DIACETYL OXIDATION BY STREPTOCOCCUS FAECALIS, A LIPOIC ACID DEPENDENT REACTION

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Received for publication June 14, 1954

The mechanism of splitting of the alpha-diketo linkage has received considerable attention in recent years. Diacetyl oxidation via the diacetyl mutase reaction discovered by Green et al. (1947) in pigeon breast muscle particulate preparations was formulated as follows:

\[
2\text{CH}_4\text{C}-\text{C}-\text{CH}_2 + 2\text{H}_2\text{O} \rightarrow \text{CH}_3\text{C} + \text{CH}_2\text{C} - \text{C} - \text{CH}_3
\]

In the over-all reaction, one molecule of diacetyl is oxidized to two molecules of acetate, and another molecule of diacetyl is reduced to acetoin. The reaction was shown to require coacarboxylase (DPT).\(^1\) Subsequently, Schweet et al. (1951) expanded this finding and pointed out the similarity between the diacetyl reaction and pyruvic acid oxidation, with respect to the metabolism of the "active aldehyde" derived from the dicarbonyl split. Juni and Heym (1953), working with *Aerobacter aerogenes*, isolated two new compounds following diacetyl metabolism. One of them, diacetylthiamymethylcarbinol, was formed by splitting diacetyl to form "active aldehyde", followed by a benzoin type of condensation of this aldehyde with another molecule of diacetyl. The other, acetylbutanediol, is a reduction product of diacetylthiamymethylcarbinol. In the *A. aerogenes* system, the active aldehyde derived from diacetyl is not oxidized, but the condensation product, diacetylthiamymethylcarbinol, undergoes further oxidation to acetate and diacetyl. Diacetyl-methylcarbinol was also found to account for a major portion of the reduced product in the pigeon breast muscle system (Juni and Heym, 1954).

The above mentioned splitting and condensation reactions are coacarboxylase dependent. Recently, Dolin (1953) in a preliminary report showed that the oxidation of diacetyl by *Streptococcus faecalis* in addition to being coacarboxylase dependent also depended upon the presence of lipoic acid,\(^4\) the biocatalyst previously known to be required for pyruvic acid oxidation (O'Kane and Gunsalus, 1948). This finding tended to generalize the function of lipoic acid by implicating it in those reactions involving the split of an alpha-dicarbonyl linkage followed by oxidation of the active aldehyde thus formed.

Reed (1953), in a review paper on lipoic acid and thiamin dependent reactions, reported that the pyruvic apoxidase of his *Escherichia coli* mutant that requires lipothiamide pyrophosphate catalyzes the cleavage of diacetyl to yield a C\(_2\) fragment at the aldehyde level which is activated either for oxidation or condensation, depending upon the acceptor. With lipothiamide pyrophosphate, the aldehyde is activated for oxidation and with DPT for condensation with free aldehyde or oxidation with ferricyanide.

The present paper is an amplification of the previous report (Dolin, 1953) on diacetyl oxidation by *S. faecalis*.

**MATERIALS AND METHODS**

**Enzyme preparations.** Vacuum-dried cell preparations of *Streptococcus faecalis*, strain 10C1, were prepared as described previously (Dolin and Gunsalus, 1951). Vacuum-dried preparations of lipoic acid deficient cells were kindly supplied by Dr. I. C. Gunsalus. Enzyme fraction I, used in the experiment shown in table 3, is one of the

\(^1\) Work performed at the Oak Ridge National Laboratory under Contract Number W-7405-Eng. 26 for the Atomic Energy Commission.

\(^4\) This observation on the lipoic acid dependency of diacetyl oxidation was made by the author in the laboratory of Dr. I. C. Gunsalus, Department of Bacteriology, University of Illinois.
previously described enzymatic components of the alpha-keto acid dismutation system of *S. faecalis* (Dolin and Gunsalus, 1952; Gunsalus, 1953).

**Manometric and chemicals.** CO₂ evolution from bicarbonate buffer was followed in the conventional Warburg apparatus at 37 C, with 100 per cent CO₂ as the gas phase. Diacetyl was added to one arm of a double side arm Warburg flask following per cent Warburg 52 side arm was tion of White procedure 50 a of the cells removed by centrifugation in the cold, in stoppered tubes.

**Diacetyl.** Diacetyl was determined by the procedure of White *et al.* (1946). Acetoin does not interfere with this method.

**Acetoin.** The following procedure was adopted for the determination of small amounts of acetoin in the presence of large amounts of diacetyl. A suitable aliquot of the acidified reaction mixture from a manometric experiment was diluted to about 5 ml with distilled water, neutralized to bromthymol blue with 1 n KOH, and rinsed into a 50 ml distilling flask to give a final volume of 10 ml. The solution was then distilled to dryness in a Stotz still (Stotz, 1943) using a 20 ml test tube calibrated at 10 ml as a receiver. The tube was then placed in a 60 C water bath, and a stream of helium was bubbled through the solution from a glass capillary placed at the bottom of the tube. Controls run through the entire procedure in the presence of enzyme blanks showed that, after about one hour, 15 μm of diacetyl could be quantitatively removed from solutions containing from 20–100 μg of acetoin, with 90–95 per cent recovery of the acetoin. The acetoin was determined by the Voges-Proskauer reaction as modified by Westerfeld (1945).

**Acetic acid.** Acetic acid was determined after steam distillation, by titration with 1 n KOH added from a Greiner ultramicroburette calibrated in 0.0001 ml. Before steam distillation, it was necessary to remove ferricyanide by precipitation with CuSO₄ since ferricyanide caused a high blank. Standard amounts of acetic acid run through the procedure showed that recoveries of 95 per cent were obtained in the range of 5–10 μm of total acetic acid distilled. Chromatography on celite (Phares *et al.*, 1952) was used to identify the volatile acid formed in the enzyme experiments.

**Ferrocyanide.** Ferrocyanide was determined by a modification (Hager, 1953) of the procedure of Horvath and Knehr (1941).

Diacetyl (Fisher Scientific Co., cp) was repurified by distillation through a fractionating column. The fraction distilling sharply at 88 C, 760 mm, was collected. The synthetic, crystalline lipoic acid was a gift from Dr. I. C. Gunsalus. Co-carboxylase was obtained from the Nutritional Biochemical Company and diphosphopyridine nucleotide, 90 per cent pure, from Sigma Chemical Co.

**EXPERIMENTAL RESULTS**

Previous work with *S. faecalis*, 10C1, demonstrated that the pyruvic acid oxidation system of this organism required an unknown cofactor (O’Kane and Gunsalus, 1948). This factor was later shown to be necessary for the oxidation of *alpha*-ketobutyrate (Dolin and Gunsalus, 1952) and *alpha*-ketoglutarate (Reed and DeBusk, 1952). The crystallization, synthesis, and characterization of this cofactor as 6,8-dimercapto octanoic acid have come about due to the joint efforts of the laboratories of Gunsalus, Reed, and the Eli Lilly group and through the independent work of the Lederle Laboratories. For complete references see the recent reviews of Reed (1953) and Gunsalus (1953, 1954). The function of this cofactor has recently been elucidated (Gunsalus, 1954; Reed, 1953); it has been shown to function in acetyl and hydrogen transfer. In some of the early work, one of the approaches used was to examine the metabolism of compounds that might be expected either to be or to yield intermediates on the keto acid pathway. Attempts were made to implicate lipoic acid in the activation of free aldehyde to “active aldehyde”; however, it was found that in *S. faecalis* neither the oxidation or dismutation of acetaldehyde (Barner and O’Kane, 1952) nor the oxidation with ferricyanide as electron acceptor (Dolin, 1953) requires lipoic acid. However, diacetyl oxidation by this organism, a process which might be expected to yield an active aldehyde similar or identical to that derived from the oxidation of keto acids, was found to be a lipoic dependent
reaction (Dolin, 1953). The reactions of diacetyl in the absence and presence of ferricyanide as electron acceptor may be formulated as follows:

\[
\begin{align*}
(1) & \quad 2 \text{CH}_4\text{C}-\text{C}-\text{CH}_4 + 2 \text{H}_2\text{O} & \rightarrow & \quad 2 \text{CH}_4\text{COOH} + \text{CH}_3\text{C}-\text{C}-\text{CH}_4 \\
(2) & \quad 2 \text{CH}_4\text{C}-\text{C}-\text{CH}_4 + \text{H}_2\text{O} & \rightarrow & \quad \text{CH}_3\text{COOH} + \text{CH}_3\text{C}-\text{C}-\text{CH}_4 \\
(3) & \quad \text{CH}_4\text{C}-\text{C}-\text{CH}_4 + 2 \text{H}_2\text{O} + 2 \text{Fe(CN)}^\text{II} & \rightarrow & \quad 2 \text{CH}_3\text{COOH} + 2 \text{Fe(CN)}^\text{III} + 2 \text{H}^+ 
\end{align*}
\]

The author is indebted to Dr. E. Juni for demonstrating the occurrence of reaction 2 in the preparations of *S. faecalis* used in the present investigation. In order to determine diacetyl-methylcarbinol, it is necessary to employ a series of colorimetric reactions that distinguish between diacetyl, acetoin, diacetylmethylcarbinol, and acetylbutanediol (Juni, *personal communication*). Reactions 1–3 may be followed manometrically by determining the rate of CO₂ evolution from bicarbonate buffer. The chemical balances for the reactions are shown in table 1. Acetic acid was determined by titration after steam distillation, as described in the methods section. In the absence of ferricyanide, the reaction resembles the diacetyl mutase reaction of Green *et al.* (1947). The stoichiometry does not conform to theory for several reasons. First, the reaction in the absence of ferricyanide is a combination of reactions 1 and 2. In *S. faecalis*, about one-third of the Voges-Proskauer reacting compound is actually diacetylmethylcarbinol. No acetylbutanediol is formed. This bacterial system then resembles the pigeon breast muscle system in forming both acetoin and diacetylmethylcarbinol, except for the fact that the animal system in the experiments of Juni and Heym (1954) forms relatively more diacetylmethylcarbinol than acetoin. As shown here, for *S. faecalis* and as confirmed by repeated experiments, the acetic acid is consistently high and the acetoin low. These results may be due to the presence of an unknown electron acceptor in the vacuum-dried cell preparations. In addition, from the known properties of diacetylmethylcarbinol (Juni, 1954, *personal communication*) it is certain that the procedure used for the acetoin determination in the present experiments (specifically the neutralization with 1 N KOH) would have destroyed diacetylmethylcarbinol without giving an equivalent amount of acetoin. The acetic acid figure is also likely to be unreliable since hydrolysis of diacetylmethylcarbinol (which would be present in the steam distillate) during the titration of acetic acid gives unpredictable results.

In the presence of ferricyanide as oxidant, the physiological aldehyde acceptor is largely re-

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**TABLE 1**

Diacetyl oxidation by vacuum-dried *Streptococcus faecalis*, strain 10C1

<table>
<thead>
<tr>
<th>Additions</th>
<th>μM Used</th>
<th>μM Formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diacetyl found calc.</td>
<td>12.9</td>
<td>15.4</td>
</tr>
<tr>
<td>Diacetyl + Fe(CN)⁶⁺ found calc.</td>
<td>6.6</td>
<td>12.1</td>
</tr>
</tbody>
</table>

KHCO₃, 74 μM; MgSO₄, 5 μM; diacetyl, 30 μM; potassium ferricyanide, 50 μM; vacuum-dried cells, 40 mg; gas phase, 100 per cent CO₂; final volume, 2 ml; pH 6.5; incubation at 37 C, 120 min.
TABLE 2
Lipoic requirement for diacetyl oxidation:
Ferricyanide as oxidant. Streptococcus
faecalis, strain 10C1

<table>
<thead>
<tr>
<th>Additions</th>
<th>µL CO₂/hr</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Apopyruvate dehydrogenase (vacuum-dried cells), 10 mg</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>2. (1) + 0.28 µg DL-α-lipoic acid</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>3. (1) + diacetyl, 30 μM</td>
<td>42</td>
<td>27</td>
</tr>
<tr>
<td>4. (3) + 0.14 µg DL-α-lipoic acid</td>
<td>171</td>
<td>153</td>
</tr>
<tr>
<td>5. (3) + 0.28 µg DL-α-lipoic acid</td>
<td>174</td>
<td>156</td>
</tr>
<tr>
<td>6. (4), but with heated cells*</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

KHCO₃, 74 μM; MgSO₄, 5 μM; potassium ferricyanide, 50 μM; dried cells, diacetyl and lipoic acid as shown; gas phase, 100 per cent CO₂; final volume, 2 ml; pH 6.5; incubation at 37 C.

* Cell suspension heated at 100 C, 10 min.

placed, acetoin formation is strongly inhibited, and the oxidation takes place as formulated in reaction 3 when the correction for acetoin formation is made. The balance in the absence and presence of ferricyanide was done in the same experiment under identical conditions, and the reaction was stopped at the same time in all flasks so that the depression of acetoin formation with ferricyanide as oxidant reflects the actual rate of acetoin accumulation under these conditions. Added acetoin is not oxidized in this system. An inhibition of acetoin formation in the presence of ferricyanide is also found in the pigeon breast muscle system (Schweet et al., 1951).

In the ferricyanide assay, presumably the first stages in the activation of keto acids (and of dicarbonyl compounds) are measured (Schweet et al., 1951; Hager et al., 1953). For a discussion of such “carboxylase” reactions see Gunsalus (1954).

The lipoic acid requirement for diacetyl oxidation, with ferricyanide as oxidant, is shown in table 2. These apopyruvate dehydrogenase cells were obtained by growth in the synthetic medium of O’Kane and Gunsalus (1948). With this particular preparation, using pyruvate or alpha-ketobutyrate as substrate, the stimulation given by saturating amounts of lipoic acid is of the order of 6 to 7-fold. With diacetyl as substrate it is apparent that an excess of synthetic lipoic acid (0.14 µg of DL-lipoic acid, corresponding to 0.07 µg of the active form) gives a stimulation of the same order of magnitude as that found for keto acids. The reaction as measured by ferricyanide reduction shows the same degree of lipoic dependency as does the reaction measured manometrically. Hager et al. (1953) have also shown the necessity for lipoic acid for ferricyanide reduction by apopyruvate cells of S. faecalis with keto acids as the substrate. These results, for reasons not yet understood, do not agree with the findings of Seaman (1952) working with Tetrahymena geleii extracts or with those of Reed and DeBusk (1953) who found no lipoic requirements for ferricyanide reduction in these systems. The lipoic stimulation of diacetyl oxidation by S. faecalis in the absence of ferricyanide is only about one-half that found in the ferricyanide system. This is due to the competing nonoxidative diacetyl methylcarbinol pathway which is largely by-passed in the presence of ferricyanide, as shown in table 1. Judging from the results obtained with the acetoin condensation in S. faecalis (Dolin and Gunsalus, 1951), the benzoin type of condensation should not be lipoic dependent.

With cell-free extracts, it is possible to show the coacarboxylase requirement for diacetyl oxidation. In table 3 the ketobutyrate and diacetyl oxidation rates are compared, using ferricyanide as oxidant. Enzyme fraction I is a component of the keto acid dismutation system of S. faecalis (Dolin and Gunsalus, 1952) corresponding in ammonium sulfate solubility to E. coli fraction A (Korkes et al., 1951). With S. faecalis fraction

TABLE 3
Diphosphothiamin dependence of diacetyl oxidation:
Ferricyanide as oxidant. Streptococcus
faecalis, strain 10C1

<table>
<thead>
<tr>
<th>Additions</th>
<th>µM Fe(CN)₆³⁻ Formed/ml Enzyme/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Enzyme fraction I</td>
<td>0.22</td>
</tr>
<tr>
<td>2. (1) + alpha-ketobutyrate, 40 µM</td>
<td>3.80</td>
</tr>
<tr>
<td>3. (1) + diacetyl, 30 µM</td>
<td>3.10</td>
</tr>
<tr>
<td>4. (3) without DPN</td>
<td>3.20</td>
</tr>
<tr>
<td>5. (3) without coenzyme A</td>
<td>3.25</td>
</tr>
<tr>
<td>6. (3) without DPT</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Potassium phosphate buffer, 200 µM, pH 6.0; MgSO₄, 5 µM; diacetyl and alpha-ketobutyrate concentrations as shown; potassium ferricyanide, 25 µM; DPT, 100 µg; DPN, 100 µg; coenzyme A, 30 µg; enzyme fraction I (see text), 1.5 mg protein; final volume, 2 ml; incubation at 37 C.
Both diacetyl and alpha-ketobutyrate are activated at similar rates. As shown, the diacetyl reaction is completely dependent upon the addition of cocarboxylase but not upon the addition of DPN or coenzyme A. It is not necessary to add lipoic acid since the enzyme contains lipoic acid in a bound form. Although the data are not shown here, a similar situation holds when keto-butyrate is the substrate. The extract, as shown in experiments on keto acid dismutation, is deficient in cocarboxylase, DPN, and coenzyme A. S. faecalis contains an enzyme that oxidizes DPNH with ferricyanide as the electron acceptor (Dolin, 1954). However, as the DPN data show, this enzyme appears to play no part in the present reaction and thus is not an obligate component of the ferricyanide assay system. The data above then are completely analogous to those obtained by Hager et al. (1953) with fraction A from E. coli and pyruvate as substrate, i.e., cocarboxylase is the only coenzyme that must be added and no further activity is obtained upon the addition of the other coenzymes of the complete keto acid dismutation system. The acyl transfer system is by-passed.

Free acetaldehyde is not oxidized in the cell-free S. faecalis system; therefore it cannot be an intermediate in diacetyl oxidation.

**DISCUSSION**

In the previous report (Dolin, 1953) on diacetyl breakdown in S. faecalis, it was suggested that hydrogen transfer was one of the functions of lipoic acid since diacetyl oxidation took place in the presence of ferricyanide, which would maintain lipoic acid in the disulfide form. Trivalent arsenicals, such as arsenite, which block the dithiol group completely inhibit lipoic catalysis (Gunsalus, 1953). It is now known, from experiments in which substrate amounts of reduced lipoic acid were employed (Hager and Gunsalus, 1953; Gunsalus, 1954), that lipoic acid functions both in hydrogen and acetyl transport, in confirmation of a reaction sequence previously proposed by Gunsalus (1952). Evidence for a similar scheme involving lipothiamide pyrophosphate was presented by Reed and DeBusk (1953).

The following scheme relates the metabolism of diacetyl to the reactions already described for keto acids (figure 1). The first step is a cleavage of the dicarbonyl bond in pyruvate to form CO₂ and H⁺, with DPT as acceptor of the aldehyde fragment. The postulated "aldehyde-DPT" intermediate has not been isolated as such. Its existence is inferred from the types of DPT dependent coupling reactions that can be demonstrated at the aldehyde level. An analogous cleavage of diacetyl would yield a similar "aldehyde-DPT" complex, while the remaining C₃ fragment (a carbonium ion) would be spontaneously converted to acetic acid. The aldehyde fragment would then be metabolized via the keto acid pathway, being transferred first to the disulfide bond of lipoic acid to form acetyl lipoic acid. Acetyl transfer from acetyl-lipoic to reduced coenzyme A yields reduced lipoic acid and acetyl coenzyme A. The reduced lipoic acid is then reoxidized by lipoic dehydrogenase, with DPN as electron acceptor. Ferricyanide is pictured as functioning as an oxidant for acetyl lipoic acid, either through a spontaneous chemical or an enzymatically catalyzed reaction. This reaction competes strongly with the normal metabolism of the C₃ fragment (acetoin formation or condensation to give diacetyl methylcarbinol), as shown by the marked inhibition of acetoin accumulation in the presence of ferricyanide coupled with the concomitant increase in acetate formation relative to the acetoin level.

Hager and Gunsalus (see Gunsalus, 1954) have shown that lipoic acid dehydrogenation is catalyzed by the previously described enzyme B of E. coli, while the transacetylation between acetyl lipoic acid and coenzyme A is catalyzed by enzyme A. Evidence for the reversibility of the reactions between the aldehyde level and reduced lipoic acid, catalyzed by enzyme A, has been presented by Chin and Gunsalus (1954). The scheme of Reed and DeBusk (1953) is similar to this except that lipothiamide pyrophosphate (the amide between lipoic acid and DPT) is reported to be the active form of lipoic acid in the reaction sequence as worked out with their mutant strain of E. coli. Lipothiamide pyrophosphate is found to be the obligate acceptor of the aldehyde fragment in the oxidative series of reactions, so that these authors would eliminate a separate cocarboxylase step. Also, as mentioned previously, they report that cocarboxylase but not lipothiamide pyrophosphate is required when ferricyanide is the electron acceptor. The pyruvate apoxidase of this strain has not been separated into individual enzymatic steps; however, the separate reactions
Figure 1. Mechanism for the cleavage and oxidation of dicarbonyl compounds. The following formulas are used: lipoic acid, disulfide form; acetyl lipoic acid, ; reduced lipoic acid, ; reduced coenzyme A, CoASH; acetyl coenzyme A, CoAS—C—CH₃.

have different heat labilities. For details of these reactions see the recent reviews of Gunsalus (1954) and Reed (1953).

Schweet et al. (1951) have previously proposed an intramolecular cleavage of diacetyl to yield one C₂ fragment at the acetate level and one at the aldehyde level of oxidation; the latter fragment would be common to the diacetyl and pyruvate pathway. The present work suggests that this common intermediate as activated for oxidation is acetyl lipoic acid. Reed (1953) mentions briefly that his E. coli mutant apo-oxidase will, in the presence of lipothiamide pyrophosphate, convert the C₂ aldehyde frag-
ment from diacetyl into pyruvate, in the presence of CO$_2$, which would be further evidence for the function of lipoic acid at the aldehyde level in these reactions.

Diacetyl oxidation as carried out by cell-free extracts of *S. faecalis* in the absence of ferricyanide has not been studied in detail because of the interference due to the nonoxidative diacetyl-lyperoxidase pathway. Diacetyl-lyperoxidase would arise by the condensation of aldehyde-DPT with diacetyl in a manner analogous to the condensation of aldehyde-DPT with pyruvate to form acetolacetate (Juni, 1952). Acetoin could be formed from diacetyl by condensation of two aldehydes or via the reduction of diacetyl with DPNH. An enzyme catalyzing the latter reaction is present in *S. faecalis*. With regard to the aldehyde condensation mechanism in this organism, it has been shown (Dolin and Gunsalus, 1951) that free aldehyde does not condense to form acetoin even in the presence of active aldehyde derived from pyruvate. The possibility that two active aldehydes condense has not been ruled out although, as pointed out above, this type of reaction should not be lipoic dependent, whereas diacetyl oxidation in the absence of ferricyanide is still partially lipoic dependent.

Diacetyl activation does not appear to be an invariable characteristic of pyruvate enzymes. The pyruvate system of *E. coli*, strain Crookes, for instance does not oxidize diacetyl (Hager, personal communication). With regard to *S. faecalis*, it is evident from the data presented in table 3 that much of the diacetyl activity fractionated along with the pyruvate "carboxylase", at least to the stage of purification of fraction I. This fraction is not pure enough to allow any conclusions to be drawn regarding the identity of the pyruvate and diacetyl "carboxylases". However, the analogy between the initial steps in pyruvate and diacetyl metabolism may serve as a model for a generalized series of reactions in which active aldehyde (aldehyde-DPT) derived from sources other than keto acids may be transferred into the oxidative pathway via acetyl lipoic acid.

**SUMMARY**

Vacuum-dried cells of *Streptococcus faecalis*, strain 10C1, catalyze the oxidation of one molecule of diacetyl of two of acetate with ferricyanide as electron acceptor. In the absence of ferricyanide, acetoin and diacetylmethylcarbinol accumulate as the reduced products.

Using the ferricyanide assay system with lipoic acid deficient vacuum-dried cells, it can be shown that diacetyl oxidation is a lipoic acid dependent reaction. Diacetyl oxidation by cell-free extracts is completely dependent upon the addition of cocarboxylase. The extracts contain bound lipoic acid.

These results indicate that lipoic acid may be involved in general in the oxidative metabolism of active aldehyde formed by the cleavage of the alpha-dicarbonyl linkage.

**REFERENCES**


Hager, L. P., Fortney, J. D., and Gunsalus, I. C. 1953 Mechanism of pyruvate and
\(\alpha\)-ketoglutarate dehydrogenase systems. Federation Proc., 12, 213.


