himbone (VI).[11] The correlation of yohimbine-type alkaloids with D/E *trans* fusion with those of D/E *cis* juncture thus is accomplished.

The above data illustrate unambiguously for the first time that the only center of asymmetry common to all yohimbine-type alkaloids is C-15.[12,13]

(11) The Schmidt reaction of tetrahydroserpentic acid and tetrahydroalstonic acid does not lead to ketones directly, but instead produces stable isocyanates. These results and further data will be discussed at a later time.

(12) This fact was pointed out by A. K. Bose, B. G. Chatterjee and R. S. Iyer [*Ind. J. Pharm.*, **18**, 185 (1956)] in connection with their discussion of the absolute configuration of yohimbine alkaloids, based on molecular rotation difference data.

(13) The authors are most grateful to Drs. Huebner, Lucas, MacPhillamy and Schlittler for a generous supply of the compounds needed for this study and to the Institute of Atomic Research, Ames, Iowa, for the use of a Baird infrared spectrophotometer.

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OBSERVATIONS OF NEW PHENOMENA IN THE FLUORESCENCE SPECTRUM OF A DIPHOSPHOPYRIDINE NUCLEOTIDE-LINKED DEHYDROGENASE

Sir:

Boyer and Theorell[1] have reported that the wave length of the maximum intensity of the fluorescence spectrum of reduced diphosphopyridine nucleotide (DPNH) shifts from 450 mμ to about 415 mμ when DPNH combines with liver alcohol dehydrogenase. Inspection of the figure presented by these authors indicates that the intensity of fluorescence of the alcohol dehydrogenase–DPNH complex is approximately 1.8 times that of DPNH alone.

We have made similar measurements with crystalline heart muscle lactic dehydrogenase using the Aminco–Bowman recording spectrophotofluorimeter.[2] The fluorescent spectra shown in Fig. 1 were measured in 0.05 *M* phosphate buffer, *p*H 6.88, at room temperature using an activating wave length of 340 mμ. The curve labelled LDH was obtained with 8.4×10^{-7} *M* lactic dehydrogenase. The molecular weight of the enzyme was taken as 135,000.[3] The curve for DPNH was recorded at a DPNH concentration of 2.5×10^{-6} *M*. When DPNH and LDH were each present at the concentration used for the measurement of their separate spectra, the curve DPNH–LDH was obtained. The wave length of maximum fluorescent emission occurs at 430 mμ as compared to 455 mμ for DPNH. Preliminary experiments indicate that the maximum shift in fluorescence occurs when the ratio of DPNH concentration to LDH concentration is approximately four.

The addition of pyruvate to the DPNH–LDH system to a final concentration of 1.2×10^{-4} *M* results in a rapid change to the spectrum of LDH alone. Surprisingly, the addition of oxamate to the same concentration results in a similar change. Since oxamate has been shown to be a powerful inhibitor for the enzymatic reaction[4] and has been

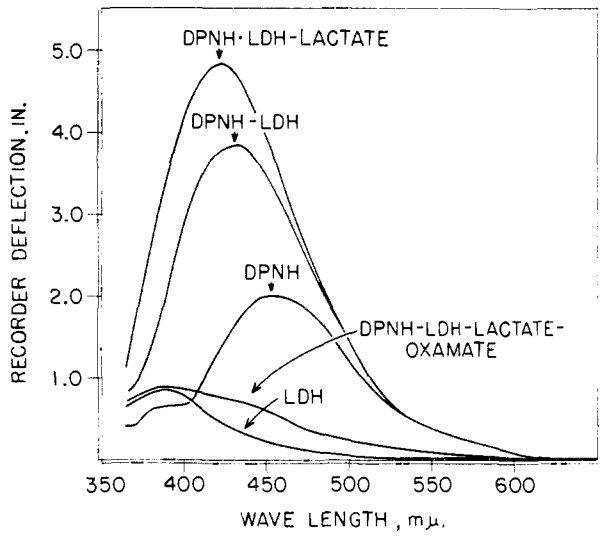

Fig. 1.

shown to be competitive with respect to pyruvate and non-competitive with respect to DPNH, it is inferred that this spectral change results from the formation of an LDH–DPNH–oxamate complex. The concentration of oxamate required to reduce the intensity of the LDH–DPNH fluorescent spectrum to half its initial value is approximately the value of K_I when oxamate is used as an inhibitor for the LDH-catalyzed reaction between DPNH and pyruvate, *i.e.*, approximately 10^{-5} *M*.

The curve labelled DPNH–LDH–lactate was obtained when Na-L-lactate, at a final concentration of 4.3×10^{-2} *M*, was added to LDH and DPNH present in the concentrations used for the other curves. The wave length of maximum emission is shifted to 420 mμ. The high concentration of lactate required to produce this effect, which presumably arises from an LDH–DPNH–lactate complex, is consistent with the observation that lactate has essentially no effect as an inhibitor of the LDH-catalyzed reaction between DPNH and pyruvate. The addition of oxamate to a concentration of 2×10^{-4} *M* results in the curve labelled DPNH–LDH–LACTATE–OXAMATE.

Results previously published from this Laboratory[5,6] indicate that a ternary LDH–DPNH–pyruvate complex is the reactive intermediate in the reaction catalyzed by LDH. Kinetic results also indicate that oxamate acts as an inhibitor by forming an unreactive LDH–DPNH–oxamate complex. Since the addition of diphosphopyridine nucleotide (DPN), which does not itself fluoresce, to LDH causes no change in the fluorescent spectrum of LDH, the present results suggest that in the LDH–DPNH–oxamate complex the bonding electrons of DPNH are constrained in a configuration resembling the aromatic ring of DPN. Moreover, since no net reaction occurs, it is possible that the LDH–DPNH–oxamate complex is an abortive complex similar in structure to the activated complex formed between LDH, DPNH and pyruvate.

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MEVALDIC ACID IN THE BIOSYNTHESIS OF MEVALONIC ACID

Sir:

Mevaldic acid (MVALD, 3-hydroxy-3-methylglutaraldehydic acid), a compound differing from mevalonic acid (MVA, 3,5-dihydroxy-3-methylpentanoic acid) by an aldehyde rather than a primary alcohol group at position 5, has been suggested as an intermediate in "isoprenoid" synthesis. The compound has now been synthesized by Shunk, *et al.*,[1] who also showed that MVALD has essentially no microbiological activity in the *Lactobacillus acidophilus* 4963 assay for MVA[2] but may be presumed to be an intermediate in "isoprenoid" biosynthesis since the counts found in cholesterol are significantly reduced by the presence of MVALD when 2-C^{14}-MVA is incubated in the rat liver system[3] that synthesizes cholesterol. On the other hand, Amdur, *et al.*,[4] found that 2-C^{14}-5-di-T-MVA is incorporated into squalene by a particle-free system of yeast with no change in the T:C^{14} ratio. If MVALD were an intermediate between MVA and squalene a decrease in the T:C^{14} ratio should have been encountered.

We have employed a technique[5] whereby the utilization of MVA by the rat liver enzyme system[6] is blocked by a preliminary incubation with ribonuclease to demonstrate that added MVALD is converted to MVA. The data of Table I show that when a liver homogenate is preincubated for 30 minutes with ribonuclease the counts found in the non-saponifiable fraction (NSF) or cholesterol (CHL) from 2-C^{14}-MVA are markedly reduced and the added MVA can be largely accounted for as such by microbiological assay of the digest. Similarly, with MVALD a sizable fraction of the highly reactive and relatively unstable compound (dehydrates and decarboxylates to 3-methylcrotonaldehyde) is found in MVA. No microbiological activity is found when liver is preincubated with ribonuclease without subsequent addition of MVA or MVALD or when ribonuclease is not used to block MVA utilization.

The accumulation of MVA from MVALD in this blocked system affords evidence that MVA is derivable biologically from MVALD. Taken in conjunction with the results of Amdur, *et al.*,[4] the data strongly suggest that MVALD is a precursor rather than a product of MVA.

TABLE I

SUMMARY OF INCUBATION EXPERIMENTS WITH MEVALDIC ACID (MVALD) AND MEVALONIC ACID (MVA)

Each experimental flask contained 1 mg. ATP, 1 mg. DPN, 5 ml. rat liver homogenate (supernatant layers after centrifuging at 200 × *g* for 3 min.), and 5 mg. crystalline ribonuclease (RNAse) where indicated. The flasks were initially aerated with a stream of oxygen, stoppered and incubated with agitation (50 oscillations per min.) at 37° for 30 min. 0.5 mg. 2-C^{14}-MVA (calculated as the DL-dibenzylethylenediammonium salt, 14,000 c.p.m.) or 1.0 mg. MVALD (calculated as the DL-dibenzylethylenediammonium salt of the 5-dimethylacetal) prepared for use as described by Shunk, *et al.*,[1] added where indicated followed by additional flushings with oxygen. Incubation continued for a total of 4.5 hours. Counts in the non-saponifiable fraction (NSF) were obtained on the petroleum ether extracts prior to preparation of the digitonides. Additional procedures employed including the preparation and counting of the cholesterol digitonides (CHL) as well as the microbiological determination of MVA have been described in detail.[6] Each experiment involves a different preparation of liver homogenate.

Expt. no.	Compd. added	Activity of fraction, c.p.m./mg. NSF Without RNAse	NSF With RNAse	CHL Without RNAse	CHL With RNAse	MVA found, mg. Without RNAse	MVA found, mg. With RNAse
1	MVA	395	33	1272	64	0	0.64
	MVALD	...	...	...	...	0	0.29
2	MVA	259	23	1158	108	0	0.51
	MVALD	...	...	...	...	0	0.30
3	MVA	369	135	780	51	0	0.50
	None	...	...	...	...	0	0

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