SPECTROPHOTOMETRIC STUDIES ON THE DECARBOXYLATION OF β-KETO ACIDS*

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The decarboxylation of some β-keto acids of biological importance in oxidative metabolism is catalyzed by specific enzymes. Some of these reactions are reversible and, in this way, play an important part in the biological assimilation of carbon dioxide. Reactions 1, 2, and 3 are catalyzed by specific enzymes from various sources.

\[
\begin{align*}
\text{H} & \quad \text{(1) } \text{COOH-CH}_2\text{.C.CO.COOH} \rightleftharpoons \text{COOH-CH}_2\text{.CH}_2\text{.CO.COOH} + \text{CO}_2 \\
\text{Oxalosuccinic acid (OSA)} & \quad \text{α-Ketoglutaric acid}
\end{align*}
\]

\[
\begin{align*}
\text{(2) } & \quad \text{COOH-CH}_2\text{.CO.COOH} \rightleftharpoons \text{CH}_3\text{.CO.COOH} + \text{CO}_2 \\
\text{Oxalacetic acid (OAA)} & \quad \text{Pyruvic acid}
\end{align*}
\]

\[
\begin{align*}
\text{(3) } & \quad \text{CH}_3\text{.CO.CH}_2\text{.COOH} \rightleftharpoons \text{CH}_3\text{.CO.CH}_2\text{.CH}_2 + \text{CO}_2 \\
\text{Acetoacetic acid (AAA)} & \quad \text{Acetone}
\end{align*}
\]

reaction 1 is catalyzed by an enzyme widely distributed in animal tissues and present in some plants (1). Oxalacetic carboxylase, which catalyzes reaction 2, has been found in bacteria (2), pigeon liver (3), and, to some extent, in other animal tissues as well as in plants (4). Mehler et al. have partially purified this enzyme from Micrococcus lysodeikticus. Acetoacetic carboxylase (reaction 3) has been obtained from acetone bacteria in highly purified form (5).

It is well known that the above β-keto acids are more or less unstable and undergo spontaneous decarboxylation in aqueous solution. Their stability decreases in the following order: acetocetic, oxalacetic, oxalosuccinic.

Krebs (6) observed that the decarboxylation of OAA, but not that of AAA, is catalyzed by polyvalent cations, notably by Zn\textsuperscript{++}, Cu\textsuperscript{++}, Fe\textsuperscript{++},

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Fe++, and Al++. Ca++, Ba++, Mg++, and Mn++ were much less effective. OSA was found in this laboratory to react toward cations in the same way as OAA (1). Speck has recently added Cd++, Co++, and Ni++ to the list of cations that accelerate the decarboxylation of OAA.

Although Mn++, when present alone, is a poor catalyst of the decarboxylation of OSA and OAA, it is required by the specific carboxylases for activity (1-4). Mg++ is much less effective. Acetoacetic carboxylase does not seem to require a metal for activity (5).

The interaction of metals, \( \beta \)-keto acids, and their carboxylases had thus far been studied by manometric methods. By the use of spectrophotometric methods we have been able to gain some understanding of the underlying mechanisms of these reactions. Our results indicate that cations like Al+++ form complexes with OSA, OAA, and AAA in the absence of a protein. The complexes formed by Al+++ with OSA, and OAA are unstable and decarboxylate at a rate faster than that of spontaneous decarboxylation of the \( \beta \)-keto acids, but the AAA complex is stable. While Mg++ and Mn++ also form complexes with OSA and OAA in the absence of protein, the extent of this complex formation or the lability of the complex is too slight to effect rapid decarboxylation. Each of these various complexes has a characteristic absorption spectrum in the ultraviolet. Our data also suggest that the effective catalysis of decarboxylation achieved by oxalosuccinic carboxylase in the presence of Mn++ is largely the result of an acceleration of the rate of complex formation. In the case of oxalacetic carboxylase, it appears that both the rate of complex formation and breakdown are influenced.

**Results**

**Effect of Metals**—The ultraviolet absorption spectra at pH 7.3 of the Na salts of OSA, OAA, AAA, and of \( \alpha \)-ketoglutaric and pyruvic acids (the decarboxylation products of OSA and OAA respectively) are shown in Fig. 1. The absorption of acetone, which is negligible, is not shown. It is evident that within a given wave-length range the decarboxylation of OSA and OAA should result in a decrease of the optical density of the solution. However, the addition of Al+++ which, as pointed out above, markedly increases the rate of decarboxylation of these acids, results at first not in a decrease, but in a sharp increase of the optical density, followed by a rapid decrease toward the level corresponding to the products of decarboxylation. This is illustrated in Fig. 2. Al+++ has no effect on the absorption of \( \alpha \)-ketoglutaric and pyruvic acids. In the case of AAA there is also a sharp increase in

2 Speck, J. F., personal communication.

3 The apparent failure of the optical density to drop to the levels corresponding to complete decarboxylation is due to turbidity contributed by aluminum hydroxide.
optical density upon the addition of Al++. However, the optical density remains stationary once the maximum is reached. It will be recalled that Al+++ does not cause a decarboxylation of this acid. When the maximum optical densities obtained at various wave-lengths after addition of Al+++

Fig. 1. Ultraviolet absorption spectra of β-keto acids and their aluminum complexes. Solutions of keto acids adjusted with NaOH to pH about 7.3. Final concentrations, \(5 \times 10^{-4} \text{M}\) oxalosuccinate and α-ketoglutarate, \(2.5 \times 10^{-4} \text{M}\) oxalacetate, pyruvate, and \(\text{Al}_2(\text{SO}_4)_3, 2.1 \times 10^{-3} \text{M}\) acetoacetate. Total volume, 2.0 cc. Quartz cells; \(d = 0.5 \text{ cm}\). Curve 1, α-ketoglutarate either with or without Al++; Curve 2, oxalosuccinate; Curve 3, oxalosuccinate and Al; Curve 4, pyruvate either with or without Al++; Curve 5, oxalacetate; Curve 6, oxalacetate and Al++; Curve 7, acetoacetate; Curve 8, acetoacetate and Al++. Curves 3, 6, and 8 were obtained by plotting the maximum densities reached at each wave-length. The expression “molecular extinction coefficient” is used only in terms of keto acid concentration and not in terms of keto acid-metal complex concentration, which is unknown.

to aqueous solutions of each of the three β-keto acids are plotted, Curves 3, 6, and 8 (Fig. 1) are obtained.

These results indicate the Al+++ forms complexes, having characteristic

This is probably a result of increased pH during decarboxylation. The addition of small amounts of acid at the conclusion of the reaction removed the turbidity and resulted in conformity of the optical densities to the theoretical conversion of the β-keto acids to the corresponding lower α-keto acids.
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ultraviolet absorption spectra, with each of the above β-keto acids. They further indicate that the aluminum complexes of OSA and OAA are unstable and that the keto acid in the complex is readily decarboxylated. On the other hand, the aluminum complex of AAA is stable. It should be recalled that Fe+++ which gives a red color with the three β-keto acids, accelerates the decarboxylation of OSA and OAA as effectively as does Al+++ This is undoubtedly due to the formation of complexes with absorption in the visible range of the spectrum. The fact that the color given by Fe+++ with OSA and OAA fades rapidly whereas that given with AAA is stable, demonstrates the similar stability of the Fe+++ and Al+++ complexes.

Since Mn++ is required for the activity of oxalosuccinic and oxalacetic carboxylases, its effect on the ultraviolet absorption of the keto acids was determined. The changes produced, although slight, are definite (Fig. 3; cf. Curves 1 and 2 4 and 5). Mn++ does not alter the absorption by α-ketoglutaric and pyruvic acids. The effect of Mg++ on the spectrum of OSA and OAA is qualitatively similar to that of equimolar concentrations of Mn++, although somewhat less marked. When followed in time at a given

![FIG. 2. Time course of spectral changes of oxalosuccinic and oxalacetic acids in the absence and presence of aluminum ions. Final concentrations, 2.5 × 10⁻⁴ M keto acid. Wave-length, oxaloacetate 252 mµ, oxalacetate 270 mµ. Other data as in Fig. 1. Curve 1a, oxalosuccinate; Curve 2a, oxalosuccinate and 1.25 × 10⁻⁴ M Al₂(SO₄)₃; Curve 1b, oxalacetate; Curve 2b, oxalacetate and 1.25 × 10⁻⁴ M Al₂(SO₄)₃.](http://www.jbc.org/)

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wave-length, the effect of Mn\textsuperscript{++} on OSA is similar to, but much less pronounced than, that of Al\textsuperscript{+++}; there is an increase followed by a decrease of the optical density (Fig. 4, Curve 1). Hence Curves 2 and 5 in Fig. 3 were obtained by plotting against the wave-length the maximum optical density reached after addition of Mn\textsuperscript{++} to the keto acids, as was done in the case of Al\textsuperscript{+++}. Therefore, Curves 2 and 5 of Fig. 3 represent the approximate absorption spectra of the manganese complexes of OSA and OAA respectively.

**Fig. 3. Ultraviolet absorption spectra of 8-keto acids and their manganese complexes.** Final concentrations, 1.67 × 10\textsuperscript{-4} M oxalosuccinate, 2.5 × 10\textsuperscript{-4} M oxalacetate. Total volume, oxalosuccinate samples 3.0 cc. (\(d = 1.0\) cm.), oxalacetate samples 2.0 cc. (\(d = 0.5\) cm.). Other data as in Fig. 1. Curve 1, oxalosuccinate; Curve 2, oxalosuccinate and 1.67 × 10\textsuperscript{-4} M MnCl\textsubscript{2}; Curve 3, oxalosuccinate, MnCl\textsubscript{2}, 0.02 cc. of enzyme (pig heart extract), and 0.134 M KCl; Curve 4, oxalacetate; Curve 5, oxalacetate and 5 × 10\textsuperscript{-4} M MnCl\textsubscript{2}. Curves 2, 3, and 5 were obtained by plotting the maximum densities reached at each wave-length.

**Oxalosuccinic Carboxylase**—As mentioned above, this enzyme has been found in manometric studies to require Mn\textsuperscript{++} for activity, Mg\textsuperscript{++} being ineffective at similar concentrations (1). Spectrophotometrically, addition of oxalosuccinic carboxylase to OSA results in a slight increase in optical density at 240 m\(\mu\), followed by a gradual decrease.\textsuperscript{4} With both enzyme and

\textsuperscript{4} This effect of the enzyme, in the absence of added Mn\textsuperscript{++}, may be due to the presence of small amounts of this metal in the crude enzyme preparations used. The effect largely disappears after the enzyme is dialyzed against pyrophosphate at pH 8.4, followed by dialysis against KCl to remove the pyrophosphate.
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Mn$^{++}$ present, there is a large and rapid increase in density, succeeded by a prompt decline to a value approximating that of α-ketoglutaric acid. These results are shown in Fig. 4 (Curves 1, 2, and 3). Potassium chloride (0.134 M) inhibits the increase in density associated with the addition of Mn$^{++}$ alone (cf. Curves 1 and 1a, Fig. 4), but it greatly augments the changes resulting from the addition of oxalo succinic carboxylase with Mn$^{++}$ and, to a lesser extent, those caused by the carboxylase without Mn$^{++}$. These striking effects of potassium chloride are shown in Fig. 4 (cf. Curves 2 and 2a, 3 and 3a); the actual peak of Curve 3a was reached too early to permit accurate measurement. The action of potassium chloride is probably an unspecific effect of increased ionic strength, since the effects of potassium
bromide (0.134 M) and sodium chloride (0.134 M) were indistinguishable from those of potassium chloride. The manometric data in Table I verify the stimulatory effect of potassium chloride on oxalosuccinic carboxylase activity.

The ultraviolet spectrum obtained by plotting maximum optical density against wave-length after the addition of OSA to carboxylase and Mn

\[\text{Effect of Potassium Chloride on Enzymatic Decarboxylation of Oxalosuccinic Acid}\]

The Warburg vessels contained 0.021 M citrate buffer of pH 5.6 and 0.007 M oxalosuccinic acid (adjusted with NaOH to pH 5.6), either with or without enzyme, 0.0013 M MnCl\(_2\), and 0.134 M KCl, as indicated. Final volume 2.8 cc. Air in gas phase. Temperature, 16°. Oxalosuccinate tipped in from side bulb after temperature equilibration.

<table>
<thead>
<tr>
<th>Additions</th>
<th>CO(_2) evolved in 5 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pig heart extract (1.6 mg. protein)</td>
</tr>
<tr>
<td></td>
<td>Change due to enzyme</td>
</tr>
<tr>
<td>Mn(^{++})</td>
<td>c.mm.</td>
</tr>
<tr>
<td>Enzyme and Mn(^{++})</td>
<td>51</td>
</tr>
<tr>
<td>Mn(^{++}) and KCl</td>
<td>138</td>
</tr>
<tr>
<td>Enzyme, Mn(^{++}), and KCl</td>
<td>190</td>
</tr>
</tbody>
</table>

oxalosuccinic carboxylase increases the rate of formation of the OSA-Mn complex.

When Mg\(^{++}\) is substituted for Mn\(^{++}\) at similar concentrations, the addition of oxalosuccinic carboxylase to a mixture of OSA and the cation, whether in the absence or presence of KCl, produces little or no spectrophotometric change beyond that obtained on mixing OSA and Mg\(^{++}\) in the absence of the enzyme. This is in agreement with the observed ineffectiveness of Mg\(^{++}\) in manometric experiments. Since Mg\(^{++}\) and Mn\(^{++}\) bring about similar changes on the absorption of OSA, it would appear that the ineffectiveness of Mg\(^{++}\) with oxalosuccinic carboxylase is due to lack of affinity between the protein and this metal.

Further evidence that the spectrophotometric changes observed on mixing oxalosuccinic carboxylase and OSA, in the presence of Mn\(^{++}\), are strictly specific and intimately related to the decarboxylation of the keto acid is
furnished by (a) the failure of oxalosuccinic carboxylase to produce any effect on OAA either in spectrophotometric or manometric (1) experiments, and (b) the absence of either spectrophotometric or manometric (1) effects of oxalacetic carboxylase on OSA. All these results indicate that there are two factors involved in the formation of an active oxalosuccinic carboxylase system: the possibility of complex formation between β-keto acid and metal, and the specific affinity of the enzyme for each of these.

Fig. 5. Spectral changes of oxalosuccinate decarboxylation at various enzyme concentrations. All samples contained 1.67 × 10⁻⁴ M oxalosuccinate, 1.67 × 10⁻⁴ M MnCl₂, and 0.134 M KCl. Total volume of reaction mixtures, 3.0 cc. (d = 1.0 cm.). Wave-length, 240 mμ. Other data as in Fig. 1. Curves 1, 2, and 3, 0.03, 0.02, and 0.01 cc. of enzyme (pig heart extract) respectively. The arrows at the lower left indicate the times at which maximum optical densities were reached in each case.

The curves in Fig. 5 demonstrate that there is some proportionality between the concentration of oxalosuccinic carboxylase and the maximum optical density in the presence of constant amounts of Mn²⁺ and keto acid. It will be observed, in addition, that the time for attainment of the maximum value is related inversely to the enzyme concentration, and also that the rate of subsequent fall of optical density roughly parallels enzyme concentration. These results suggest a rapid and sensitive optical test for oxalosuccinic carboxylase.

Determination of the optimum concentrations of OSA and Mn²⁺ was made with a constant amount of enzyme with the maximum density as a measure of activity. Under these conditions the optimum concentration of
Comparative Effectiveness of Mg\textsuperscript{++} and Mn\textsuperscript{++} on Oxalacetic Carboxylase of Micrococcus lysodeikticus

The Warburg vessels contained 0.025 M acetate buffer, pH 5.0, 0.0095 M oxalacetic acid (adjusted with NaOH to pH 5.0), and 0.5 mg. of enzyme. Other additions as indicated. Final volume 2.0 cc. Air in gas phase. Temperature, 25°.

<table>
<thead>
<tr>
<th>MgCl\textsubscript{2}</th>
<th>MnCl\textsubscript{2}</th>
<th>CO\textsubscript{2} evolution*</th>
<th>Change due to metal</th>
</tr>
</thead>
<tbody>
<tr>
<td>(5 \times 10^{-4})</td>
<td>(8 \times 10^{-6})</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>(1 \times 10^{-3})</td>
<td>(2 \times 10^{-4})</td>
<td>48</td>
<td>32</td>
</tr>
<tr>
<td>(4 \times 10^{-3})</td>
<td>(5 \times 10^{-4})</td>
<td>64</td>
<td>48</td>
</tr>
</tbody>
</table>

* During 5 to 15 minutes after tipping oxalacetate from the side bulb.

Fig. 6. Spectral changes connected with the enzymatic decarboxylation of oxalacetic acid. Final concentrations, \(2.5 \times 10^{-4}\) M oxalacetate, \(2.5 \times 10^{-4}\) M MnCl\textsubscript{2}. Total volume of reaction mixtures, 2.0 cc. (\(d = 0.5\) cm.). Wave-length, Curves 1a and 2a, 230 mp; Curves 1b and 2b, 290 mp. Other data as in Fig. 1. Curves 1a and 1b, oxalacetate and MnCl\textsubscript{2}; Curves 2a and 2b, oxalacetate, MnCl\textsubscript{2}, and 0.5 mg. of enzyme from Micrococcus lysodeikticus.
OSA was about $2 \times 10^{-4}$ M, while concentrations over $4 \times 10^{-4}$ M were inhibitory. Optimum concentrations of Mn$^{++}$ were also reached at about $2 \times 10^{-4}$ M.

**Oxalacetic Carboxylase**—Krampitz and Werkman reported that Mg$^{++}$ and Mn$^{++}$ were approximately equally effective in stimulating the decarboxylation of OAA in the presence of crude lysed preparations of *Micrococcus lysodeikticus* (2). With a partially purified preparation from the same source we found, as in the case of oxalosuccinic carboxylase, that Mn$^{++}$ is much more effective than Mg$^{++}$. This is shown in Table II.

The curves in Fig. 6 show the spectral changes observed on mixing OAA with Mn$^{++}$, either with or without oxalacetic carboxylase, at two different wave-lengths, 230 and 290 mμ. It should be recalled that at 230 mμ, Mn$^{++}$ does not alter the absorption of OAA, whereas at 290 mμ it produces its maximum absorption increase (Fig. 3, Curves 4 and 5). It is apparent that the two curves representing enzymatic decarboxylation differ markedly. There is a prompt and steady decline at 230 mμ, while a transitory plateau, indicating formation of a complex, precedes the decline at 290 mμ. The activity of the oxalacetic enzyme is not enhanced by increased salt concentration.

**DISCUSSION**

Since metal complexes are probably formed with the enol form of keto acids, the non-enzymatic reactions of OSA and OAA studied in this paper might involve the following steps.

1. $\beta$-Keto acid $\leftrightarrow$ enol
2. Enol + cation $\leftrightarrow$ complex
3. Complex $\leftrightarrow$ α-keto acid + CO$_2$ + cation

None of the above is an instantaneous reaction. Each proceeds at a rate depending upon the nature of both the keto acid and the cation. When the cation is Al$^{+++}$, and presumably any which have been shown to accelerate the decarboxylation of the two keto acids, the above set of reactions would proceed rapidly in the absence of an enzyme, since aluminum complexes are either formed rapidly or are very unstable or both.

Enzymatic catalysis of the over-all reaction, $\beta$-keto acid $\leftrightarrow$ α-keto acid + CO$_2$, might be due to acceleration by the enzyme of one of the above reactions (Nos. I, II, or III), of two of them, or of all three. Since the enzymes are active only in the presence of Mn$^{++}$ and since this cation by itself is not very effective in accelerating decarboxylation, the enzyme might be considered to catalyze Reaction I, i.e. the formation of enol. However, since, as previously mentioned, the spectrophotometric changes produced by Mn$^{++}$ and Mg$^{++}$ on OSA, in the absence of enzyme, are about the same, it
would seem that the affinity of the keto acid for each of these two cations is approximately equal. This eliminates the possibility that the enzyme merely acts by catalyzing Reaction I, since one would then expect equal effectiveness of Mg$$^{++}$$ and Mn$$^{++}$$ in the presence of enzyme.

The mechanism of the enzyme-catalyzed reactions may be conveniently discussed with the accompanying scheme as a basis.

\[
\begin{align*}
\beta\text{-Keto acid} & \xrightleftharpoons{a} \text{enzyme complex} \xrightleftharpoons{b} \alpha\text{-keto acid} + \text{CO}_2 \\
& + \text{Mn}^{++} \quad \quad \quad \quad \quad + \text{Mn}^{++} \\
& + \text{Enzyme} \quad \quad \quad \quad \quad + \text{enzyme} \\
& \text{Enzyme} \quad \quad \quad \quad \quad + \text{complex}
\end{align*}
\]

In the case of OSA decarboxylation, our data are in agreement with the view that the enzyme catalyzes the formation of an OSA-Mn complex. It is difficult to interpret the increase in optical density caused by the enzyme as due to anything but increased complex formation. While it is clear that this increase in complex formation can account for a major part of the overall catalysis of decarboxylation, it cannot be determined from our data whether the enzyme-OSA-Mn complex dissociates into enzyme and OSA-Mn complex, which then decarboxylates spontaneously (Reactions $a \rightarrow b' + c$), or whether the over-all reaction proceeds mainly by way of the partial reactions, $a$ and $b$.

The ability of the enzyme to catalyze the reaction in the direction of carboxylation of $\alpha$-ketoglutaric acid has been demonstrated by linking the synthesis of the $\beta$-keto acid with reduction to isocitrate. This reversal is most likely to proceed by Reactions $b$ and $a$, since a non-enzymatic trimolecular reaction to form OSA-Mn complex from $\alpha$-ketoglutaric acid, CO$$_2$$, and Mn$$^{++}$$ is very unlikely.

In the case of oxalacetic carboxylase the time curve with enzyme, Mn$$^{1^+}$$, and OAA at 290 m$$\mu$$ (Fig. 6) does not show much increase in complex over that formed by Mn$$^{++}$$ and OAA in the absence of enzyme but it indicates that, in the presence of the enzyme, the complex decarboxylates more rapidly. Comparison with the curves obtained at 230 m$$\mu$$, at which the OAA-Mn complex has the same absorption coefficient as OAA, shows that the enzyme must increase the rate of complex formation because the curve at 290 m$$\mu$$ (Curve 2b) shows no decrease in density at a point when that at 230 m$$\mu$$ (Curve 2a) indicates that the OAA is already decarboxylated to some extent. It would then seem that, in this case, the reaction is proceeding by way of the partial reactions, $a$ and $b$.

The action of acetoacetic carboxylase seems to involve an entirely different mechanism from that operating in the case of the oxaloacetic and oxalacetic enzymes. The decarboxylation of AAA is unaffected by metals and,
although complexes can be formed with polyvalent cations, such complexes are stable. It would appear that the nature of the \( \beta \)-keto acid, and especially its degree of stability and its ability to form unstable metal complexes, largely determines the mechanism by which enzymatic decarboxylation is carried out. With the very unstable OSA there are no indications of the need for a prosthetic group besides Mn\(^{++} \). With the more stable OAA a prosthetic group might well be involved in addition to Mn\(^{++} \). Recent work suggests a possible function of biotin in some enzymes concerned with OAA and dicarboxylic acid metabolism (8). In the case of AAA, much more stable than the other two \( \beta \)-keto acids, the work of Davies suggests the presence of an as yet unknown prosthetic group in the carboxylase, and no metal is involved (5).

It is well known that amines have a marked catalytic effect on the decarboxylation of \( \beta \)-keto acids; aniline (9) is widely used for their quantitative estimation. This might lead one to believe that amino groups on side chains of a number of proteins might generally catalyze \( \beta \)-keto acid decarboxylation in an unspecific manner. While this seems to be true to some extent (10), it is doubtful whether this unspecific catalysis is of any significance in the biological reactions involving \( \beta \)-keto acids.

Methods

Spectrophotometric Measurements—To each of two quartz cells there were added equal amounts of the various components, with the exception of the keto acids. These were added only to one cell, while the other cell served as control for 100 per cent light transmission. The addition of keto acid was always made last and was immediately preceded by a zero time reading of the optical density of the experimental solution. No buffers were used other than the keto acids themselves, although, as already mentioned, an increase in pH may cause some turbidity in the presence of aluminum ions. Measurements were made in air at room temperature in the Beckman spectrophotometer. Other experimental details are given in the legends to the figures.

Enzyme Preparations—The oxalosuccinic carboxylase was a dialyzed extract of washed, acetone-dried pig heart (1) containing from 3 to 5 mg. of protein per cc. In some cases the ice-cold extract was precipitated with 3 volumes of acetone at 0\(^\circ\). An aqueous solution of the dried acetone precipitate, after removal of an insoluble residue by centrifugation, was then used as the enzyme. This is referred to as “acetone enzyme.” The oxalacetic carboxylase was a partially purified preparation from Micrococcus lysodeikticus.\(^1\) This was kept as a dry acetone powder and was dissolved in water before use.

Chemical Preparations—The preparation of oxalosuccinic acid has already
been described (1). The barium salt was dissolved in water, with the aid of some dilute hydrochloric acid, and converted to the sodium salt just before use. Oxalacetic acid was obtained by hydrolysis of diethyl oxalacetate according to Krampitz and Werkman (2), with modifications suggested by Dr. Fritz Lipmann. The preparation of acetoacetic acid was made according to Davies (5). Crystalline sodium pyruvate and α-ketoglutaric acid were prepared as previously described (11).

**SUMMARY**

The catalytic decarboxylation of oxalosuccinic and oxalacetic acids by aluminum ions is preceded by the formation of unstable intermediates having characteristic ultraviolet absorption spectra. The peaks are at 252 and 274 μm respectively. The intermediates are assumed to be β-keto acid-aluminum complexes which undergo rapid decarboxylation. Acetoacetic acid forms a similar but stable complex with Al+++. Spectral changes occurring with Mg+++ and Mn+++ also suggest the formation of complexes with these cations.

In the presence of Mn+++ and oxalosuccinic acid, oxalosuccinic carboxylase causes a pronounced increase in absorption at 240 μm, presumably as a result of increased formation of an intermediate oxalosuccinate-manganese complex; this increase is followed by a rapid drop, indicating decarboxylation. These effects of the enzyme are markedly augmented, as is the decarboxylation of oxalosuccinate followed manometrically, by an increase of the ionic strength of the reaction mixtures to 0.134 M.

In the presence of Mn+++ and oxalacetic acid, oxalacetic carboxylase brings about a rapid decrease in absorption, indicating decarboxylation, at 230 μm. At 290 μm this decrease is preceded by a small transient increase. In this case, if the carboxylase brings about an increased formation of an intermediate oxalacetic-manganese complex, the decarboxylation of this complex must be increased to a similar extent.

The spectrophotometric and manometric changes produced by oxalosuccinic and oxalacetic carboxylases on their respective substrates are strictly specific with regard to β-keto acid and metal.

The above results are discussed in connection with the mechanism of the reactions catalyzed by cations and by β-keto acid carboxylases.

We wish to thank Dr. Isidor Greenwald and Dr. Milton Levy for helpful suggestions. We are also indebted to Mr. Morton C. Schneider for skilful technical assistance.

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5 Personal communication. We are very indebted to Dr. Lipmann for this information.
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   2082 (1935).
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