THE SYNTHESIS OF MEVALONIC ACID BY NON-PARTICULATE AVIAN AND MAMMALIAN ENZYME SYSTEMS.*

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Reports by Rabinowitz and Gurin (1954) on the synthesis of β-hydroxy, β-methyl glutaric acid (HMG), presumably as the CoA ester, in a soluble rat liver enzyme system, by Rudney (1957) on the reactions involved in the synthesis of HMGCoA from acetate in a similar system, and by Knauss, Porter and Wasson (1959) on the synthesis of mevalonic acid (MVA) from acetate by a combination of rat liver soluble enzymes and microsomes have greatly clarified our understanding of the initial reactions in the biosynthesis of sterols in animal systems. Information on the details of the reduction of HMGCoA to MVA in animal systems is, however, still quite meager, mainly because solublization of an animal HMGCoA reductase has not been reported.

In the present communication a report is made of soluble avian and mammalian enzyme preparations which convert acetate to HMGCoA and MVA. In addition, a report is made of the enzymatic synthesis of mevaldic (MVALD) acid, a compound frequently postulated as an intermediate in the reduction of HMGCoA to MVA. This finding is then discussed in relationship to the conversion of HMGCoA to MVA in the animal system.

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The pigeon liver supernatant solution described by Wakil, Porter and Gibson (1957) was fractionated with solid ammonium sulfate. The protein precipitating between 50-65% of saturation was dissolved in phosphate-bicarbonate buffer (0. 1 M in phosphate and . 07 in bicarbonate), pH 7. 0 and then dialyzed. A second enzyme system which converts acetate to MVA was obtained through treatment of rat liver microsomes with phosphate-bicarbonate

TABLE I

SPECIFIC RADIOACTIVITIES OF DERIVATIVES OF HMG, MVALD ACID AND MVA*

Recrystallization	HMG		MVALD ACID		MVA	
	Counts/min. /mg.					
	(a)	(b)	(c)	(d)	(e)	(f)
1	138	82. 0	41. 7	45. 7	133	212
2	127	56. 5	10. 1	12. 5	81. 5	100
3	124	59. 5	8. 0	11. 5	83. 5	66. 5
4	125	58. 8	7. 1	10. 0	82. 5	53. 4
5	---	----	6. 1	9. 6	----	52. 5
6	---	----	5. 9	9. 8	----	51. 5
7	---	----	6. 0	9. 8	----	----
8	---	----	5. 9	----	----	----

* Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer.

(a) Incorporation = 28, 000 cpm (8. 4 mμM) of 8. 8 x 10^6 cpm (8 μM) acetate.

(b) Incorporation = 13, 000 cpm (3. 9 mμM) of 4. 4 x 10^6 cpm (4 μM) acetate.

(c) Incorporation = 950 cpm (0. 3 mμM) of 4. 4 x 10^6 cpm (4 μM) acetate.

(d) Incorporation = 1440 cpm (3 mμM) of 9. 5 x 10^4 cpm (. 196 μM) HMGCoA.

(e) Incorporation = 10, 000 cpm (3. mμM) of 4. 4 x 10^6 cpm (4 μM) acetate.

(f) Incorporation = 6800 cpm (2. mμM) of 4. 4 x 10^6 cpm (4 μM) acetate.

buffer. This system, Anderson, Rice and Porter, (unpublished) also converts farnesyl pyrophosphate to squalene.

Radioactivity of 1-C^{14} acetate is incorporated into MVALD and MVA acids in the presence of ATP, Mg^{++}, GSH, CoASH, TPN, isocitrate, phosphate buffer, pH 7.0 and the soluble pigeon liver enzyme system. The TPNH generating system is omitted from the incubation mixture when HMGCoA is to be isolated. Values obtained, in radioactivity per mg. of derivative of HMG, MVALD and MVA, are given in Table I.

HMGCoA was isolated through paper chromatography in an ammonia: isobutyric acid: versene: water system (12:62:1:28), R_f 0.58-0.62. After elution and addition of carrier HMG, HMGCoA was cleaved with mild alkali at room temperature. The solution was acidified and then HMG was extracted with ethyl ether and esterified with α, p-dibromacetophenone. The resultant p-bromophenacyl ester was recrystallized from aqueous ethanol to constant specific radioactivity (m.p. 126-7°). After it was established that HMGCoA is present in incubation mixtures the chromatographic separation of HMGCoA was omitted in the preparation of the p-bromophenacyl ester.

Radioactive mevaldic acid was obtained from incubation systems that contained 90 μM MVALD acid and 20 μM MVA. Additional carrier MVALD acid was added prior to acidification and extraction of MVALD acid with ether. The ether solution was then evaporated over water, 4-phenyl semicarbazide was added, and the solution was refluxed gently for one hour at pH 5. The resultant 4-phenyl semicarbazone was recrystallized from aqueous ethanol to constant specific radioactivity (m.p. 173-4°).

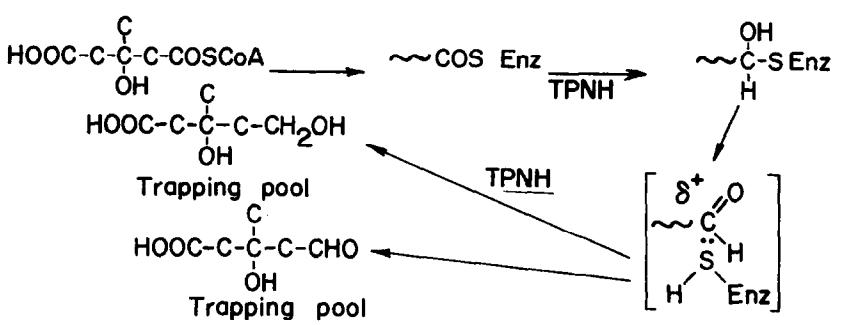
Radioactive MVA was isolated in the presence of non-radioactive MVA and in the presence and absence of a MVALD acid trap. MVA was converted to the lactone with acid, extracted with ether and then reacted with benzhy-

drylamine. The resultant benzhydrylamide was recrystallized from benzene-petroleum ether to constant specific radioactivity (m. p. 97. 5-98. 5).

An HMGCoA reductase was found in the soluble pigeon liver system, and in the soluble enzyme system obtained from rat liver microsomes. The reductase was not present in the non-microsomal rat liver soluble system used by Knauss, Porter and Wasson (1959) in combination with microsomes for the synthesis of MVA.

The present report is the first description of the biosynthesis of MVA from acetate in a soluble avian or animal enzyme system. It is also the first report of the enzymatic synthesis of MVALD acid. The amount of MVALD acid formed is, however, not large enough to justify the suggestion that free MVALD acid is an intermediate in the biosynthesis of MVA, a conclusion which is in agreement with those of Knappe, et al. (1959) and Durr, et al. (1959). The presence of radioactivity in MVALD acid does indicate, however, that the MVALD acid moiety does appear at one stage in the conversion of HMGCoA to MVA. Since incorporation into MVALD acid occurred in the presence of a trapping pool of MVA, the possibility of the incorporation of radioactivity into MVALD acid by the action of MVA dehydrogenase is precluded.

A possible interpretation of the reaction pathway in the conversion of HMGCoA to MVA which is consistent with our findings is the following:



In this proposed scheme the initial reduction product is hydrolyzed on the enzyme surface to enzyme bound MVALD acid. The latter is then reduced to MVA. The appearance of a small amount of radioactivity in the trapping pool would then be due to a slow loss of MVALD acid from the enzyme surface. A charge-transfer complex is a possible representation of the nature of the protein-MVALD acid interaction. This sequence of reactions also alleviates the necessity of proposing the unlikely hydrogenolysis of the carbon sulfur bond in the reduction process. The presence of an active MVALD reductase in mammalian liver, reported by Knauss and Porter (1960) and Schlesinger (1959), would then have the function of converting MVALD acid lost from the surface of the HMGCoA reductase to MVA.

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