Pyruvate Carboxylase

I. Nature of the Reaction*

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The direct carboxylation of pyruvate to oxaloacetate (Wood-Werkmans reaction) was postulated more than 25 years ago as a probable mechanism for the formation of dicarboxylic acids by CO₂ fixation (1) and a considerable body of evidence on the interrelationship of dicarboxylic acids and CO₂ during bacterial growth was interpreted in this light (2-4). There was no direct evidence for the existence of such a reaction, however, and as alternative pathways of dicarboxylic acid formation from CO₂ were provided by enzymic studies (5-7), the hypothesis of the direct carboxylation of pyruvate assumed less significance. Recently, Woronick and Johnson (8) reported that extracts from Aspergillus niger formed aspartate and malate from pyruvate, CO₂, and adenosine triphosphate. These authors again suggested that a direct carboxylation of pyruvate to form oxaloacetate might be involved. Our interest in the possibility that such a reaction might occur in animal liver arose from the observations that whereas liver mitochondria can form phosphoenolpyruvate from pyruvate (9-11), studies of the intracellular distribution of the pertinent enzymes suggested that neither of the previously considered pathways for the formation of phosphoenolpyruvate could be responsible for the observed results with mitochondria. Pyruvate can be converted to phosphoenolpyruvate by a reversal of the pyruvic kinase reaction (12) or by a dicarboxylic acid pathway involving malic enzyme (13, 14) but neither of these enzymes is present in significant amounts in liver mitochondria (15). Since the apparent precursor of phosphoenolpyruvate in liver mitochondria is oxaloacetate we therefore sought a more direct pathway for the formation of this substance from pyruvate. As described in this communication, an enzyme has been detected in and concentrated from liver mitochondria which carries out the following reaction.

\[
\text{Pyruvate} + \text{ATP} + \text{CO}_2 \rightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i \quad (1)
\]

This enzyme has been given the tentative trivial name "pyruvate carboxylase" but is systematically classified as pyruvate:CO₂ ligase (ADP), EC 6.4.1.1. As indicated by the reaction, the formation of oxaloacetate by this enzyme is dependent on the presence of acetyl coenzyme A (or propionyl coenzyme A). The succeeding sections describe methods for measuring and concentrating the active protein and present stoichiometric and isotopic data in support of Reaction 1. The relationship of this enzyme to other reactions leading to the formation of dicarboxylic acids is also discussed. A preliminary account of some of these results has appeared previously (16).

Experimental Procedure

Measurement of Enzymes—Acetyl- and propionyl-CoA carboxylases were assayed by the conversion of C⁴₀₂ to a non-volatile form in the presence of the appropriate acyl-CoA. The reactions were carried out in small stopped test tubes containing the following constituents (micromoles in 0.5 ml); acetyl- or propionyl-CoA, 0.35; ATP, 1.25; MgCl₂, 5; Tris-HCl buffer, pH 7.4, 30; and NaHCO₃, 15, containing 12 x 1⁰ c.p.m. per μmole. The reaction was started immediately after addition of the NaHCO₃ by introducing the enzyme and incubation for 5 minutes at 30°. After deproteinization with trichloroacetic acid, the reaction filtrate was neutralized and the radioactivity measured. P-Enolpyruvate carboxykinase was determined spectrophotometrically by coupling the synthesis of oxaloacetate from P-enolpyruvate, IDP, and CO₂ with malate dehydrogenase (13) and conventional optical methods were employed for measuring malic enzyme (5), lactate and malate dehydrogenases, and pyruvate kinase. The latter assay was also adapted for the determination of ATPase and adenylate kinase activities. Oxaloacetate propionyl CoA transcarboxylase was estimated by measuring oxaloacetate formation from methylmalonyl-CoA and pyruvate (17).

Analytical—Oxaloacetate and pyruvate were measured spectrophotometrically with malate and lactate dehydrogenases, respectively. ADP was measured by the pyruvic kinase reaction as described previously (18). Acetyl-CoA was measured colorimetrically as acetylhydroxamate (19), or enzymatically with condensing enzyme in combination with malate, malate dehydrogenase, and NAD (20). Protein (21) and Pi (22) were determined by colorimetric means.

Spectrophotometric and Counting Procedures—The various spectrophotometric assays were carried out in cuvettes with a 5-mm light path with a Zeiss spectrophotometer as modified by Gilford Instrument Company, equipped with a recorder and an automatic four-place cuvette changer. Successive readings on any single cuvette were taken at approximately 25-second intervals.

Counting procedures employed stainless steel planchets and an end window counter with an efficiency of approximately 5%. Results are reported as counts per minute for infinitely thin samples.

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Materials. Lactate dehydrogenase with a specific activity* of 200 was prepared by a modification of the method of Kornberg and Pricer (23) and condensing enzyme with a specific activity of 4 was prepared according to Ochoa (20). The pyruvate kinase and malate dehydrogenase used were commercial products (California Corporation for Biochemical Research). Acetyl- and propionyl-CoA were prepared from CoA (Pabst Brewing Company) and the respective acid anhydrides (24). Methylmalonyl-CoA prepared from the mixed anhydride (25) was kindly supplied by Dr. H. G. Wood and acetyl-1-C14-CoA by Dr. H. Rudney. Pyruvate-1- and -2-C14 were purchased as the commercial sodium salts but were found to be contaminated with substantial amounts of parapyruvate-C14 which interfered with some of the isotopic procedures. The latter compound was removed after dilution with carrier pyruvate by passage through a small Dowex 1 (Cl-) column with just sufficient 0.01 or 0.02 M HCl to remove about 90% of the pyruvate. Small amounts of parapyruvate are formed on storage of the chromatographed material and it was never possible to ignore this factor in isotopic procedures, as is discussed in connection with the experiments of Table V. Oxaloacetic acid was prepared from commercial sodium diethyl oxaloacetate (26) and recrystallized three times from acetone-benzene before use.

RESULTS

Preparation of Pyruvate Carboxylase. The enzyme has been concentrated approximately 90-fold from chicken liver by procedures summarized in Table I. The extract of washed, lyophilized mitochondria was used as the starting material. Fresh livers were cut into small pieces and homogenized in 4 volumes (milliliters per g) of 0.25 M sucrose in a Waring Blender at reduced speeds for 45 seconds. The homogenate was centrifuged at 700 × g for 15 minutes and the supernatant layer carefully removed and discarded. The heavy mitochondrial layer can then be decanted as a poorly packed layer, leaving behind the more firmly sedimented unbroken cells and other debris. The mitochondrial layer was diluted to the original volume of the homogenate with water and centrifuged for 20 minutes at 12,000 × g. The resulting precipitate was suspended evenly in 0.25 M sucrose and lyophilized. All of the above procedures were carried out at 0–3°C. This treatment not only removes much of the water-soluble protein from the mitochondria but also renders pyruvate carboxylase readily extractable by mild procedures. The enzyme appears to be released only slowly from mitochondria kept in sucrose when subjected to sonic disintegration. As shown in Table I, little activity is lost in the preparation of the washed mitochondrial fraction. The lyophilized material is stable for several weeks when stored in a vacuum at −20°C.

Two grams of the lyophilized material were extracted twice at 2°C with 10 volumes of 0.04 M Tris-HCl buffer, pH 7.8, and the clear supernatant layers obtained by centrifuging at 20,000 × g for 10 minutes were combined. The protein fraction precipitating between 25 and 33% saturation with ammonium sulfate at 2°C was collected by centrifugation and dissolved in 10 ml of 0.04 M Tris-HCl, pH 7.8. The precipitate contains essentially all of the active material from the original extract but is quite unstable at this stage, particularly in solution. Some protective effect was exerted by the presence of ATP, MgCl2, acetyl CoA, and NaHCO3 added in the approximate concentrations used in the spectrophotometric assay described under Fig. 1.

Aged Ca3(PO4)2 gel (27) was added to the solution (0.7 mg of gel, dry weight, per mg of protein) and the pH adjusted to 6.8 by m acetate buffer (pH 5). After stirring for 15 minutes, the suspension was centrifuged and the precipitate discarded. Most of the enzyme was then removed from the supernatant fraction by the addition of more gel (0.2 mg per mg of protein in the starting material) and adjustment of the pH to 6.2. The enzyme was eluted from the gel with 8 ml of 0.03 M Tris-HCl (pH 7.8) containing 0.5 M sucrose. The procedure just described is a typical one, but the exact amounts of gel required and the most satisfactory pH values for the negative and positive gel steps varied according to the age and state of the Ca3(PO4)2 gel and these factors had to be adjusted accordingly with each batch of gel. In the absence of sucrose, the enzyme has a half-life of 1 to 2 hours, but with sucrose concentrations of 0.5 to 1.0 M, the enzyme maintains its activity reasonably well for several hours at 0°C or for several days after quick freezing and storage at −20°C.

The level of activity reached in the preparation described in Table I is typical although with minor modifications in the gel treatment it has been possible to obtain occasional preparations with specific activities at least twice as high as the final fraction shown in Table I.

Pyruvate carboxylase was ordinarily assayed spectrophotometrically by measuring oxaloacetate formation from pyruvate and CO2 with the aid of malic dehydrogenase and NADH. A typical assay showing the effect of the concentration of enzyme on reaction rate is shown in Fig. 1. When the presence of lactate dehydrogenase was suspected, a cuvette from which acetyl-CoA had been omitted was used as a control. In initial extracts in which lactate dehydrogenase activity was quite high, the optical assay was replaced by one involving the fixation of CO2 as described in Table I. In this assay, controls from which pyruvate had been omitted were used to permit correction for acetyl-CoA carboxylase activity. The product formed in the isotopic assay with crude fractions is mainly citrate rather than oxaloacetate since condensing enzyme is present as a contaminant in the crude fractions and acetyl-CoA is present in the assay mixture.

Because of possible relationships of the present reaction with other CO2-fixing reactions, the level of activity of several of these enzymes has been followed during fractionation procedures. A summary of these results based on the initial extract from the washed and lyophilized mitochondria and the stage of highest purity is shown in Table II. Since either acetyl- or propionyl-CoA is required for oxaloacetate formation it was of special interest to examine the two fractions for the presence of acetyl-CoA carboxylase (28, 29) and propionyl-CoA carboxylase (30). The extract from the washed mitochondria contains a significant amount of acetyl-CoA carboxylase but the purification procedures are much more efficient in concentrating pyruvate carboxylase and the specific activity of acetyl-CoA carboxylase in the final fraction is less than 2% of that of pyruvate carboxylase. The initial extract shows a considerable ability to fix CO2 with propionyl-CoA as the acceptor but this activity is lost during purification. The disparity in behavior during fractionation suggests that the carboxylation of acetyl- and propionyl-CoA is catalyzed by different protein moieties at
least in this preparation. The initial extract contains about twice as many units of P-enolpyruvate carboxykinase as pyruvate carboxylase but the former is concentrated only slightly during the purification process. Thus far we have not been successful in preparing pyruvate carboxylase fractions which are completely free of P-enolpyruvate carboxykinase but, as is shown in the succeeding paper (31), P-enolpyruvate carboxykinase fractions entirely devoid of pyruvate carboxylase activity can be obtained. No evidence could be obtained for the presence of the transcarboxylation reaction between methylmalonyl-CoA and pyruvate described by Swick and Wood (17) in extracts from Propionibacterium. Since the absence of transcarboxylase activity is an important factor in considerations of the mechanism of the pyruvate carboxylase reaction, special pains were taken to establish the validity of this negative observation. These included parallel and internal control experiments with bacterial extracts which showed that the techniques and reagents were adequate to detect the presence of the transcarboxylation reaction if present.

Several enzymes such as ATPase, adenylate kinase, and condensing enzyme which might interfere with stoichiometric studies were shown to be absent from the fraction with the highest level of purity.

_Nature of the Reaction._—The effects of the omission of various components from the assay medium are shown in Table III. Oxaloacetate formation is completely dependent on the presence of ATP, 0.35 moles of acetyl-CoA, 30 NaHCO₃, 0.35 NADH, 1 unit of malate dehydrogenase and pyruvate carboxylase as follows: Cuvette A, 12 μg of protein; Cuvette B, 6 μg of protein; Cuvette C, 4 μg of protein. Optical density changes were measured at 340 μm in a cell with a 5-mm light path at room temperature.

### TABLE I

**Purification of pyruvate carboxylase**

Assays for Stages 1 through 3 were carried out with C¹⁴O₂ as follows. A total volume of 0.5 ml contained 50 micromoles of Tris-HCl (pH 7.4), 5 micromoles of K pyruvate, 5 micromoles of MgCl₂, 1.25 micromoles of ATP, 0.39 micromole of acetyl-CoA, and 15 micromoles of NaHCO₃ (12,500 c.p.m. per mole). After incubation for 5 minutes at 30° and deproteinization with 5% trichloroactic acid, and the filtrate was freed of C¹⁴O₂ and neutralized; BaCl₂ (10 micromoles) was added and radioactivity determined after drying under a heat lamp. Assays for Stages 3 through 5 were carried out spectrophotometrically as described in Fig. 1. The Stage 3 material had a specific activity of 1.6 by the spectrophotometric test and 0.8 by the C¹⁴O₂ assay; accordingly, the specific activity and units for Stages 1 and 2 have been corrected by multiplying by a factor of 2.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Protein</th>
<th>Total activity</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extract from whole liver†</td>
<td>mg</td>
<td>units</td>
</tr>
<tr>
<td>2</td>
<td>Extract from washed mitochondria</td>
<td>2140</td>
<td>128</td>
</tr>
<tr>
<td>3</td>
<td>(NH₄)₂SO₄ precipitate (25 to 33%)</td>
<td>380</td>
<td>106</td>
</tr>
<tr>
<td>4</td>
<td>Ca₃(PO₄)₂ gel, negative adsorption</td>
<td>04</td>
<td>103</td>
</tr>
<tr>
<td>5</td>
<td>Ca₃(PO₄)₂ gel, adsorption and elution</td>
<td>32</td>
<td>83</td>
</tr>
</tbody>
</table>

*Expressed as micromoles of oxaloacetate formed per minute per mg of protein. The assay procedures are described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Specific activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate carboxylase</td>
<td>0.22 5.35</td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase</td>
<td>0.018 0.087</td>
</tr>
<tr>
<td>Propionyl CoA carboxylase</td>
<td>0.029 0</td>
</tr>
<tr>
<td>P-Enolpyruvate carboxykinase</td>
<td>0.40 0.69</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>0.18 0</td>
</tr>
<tr>
<td>Malate enzyme</td>
<td>0</td>
</tr>
</tbody>
</table>

* Specific activities are expressed as micromoles per minute per mg of protein. The assay procedures are described under "Experimental Procedure."

of pyruvate, ATP, and NaHCO₃ although in order to demonstrate dependence on added NaHCO₃, it is necessary to remove traces of CO₂ from the reaction mixture by subjecting it to mild vacuum and gassing procedures. The small activity observed in the absence of added MgCl₂ and acetyl-CoA may be due to a carrying along of these constituents in the enzyme fraction since the protective mixture added before the gel steps includes these substances.

The stoichiometry of the pyruvate carboxylase reaction is shown in an experiment summarized in Table IV in which oxaloacetate has been formed in the absence of malate dehydrogenase. The results show: (a) good correspondence between oxaloacetate formed and pyruvate utilized, (b) that ADP and P₁ are the products of ATP utilization, and (c) that acetyl-CoA plays only a catalytic role in this reaction. In this experiment, acetyl-CoA levels were measured by the hydroxamate assay but similar...
The complete system contained the following components (as micromoles) in a total volume of 1.0 ml: 100 Tris-HCl (pH 7.4), 1.0 pyruvate, 2.5 ATP, 10 MgCl₂, 0.35 acetyl-CoA, 30 NaHCO₃, and 250 μg of pyruvate carboxylase (Stage 4, cf. Table I). The system was incubated for 5 minutes at 30°. The various analyses are described in the text under "Experimental Procedure."

### Table III

**Components of assay system for pyruvate carboxylase**

The activity is expressed as decrease in optical density at 340 μM per minute with the system as described in Fig. 1 and with 12 μg of protein (Stage 5).

<table>
<thead>
<tr>
<th>System</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>0.126</td>
</tr>
<tr>
<td>No pyruvate</td>
<td>0</td>
</tr>
<tr>
<td>No ATP</td>
<td>0</td>
</tr>
<tr>
<td>No acetyl-CoA</td>
<td>0.008</td>
</tr>
<tr>
<td>No MgCl₂</td>
<td>0.015</td>
</tr>
<tr>
<td>No NaHCO₃</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table IV

**Stoichiometry of oxaloacetate formation**

The complete system contained the following components (as micromoles) in a total volume of 1.0 ml: 100 Tris-HCl (pH 7.4), 1.0 pyruvate, 2.5 ATP, 10 MgCl₂, 0.35 acetyl-CoA, 30 NaHCO₃, and 250 μg of pyruvate carboxylase (Stage 4, cf. Table I). The system was incubated for 5 minutes at 30°. The various analyses are described in the text under "Experimental Procedure."

<table>
<thead>
<tr>
<th>System</th>
<th>Oxaloacetate Change</th>
<th>Pyruvate</th>
<th>ADP</th>
<th>Pi</th>
<th>Acetyl-CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>+0.81</td>
<td>-0.78</td>
<td>+0.92</td>
<td>+0.87</td>
<td>-0.08</td>
</tr>
<tr>
<td>No pyruvate</td>
<td>0</td>
<td></td>
<td>+0.07</td>
<td>0</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

### Table V

**Formation of oxaloacetate from labeled precursors by pyruvate carboxylase**

Each experiment contained in a total volume of 1.0 ml (as micromoles): Tris (pH 7.4), Na pyruvate, 2.5 ATP, 3 MgCl₂, 0.7 acetyl-CoA, 30 NaHCO₃, and 50 μg of pyruvate carboxylase (Stage 3). Various components were labeled as indicated. The system was incubated for 5 minutes at 30°. Deproteinization and separation procedure is described in the text.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Oxaloacetate formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>Specific activity*</td>
</tr>
<tr>
<td>C₄O₄</td>
<td>58</td>
</tr>
<tr>
<td>CH₃CO·C₄O⁴⁻</td>
<td>32</td>
</tr>
<tr>
<td>CH₃(CH₃CO)₂·COO⁻</td>
<td>15</td>
</tr>
<tr>
<td>Acetyl-1-C₄O₄-CoA</td>
<td>110</td>
</tr>
</tbody>
</table>

* Specific activity is given as thousands of counts per minute per μmole of compound or moiety after correction for dilution.
oacetate during the isolation procedures with Celite as applied here and accordingly zero time controls were included for each of the experiments. Corrections based on these controls have been applied where necessary in Table V.

**Discussion**

At least six pathways have now been described which lead to the formation of 4 carbon dicarboxylic acids by CO₂ fixation. These include the carboxylation of P-enolpyruvate by P-enolpyruvate carboxykinase (7), by P-enolpyruvate carboxylase (6), and by the enzyme recently reported by Sin, Wood, and Stjernholm (35) which requires P₃ as the phosphate acceptor. Pyruvate can be reductively carboxylated by malate enzyme or converted to oxaloacetate by the enzyme described here. Propionyl-CoA can be converted to succinate by a series of reactions initiated by carboxylation (30, 36). In view of the multiplicity of pathways, it is of some importance to consider the relationship of the pyruvate carboxylase reaction to the other enzymes of the group. The distribution of P-enolpyruvate carboxylase appears to indicate that this enzyme is confined to plants and certain autotrophic bacteria. As mentioned earlier, although P-enolpyruvate carboxykinase is found in close association with pyruvate carboxylase in liver and kidney mitochondria, there is considerable evidence that the two reactions are catalyzed by different protein moieties (cf. Table II). In addition, as described elsewhere (31), the nucleoside phosphate specificity, Kₐₐ values for CO₂, and other properties of the two enzymes are very different. The enzyme from Propionibacterium shermanii which forms oxaloacetate from P-enolpyruvate and CO₂ with P₃ as the acceptor appears to be different both in substrate requirements and distribution from any of the other CO₂-fixing reactions described here (37). Thus, it is unlikely that the reaction discussed here has any relationship to the other pathways described previously with the possible exception of propionyl carboxylase as discussed below.

The observation that the carboxylation of pyruvate is entirely dependent on the presence of acetyl-CoA or propionyl-CoA might suggest that these substances are serving as CO₂ carriers by taking part in a carboxylation and transcarboxylation sequence, since the sum of these reactions is expressed by Reaction 1. Although this hypothesis is attractive from the standpoint of unification, the purified fractions studied here catalyze neither the carboxylation of propionyl-CoA nor the transcarboxylation reaction between methylmalonyl-CoA and pyruvate and the carboxylation of acetyl-CoA is very slow. Hence, if such a mechanism is to be considered it must occur via enzyme-bound CoA intermediates with the individual steps not readily demonstrable. The present data do not rigorously exclude such a mechanism but lend no support to this hypothesis.

The possibility must also be considered that acetyl-CoA does not participate directly in the metabolic events but acts indirectly by affecting the protein in some manner which confers or maintains an active configuration. The possibility that these substances which are not themselves substrates may influence the catalytic activity of enzymes by acting at noncatalytic sites and thereby influencing the conformation of the protein is receiving increased attention. The best established case is that of glutamic acid dehydrogenase in which a number of nonsubstrates have been shown to cause association or dissociation of the subunits of the enzyme with concomitant changes in catalytic activity (38–40). Vagelos, Alberts, and Martin (41) have reported that citrate acts in a similar fashion on acetyl-CoA carboxylase. The inhibition of aspartate transcarbamylase by CTP occurs through action of the latter compound with a noncatalytic site (42). Several other examples, e.g. acetylglutamate for carbamyl phosphate synthetase (43), ATP for adenylic deaminase (44), and ATP for 5-AMP ribosidase (45), suggest that conformational changes induced by nonsubstrates might be common phenomena. The hypothesis that the action of acetyl-CoA on pyruvate carboxylase does not involve direct participation in the reaction is strengthened by the recent finding of Seubert and Remberger (46) that Pseudomonas citronella contains a pyruvate carboxylase which does not require acetyl-CoA for activity although the over-all reactions catalyzed by the avian and bacterial enzymes appear to be identical.

The pyruvate carboxylase reaction as described here is an elaborated version of the Wood-Werkman reaction postulated many years ago to account for dicarboxylic acid formation. This hypothesis was invoked to explain numerous observations on interrelationships of dicarboxylic acid and CO₂ in bacterial growth (1–4) or, in a few cases, to explain studies with cell-free systems (47, 48). With the elucidation of other pathways for dicarboxylic acid formation by CO₂ fixation, i.e. malate enzyme, P-enolpyruvate carboxykinase, and P-enolpyruvate carboxylase, it became clear that many of the above observations could be explained on the basis of one or more of these pathways. There were also numerous observations linking the synthesis of dicarboxylic acids to biotin (3, 4, 49, 50) and it should be noted that none of the three enzymes mentioned above appears to involve biotin directly in its action. Pyruvate carboxylase does contain biotin (31) but this is insufficient evidence to suggest that the earlier studies on biotin and CO₂ fixation reflect the presence of this reaction. Evidence has been presented that the synthesis of malate enzyme is related to biotin levels (51) even though the enzyme itself does not contain biotin and the enzyme is not inhibited by avidin.² In addition, when the pathways of dicarboxylic acid synthesis are enlarged to include propionyl-CoA carboxylase and oxaloacetate-propionyl-CoA transcarboxylase, the requirement for biotin can be attributed to one of these enzymes since both contain this substance (52, 53). It is apparent that information at an enzymic level will be required to establish possible pathways which may be involved in the earlier observations on bacterial growth. As mentioned, Seubert and Remberger have found an enzyme in P. citronella which appears to be similar to the one described here (46). A preliminary report by Fuller and Kornberg (54) of a carboxylation of pyruvate which is stimulated by acetyl-CoA in a species of Chlamydomonas and the previously mentioned finding of Woronick and Johnson with Aspergillus niger (8) also suggest that the pyruvate carboxylase reaction may occur in microorganisms.

**Summary**

An enzyme has been detected in and concentrated from avian liver which catalyzes the formation of oxaloacetate from pyruvate, adenosine triphosphate, and CO₂. The reaction appears not to be related to any other CO₂-fixing reaction previously described. The reaction depends on the presence of acetyl coenzyme A (CoA), or propionyl-CoA, although the acetyl CoA performs only a catalytic function. Isotopic experiments show that the product is oxaloacetate and that this substance is

² M. F. Utter, unpublished results.
formed by the direct carboxylation of pyruvate with labeled acetyl-CoA contributing no radioactivity to the oxaloacetate formed. Stoichiometric experiments indicate that the products of the reaction other than oxaloacetate are adenosine diphosphate and Pi.

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Pyruvate Carboxylase: I. NATURE OF THE REACTION
Merton F. Utter and D. Bruce Keech


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