

cm.⁻¹, accompanying the strong maximum at 1703 cm.⁻¹ (0.01 *M* soln. in CCl₄)⁷ corresponding to "non-interacted" conformations of I. By contrast, under the same conditions, tetrahydro-4H-1-thiapyran-4-one (II)⁸ and 1-thiacycloheptan-4-one (III)⁹ exhibited single maxima at 1716 and 1711 cm.⁻¹, respectively. The dipole moment of the eight-membered ring compound (I), 3.81 *D* in benzene, was higher than that of the seven-membered ring compound (III) (3.04 *D*; 1.73 *D* for II).^{2b} It is important to note that S-C_{CO} interaction occurs to a lesser extent than N-C_{CO} transannular interaction in the electronic *ground state* by comparison (infrared especially) of 1-thiacyclooctan-5-one with 1-methyl-1-azacyclooctan-5-one.⁷

Finally, the ultraviolet absorption maxima of I in cyclohexane, at 226 mμ (ε 2445) and ~232 mμ (ε 2150), are associated with *excitation* of the interacting S-C_{CO} system (λ_{max}^{II} 223 mμ (ε 695), λ_{max}^I 233 mμ (ε 507)).¹⁰

(7) N. J. Leonard, M. Ōki, J. Brader and H. Boaz, *THIS JOURNAL*, **77**, 623 (1955).

(8) E. A. Fehnel and M. Carmack, *ibid.*, **70**, 1813 (1948).

(9) C. G. Overberger and A. Katchman, *ibid.*, **78**, 1965 (1956).

(10) E. Fehnel and M. Carmack (*ibid.*, **71**, 84 (1949)) have suggested earlier that the difference between the ultraviolet spectrum of tetrahydro-4H-1-thiapyran-4-one (II) and those of its acyclic analogs is attributable to direct interaction between the 1,4-atoms in the excited state. (See also V. Georgian, *Chemistry and Industry*, 1480 (1957).) If this is correct, the four- to five-fold increase in intensity for the eight membered ring over the six-membered ring may be regarded as manifestation of the greater contribution of transannular interaction in the medium-ring compound.

(11) Sinclair Refining Co. Fellow in Organic Chemistry, 1957-1958. Work done under the sponsorship of the Sinclair Research Laboratories, Inc.

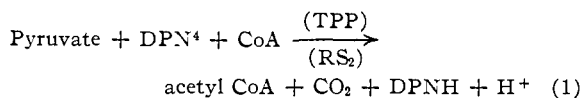
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RECEIVED DECEMBER 4, 1958

ON THE MECHANISM OF OXIDATIVE DECARBOXYLATION OF PYRUVATE

Sir:

Extracts of *Escherichia coli* contain an enzyme system which catalyzes an oxidative decarboxylation of pyruvate represented by reaction 1.^{1,2,3}



We have obtained highly purified preparations (250-fold purification) of this system from extracts of the Crookes strain. It is apparently an enzyme complex,⁵ and sediments in the ultracentrifuge (1 to 2 hr. at 144,000 × *g*) as a dark yellow, fluorescent pellet. The complex contains a flavin which has been tentatively identified as FAD. Release of the flavin by precipitation of the enzyme complex with ammonium sulfate at pH 3.6 resulted in a decrease in the enzymatic activities: dihydrolipoic

(1) S. Korkes, *et al.*, *J. Biol. Chem.*, **193**, 721 (1951).

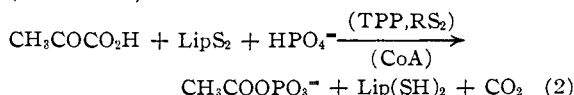
(2) I. C. Gunsalus, in "The Mechanism of Enzyme Action," The Johns Hopkins Press, Baltimore, Md., 1954, p. 545.

(3) L. J. Reed, *et al.*, *J. Biol. Chem.*, **232**, 123, 143 (1958).

(4) Abbreviations: DPN, diphosphopyridine nucleotide; CoA, coenzyme A; TPP, thiamine pyrophosphate; FAD, flavin adenine dinucleotide; LipS₂, free lipoic acid; Lip(SH)₂, free dihydrolipoic acid; RS₂, protein-bound lipoic acid.

(5) R. S. Schweet, *et al.*, *J. Biol. Chem.*, **196**, 563 (1952); D. R. Saadi, *et al.*, *ibid.*, **197**, 851 (1952).

dehydrogenase, DPN reduction (reaction 1), pyruvate dismutation, and reduction of free lipoic acid (reaction 2).^{3,6} These activities were restored by



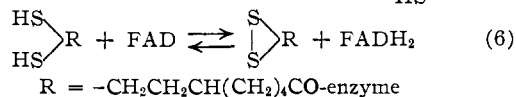
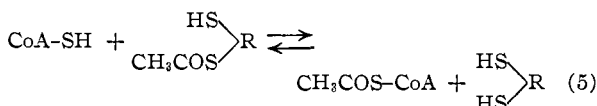
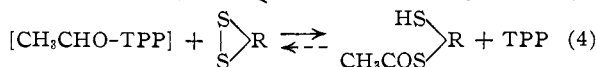
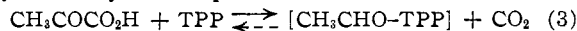
addition of FAD, but not of FMN (Table I). The dihydrolipoic transacetylase activity of the preparation was not affected by removal of flavin.

TABLE I
REACTIVATION OF SPLIT PYRUVATE DEHYDROGENATION
SYSTEM WITH FAD

Assay system	Before splitting	Specific activities ^a		
		Without FAD	After splitting With FAD ^b	With FMN ^c
Lipoic DeH ^b	870	214	544	228
Dismutation ^c	870	214	486	144
Reaction 1 ^d	156	20	62	20
Reaction 2 ^e	90	32	64	32
Lip. transac. ^f	112	100	110	100

^a Expressed as μmoles/hr./mg. protein based on assays described previously. ^b Ref. 7, pH 7. ^c Ref. 3. ^d Ref. 8. ^e Ref. 6, pH 7, 5 μmoles DL-lipoamide employed. ^f Ref. 8. ^g Aliquots of split complex incubated 10 min. at 30° with FAD or FMN before assay. Final concentration of added flavin in assays was 10⁻⁵ to 10⁻⁶ *M*.

These data indicate that FAD is an essential component of the enzyme complex, presumably associated with dihydrolipoic dehydrogenase. The data are consistent with the reaction sequence^{2,3} shown for oxidative decarboxylation of pyruvate by the enzyme complex.



The reduced flavoprotein produced in reaction 6 apparently can interact with DPN (*cf.* reaction 1) and free lipoic acid (*cf.* reaction 2).

(6) I. C. Gunsalus, *Federation Proc.*, **13**, 715 (1954).

(7) L. P. Hager and I. C. Gunsalus, *THIS JOURNAL*, **75**, 5767 (1953).

(8) L. P. Hager, Thesis, University of Illinois, 1953.

(9) During this investigation Dr. V. Massey, *Biochim. et biophys. acta*, **30**, 205 (1958) communicated to us his significant finding (ref. 9) that highly purified diaphorase exhibited strong dihydrolipoic dehydrogenase activity.

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RECEIVED NOVEMBER 25, 1958

CHELATION AS A DRIVING FORCE IN SYNTHESIS. A NEW ROUTE TO α-NITRO ACIDS AND α-AMINO ACIDS

Sir:

Dibasic α-nitro acids (I) are converted in weakly basic media to acid salts which rapidly decarbox-