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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 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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