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## Diacetyl as Acetyl Donor

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# Diacetyl as Acetyl Donor\*

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## Abstract

Diacetyl can serve as an acetyl donor for the formation of citrate and the acetylation of sulfanilamide in the dog heart homogenate. Diphosphothiamine and coenzyme A are essential for these reactions.

## DIACETYL AS ACETYL DONOR

By

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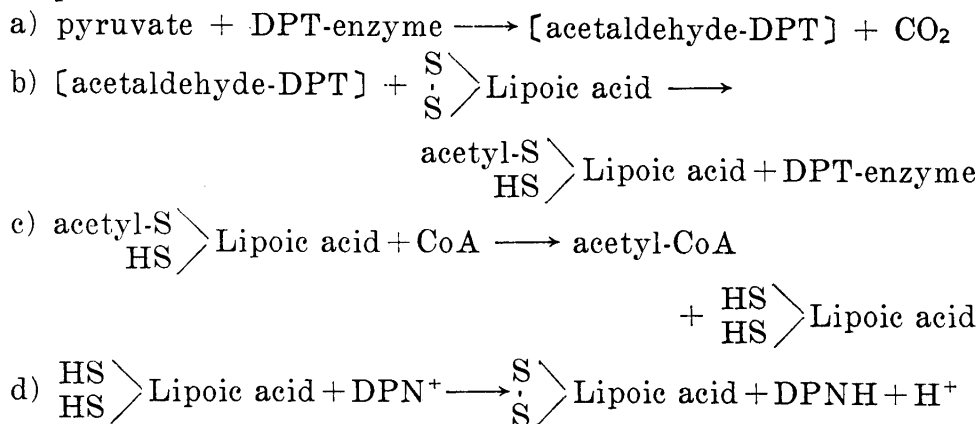
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### Introduction

Since diacetyl mutase was found in pigeon breast muscle<sup>1)</sup>, some proposed mechanisms for this reaction have been presented<sup>2)3)4)</sup>. A hypothetical C<sub>2</sub>-intermediate generated from diacetyl is believed to be an acetaldehyde-enzyme complex which is similar in behavior to the C<sub>2</sub> unit of acetaldehyde level derived from pyruvate by thiamine enzyme. It has been suggested that the synthesis of acetyl coenzyme A from acetaldehyde enzyme complex generated from pyruvate involves the following steps<sup>5)</sup>:



It should appear, therefore, that diacetyl can serve as an acetylating agent. *Doisy & Westerfeld*<sup>6)</sup> reported that acetoin could act as an acetyl donor, although a large proportion of acetoin were utilized to form acetoacetic acid<sup>7)</sup>. The present paper is concerned with the formation of citrate and the acety-

The following abbreviations are used: DPT, diphosphothiamine; CoA, coenzyme A; DPN, diphosphopyridine nucleotide; ATP, adenosine triphosphate; SAM, sulfanylamide; OAA, oxaloacetic acid.

lation of SAM by means of diacetyl in the dog heart homogenates.

### Experimental

Materials: Diacetyl was prepared by the method of *Pechmann*<sup>8)</sup>. Oxaloacetic acid was synthesized by the method of *Wislicenus*<sup>9)</sup> and of *Simon*<sup>10)</sup>. Coenzyme A was prepared from a hog liver by the method of *Lipmann*<sup>11)</sup>.

Na-ATP, DPN, DPT were kindly furnished by Dr. P. Handler of Duke University (U. S. A.). D. L- $\alpha$ -Lipoic acid was a generous gift from Dr. L. J. Reed of Texas University (U. S. A.).

Methods: 1 ml of 20% dog heart homogenate prepared in an alkaline isotonic KCl was pipetted into each Warburg flasks containing potassium phosphate buffer, MgCl<sub>2</sub>, cofactors, and acetyl-acceptors. 0.2 ml of 5% KOH was placed in the center well. Air was used as a gas phase. After equilibrium (10 min., 37°C) diacetyl was introduced into main chamber from the side arm. The reaction was stopped by the addition of 10% Trichloroacetic acid.

Citrate in the filtrate was determined by the method of *Krebs & Eggleston*<sup>12)</sup>. SAM was estimated by the method of *Tsuda*<sup>13)</sup>. Diacetyl determination was carried out by the method of *White et al.*<sup>14)</sup>.

### Results

#### a) Acetylation of Sulfanilamide

The data of Table 1 show that the complete system can acetylate about 25 per cent of added SAM, although the contribution of diacetyl to the acetylation of SAM is small. Both DPT and CoA were indispensable for this acetylation reaction. Oxygen consumption was very small in all Warburg flasks. In the presence of ATP and DPN, the acetylation of SAM was not accelerated, but the oxygen consumption was increased.  $\alpha$ -Lipoic acid added in this system had no effect on the acetylation of SAM.

#### b) Citrate Formation.

The results are shown Table 2. The citrate formation from

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Table 1. Acetylation of Sulfanilamide

The complete system contained 20% dog heart homogenate - 1 ml, potassium phosphate buffer (pH 7) - 100  $\mu$  M, MgCl<sub>2</sub> - 10  $\mu$  M, Cystein - 10  $\mu$  M, CoA - about 10 units, DPT - 100  $\gamma$ , SAM - 50  $\gamma$ , Diacetyl - 50  $\gamma$ . Final volume 3.0 ml., one hour incubation at 37°C.

	SAM acetylated	Diacetyl disappeared
Complete system	12 — 13 $\gamma$	18 — 24 $\gamma$
" minus DPT	5 — 6 $\gamma$	10 — 13 $\gamma$
" minus CoA	6 — 7 $\gamma$	negligible
" minus DPT & CoA	negligible	negligible

Table 2. Citrate formation

The complete system contained 20% homogenate - 1 ml., potassium phosphate buffer (pH 7) - 100  $\mu$  M., MgCl<sub>2</sub> - 10  $\mu$  M, Cystein - 10  $\mu$  M, CoA - about 10 units, DPT - 100  $\gamma$ , Diacetyl - 5  $\mu$  M~10  $\mu$  M, OAA - 10~20  $\mu$  M. Final volume 3.0 ml., 15~20 min. incubation at 37°C.

Experiment 1. diacetyl - 5  $\mu$  M, oxaloacetate - 10  $\mu$  M, 15 min. incubation

	Citrate formed	Diacetyl disappeared
Complete system	205 $\tau$	110 $\tau$
" minus DPT	120 $\tau$	30 $\tau$
" minus CoA	110 $\tau$	60 $\tau$
" minus CoA & DPT	90 $\tau$	40 $\tau$

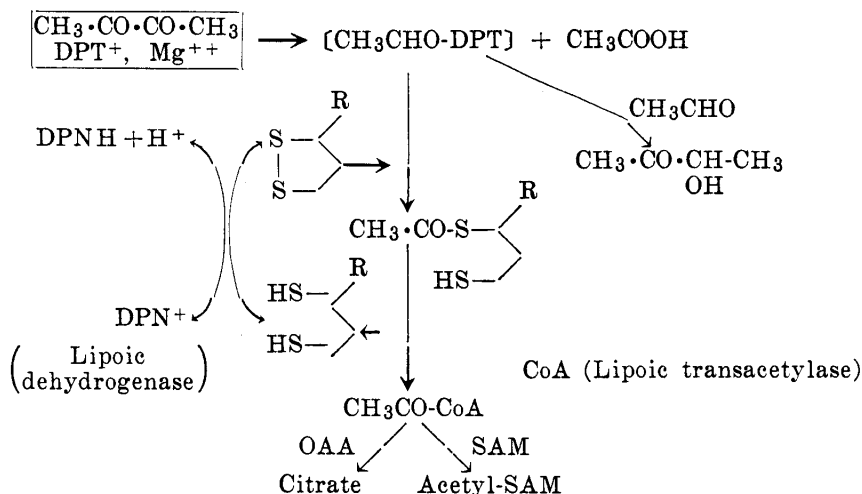
Experiment 2. diacetyl - 10  $\mu$  M, oxaloacetate - 20  $\mu$  M, 20 min. incubation

	Citrate formed	Diacetyl disappeared
Complete system	386 $\tau$	250 $\tau$
" minus Diacetyl	330 $\tau$	—
" minus OAA	100 $\tau$	220 $\tau$
" minus Diacetyl & OAA	50 $\tau$	—

diacetyl and oxaloacetate also requires both DPT and CoA. Although the contribution of diacetyl to the citrate formation is also small in the experimental condition used, it appears to be certain that some portion of citrate formed is derived from diacetyl. Oxygen consumption was negligible. Malonate, ATP and DPN had not any measurable effect on the formation of citrate.

### Discussion

From the data presented, it is apparent that the diacetyl can act as an acetyl donor in the dog heart homogenate. The acetylation of SAM and the formation of citrate in this experiments may be carried out follows :



Although the fate of acetic acid generated from diacetyl at the first step cleavage by thiamine enzyme is not clear, in this dog heart system acetic acid acts as an acetyl donor only in the presence of ATP. This fact is the point different from  $\text{C}_2$ -unit generated from diacetyl. Lipoic acid and DPN may be present enough in this system.

### Summary

Diacetyl can serve as an acetyl donor for the formation of citrate and the acetylation of sulfanilamide in the dog heart homogenate. Diphosphothiamine and coenzyme A are essential for these reactions.

Grateful acknowledgement is made to *Y. Takeuchi* for technical assistance, to *Dr. S. Mizuhara* for valuable advice, and to the Department of Education for a grant.

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