concentrations of sulfhydryl groups are known to occur.

Further studies in the distribution of sulfhydryl and disulfide groups and their changes in various physiological states will be published elsewhere.

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Role of Coenzyme A and DPN in the Oxidation of α-Ketoglutaric Acid[1]

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From recent work it has become apparent that diphosphopyridine nucleotide (DPN) and coenzyme A (CoA) participate in the oxidation of α-ketoglutarate (*1–4*). Succinyl CoA has been postulated as an intermediate in the reaction. Using α-ketoglutaric oxidase (*5*) of over 90% purity, as shown by electrophoretic and ultracentrifuge analyses, we have found that the primary reaction is as follows:

$$\alpha\text{-Ketoglutaric acid} + \text{CoA} + \text{DPN}^+ \rightarrow \text{Succinyl CoA} + \text{DPNH} + CO_2 + H^+.$$

For the first time an acylated derivative of CoA has been obtained from the enzymatic oxidation of an α-keto acid.

In the presence of CoA[3] and cysteine (or glutathione or borohydride instead of cysteine), the purified α-ketoglutaric oxidase (*5*) catalyzes the reduction of DPN by α-ketoglutaric acid (Fig. 1). The stoichiometry of the reaction with CoA (Table 1) is consistent with the above equation. The disappearance of —SH group and CoA in amounts equivalent to formation of acid anhydride[4] (as shown by the reaction with hydroxylamine) suggests strongly that the product is succinyl CoA. As in the case of acetyl CoA (*10*), the anhydride bond appears to be formed between the —SH of CoA and one of the carboxyls of succinate. Succinyl CoA is obtained from the reaction mixture by adsorption on Nuchar C190 followed by

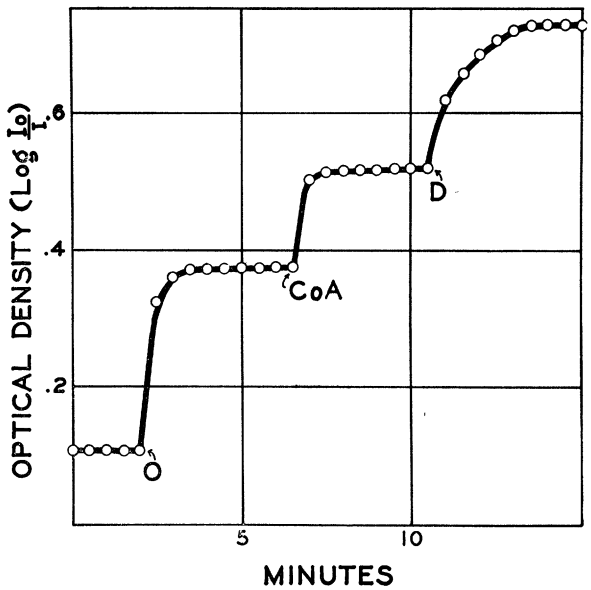

FIG. 1. Reduction of DPN by α-ketoglutarate and CoA. Experimental conditions: cysteine (0.1 ml 0.5 *M* neutralized solution) and CoA (0.1 ml containing 0.3 mg of preparation with an activity of 340 u/mg) were incubated for 3 min at 30°. α-Ketoglutarate (5 μM), DPN (0.3 μM), glycylglycine at pH 7.2 (100 μM), and water to a total vol of 3.0 ml were added to the cysteine-CoA mixture. After 2 min 10 u α-ketoglutaric oxidase in 0.01 ml were added to start the reaction. A mixture of cysteine (0.1 ml) and CoA (0.05 ml) was added at 6.5 min, and purified succinyl CoA deacylase (*4*) at 10.5 min. Optical density ($\log \frac{Io}{I}$) was measured at 340 mμ in a Beckman DU spectrophotometer.

TABLE 1

STOICHIOMETRY OF THE REACTION OF α-KETOGLUTARATE WITH DPN AND CoA*

Expt	Δ α-Ketoglutarate	Δ CoA	Δ —SH	DPN reduced	Hydroxamic acid formed
1	7.0	4.5	—	4.6	4.8
2	2.4	1.5	—	1.4	1.7
3	0.76	—	0.47	—	0.51

* The data are expressed in μM. Three to 7 min after the reaction was started by the addition of oxidase, an aliquot was reacted with hydroxylamine (*6*) for 1 hr. Another aliquot was treated with methanol, and the DPNH determined by the change in optical density at 340 mμ produced by acetaldehyde and crystalline alcohol dehydrogenase. The free sulfhydryl group was determined by the nitroprusside reaction (*7*), CoA by arsenolysis with phosphotransacetylase (*8*), and α-ketoglutarate as the 2,4-dinitrophenylhydrazone (*9*). Cysteine was not present in Expt 3.

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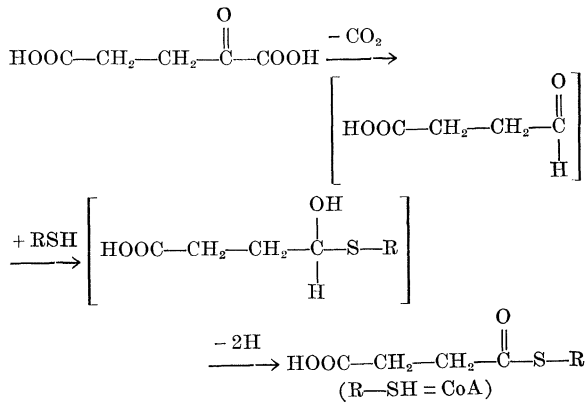
[3] We are greatly indebted to H. Beinert and others for generous gifts of CoA (*J. Am. Chem. Soc.*, **74**, 854 [1952]), which made this investigation possible.

[4] Using S-acetyl-β-mercaptoethylamine as a model, Novelli has presented evidence that thioesters behave like acid anhydrides in that the activated carboxyl reacts with hydroxylamine at acid pH (*1*).

elution with aqueous pyridine, or by extraction with a phenol-benzyl alcohol mixture. The isolated sample reacted with sulfanilamide in the presence of crude pigeon liver acetone powder extract (*3*) under conditions where succinate, CoA, and cysteine were inactive. The hydroxamic acid derived from the sample has been identified by paper chromatography.

The equilibrium of the reaction is far to the right and is independent of pH. Triphosphopyridine nucleotide will not replace DPN in the reaction. Although pantetheine (synthetic LBF) (*11*) is inactive at low levels (1.3×10^{-4} *M*), high concentrations (6.7×10^{-3} *M*) can replace CoA. Protogen B,[5] α-lipoic acid,[5] and boiled extracts of the oxidase containing protogen in coenzyme form (*5*) are inactive.

From the data presented, both DPN and CoA may be considered as prosthetic groups of the α-ketoglutaric oxidase. The reaction mechanism in which the brackets refer to enzyme complexes may be represented as follows:



The initial decarboxylation has been indicated previously (*5*) and is further supported by the rapid incorporation of radioactive CO_2 into α-ketoglutarate in the absence of DPN or CoA (*12*).

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[5] Kindly supplied by T. H. Jukes and L. J. Read.

Direct Determination of Maximal Daily Metabolism of Alcohol

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Not long ago one of us (*1*) estimated that the maximal daily metabolism of alcohol by a man of average weight was represented by a quart of 100-proof liquor. This estimate was based on prolonged feeding experiments in dogs and the demonstrable similarity in the rate of alcohol metabolism in the dog and in man.

In spite of the simplicity of the direct experimental approach to this problem in man, search of the literature failed to reveal any objective information, and for this reason the present work was undertaken. Four subjects were used, three men and one woman. Two were moderate drinkers, and two had drunk to excess sufficiently to classify them as chronic alcoholics. They varied over a wide range as to age and body build. The experimental procedure was essentially the same in all cases. A test dose of 1.0 ml/kg of 95% alcohol diluted to about 20% with water was administered by mouth on an empty stomach, after which samples of venous blood were taken hourly and analyzed for alcohol by a modification of Winnick's method (*2*). From the length of time alcohol remained in the blood the amount metabolized per hour was calculated. Subsequently this amount was administered hourly, either as diluted alcohol or as an acceptable alcoholic beverage, and the blood or saliva alcohol concentration was determined at suitable intervals. To avoid undue inconvenience to the subject, during the night three doses three times the size of the hourly dose were given, which required only two awakenings. The results can best be presented for each individual subject.

Subject 1 was a moderate drinker, male, age 44, weight 61.4 kg, height 168 cm, body surface (Dubois) 1.70 m^2. The estimated hourly dose of 12.0 ml 95% alcohol resulted in 71 hr in a blood alcohol concentration of 17 mg %. Increasing the dose to 15.0 ml resulted in 48 hr in a rise to 159 mg %. According to Widmark's formula (*3*), it can be calculated that this rise in blood alcohol concentration would require 81 ml 95% alcohol; actually the subject received during this time 720 ml. This, less the amount required to produce the rise in blood alcohol concentration, amounts to 639 ml in 48 hr, or 320 ml daily.

Subject 2 was a moderate drinker, male, age 60, weight 65.0 kg, height 173 cm, body surface 1.79 m^2. The estimated hourly dose was 10.0 ml, which resulted in 54 hr in a blood alcohol of 8 mg %. Increasing the dose to 12.5 ml for 51 hr raised the blood alcohol to 91 mg %, which rise would require 52 ml alcohol. The actual amount given, 638 ml, less this excess, amounts to 586 ml in 51 hr, or 276 ml daily.

Subject 3 was a chronic alcoholic, female, age 32,