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.

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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 ($DPNH_2$) 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 $DPNH_2$[1] and an aqueous extract of an acetone powder of rabbit muscle at pH 7.2, the characteristic absorption band of these compounds at 2900 A and the band due to $DPNH_2$ at 3400 A 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 $DPNH_2$, 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 $DPNH_2$ and diketo acid, respectively, disappeared after 12 min of incubation at 25° C. Neither $DPNH_2$ 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,

$$RCCH_2CCOOH + DPNH_2 \rightarrow RCCH_2CHCOOH + DPN$$
$$\quad\;\; \| \quad \| \qquad\qquad\qquad\qquad\quad \| \quad |$$
$$\quad\;\; O \quad O \qquad\qquad\qquad\qquad\quad O \quad OH$$

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 followed spectrophotometrically by measuring the rate of decrease of the $DPNH_2$ band at 3400 A. All of the normal 2,4-diketo acids from valeric to undecylic were reduced in the system, as shown in Table 1. Under the same experimental conditions, neither 4-keto valeric acid nor 3,5-diketohexanoic acid was reduced. The nature of the enzyme involved and its possible relationship to lactic dehydrogenase is under investigation.

TABLE 1

Enzymatic Reduction of 2,4-Diketo Acids*

2,4-Diketo acid	Disappearance of $DPNH_2$
	(Moles $\times 10^{-7}$ per min)
n-Valeric	1.83
n-Hexanoic	1.74
n-Heptanoic	1.45
n-Octanoic	1.48
n-Nonanoic	1.36
n-Capric	1.33
n-Undecylic	1.45

* Composition of system in moles per 3 ml was 5×10^{-7} $DPNH_2$, 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.

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A Convenient Quick Method of Obtaining Vitamin B_{12} Concentrate[1]

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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 B_{12}. 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 1*N* 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 × 300-mm column with the 1: 2: 1 mixture as solvent.

[1] This work is part of that done under a joint contract with the Office of Naval Research, United States Navy Department, and the United States Atomic Energy Commission.

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