cyclic AMP is probably due to the well known increase in corticosterone secretion (38).

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