

administered intraperitoneally, so that initial high sulfadiazine levels could be obtained. Following the intraperitoneal injection, the mice were placed on the sulfadiazine diet for 24 hr. Similar results were obtained whether sulfonamide was administered only on the 6th or only on the 7th day following appearance of the vaginal plug. No data have been obtained concerning the period from the 1st to 6th day.

References

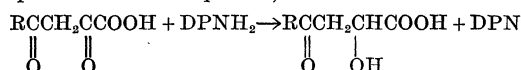
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Enzymatic Reduction of 2,4-Diketo Acids Catalyzed by Dihydrodiphosphopyridine Nucleotide

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The reduction of pyruvic acid to lactic acid, catalyzed by dihydrodiphosphopyridine nucleotide (DPNH₂) in muscle extracts is well known. An apparently analogous reaction in which 2,4-diketo acids are reduced has been observed. When a series of homologous 2,4-diketo acids was incubated with 90% pure DPNH₂¹ and an aqueous extract of an acetone powder of rabbit muscle at pH 7.2, the characteristic absorption band of these compounds at 2900 Å and the band due to DPNH₂ at 3400 Å disappeared progressively. Equimolar amounts of coenzyme and diketo acid were utilized, suggesting the reduction of one keto group. In a typical experiment using a system containing 5×10^{-7} moles each of 2,4-diketovalerate and DPNH₂, 10^{-4} moles of phosphate buffer at pH 7.2, and 80 γ of protein nitrogen per 3 ml, 2.48×10^{-7} and 2.44×10^{-7} moles of DPNH₂ and diketo acid, respectively, disappeared after 12 min of incubation at 25° C. Neither DPNH₂ nor substrate disappeared when one of these was omitted from the system or in the absence of enzyme. No lactate was formed as determined by the method of Barker and Summerson (1), ruling out prior hydrolysis of the diketo acid to pyruvic acid (3). The facts are compatible with the equation,



whereby the product is considered tentatively to be the 2-hydroxy-4-keto acid.

The reaction proceeded more rapidly with increasing concentrations of substrate and was conveniently fol-

¹ DPN was purified by countercurrent distribution as described by Hogeboom and Barry (2). Cruder preparations tended to interfere with measurements made at 2900 Å. *m* = calibration factor for adrenalin

lowed spectrophotometrically by measuring the rate of decrease of the DPNH₂ band at 3400 Å. All of the normal 2,4-diketo acids from valeric to undecylic were reduced in the system, as shown in Table 1. Under the

TABLE 1
ENZYMATIC REDUCTION OF 2,4-DIKETO ACIDS*

2,4-Diketo acid	Disappearance of DPNH ₂ (Moles $\times 10^{-7}$ per min)
<i>n</i> -Valeric	1.83
<i>n</i> -Hexanoic	1.74
<i>n</i> -Heptanoic	1.45
<i>n</i> -Octanoic	1.48
<i>n</i> -Nonanoic	1.36
<i>n</i> -Capric	1.33
<i>n</i> -Undecylic	1.45

* Composition of system in moles per 3 ml was 5×10^{-7} DPNH₂, 9×10^{-6} diketo acid, 1×10^{-4} phosphate buffer (pH 7.2); and 0.1 ml enzyme preparation (100 γ protein nitrogen) per 3 ml; 25° C.

same experimental conditions, neither 4-keto valeric acid nor 3,5-diketoheptanoic acid was reduced. The nature of the enzyme involved and its possible relationship to lactic dehydrogenase is under investigation.

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A Convenient Quick Method of Obtaining Vitamin B₁₂ Concentrate¹

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The nonprotein filtrate of liver homogenate (proteins coagulated by boiling at pH 5.0) chromatographed on starch columns by the method of Moore and Stein (1) gave a reddish brown fraction in the first portions of the effluent.

The behavior and color of this fraction suggested a possible relation to B₁₂. This was tested as follows: 0.5 ml of liver injection, USP (Lederle Solution Extract, from beef liver, 15 u per ml) was dried by blowing air across it at room temperature. To the residue 0.1 ml 1N HCl was added, and then 0.5 ml of a mixture consisting of 0.1 N HCl, *n*-propanol, and *n*-butanol in the proportions 1:2:1. The solution was chromatographed on 25 g starch in a 10-mm \times 300-mm column with the 1:2:1 mixture as solvent.

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