

give pregnan-3 α ,17 α -diol-11,20-dione 3-acetate, m.p. 195–198°, $[\alpha]_D^{25} +50.3^\circ$ (chloroform), and by hydrolysis, pregnan-3 α ,17 α -diol-11,20-dione, m.p. 199.5–200.5°, $[\alpha]_D^{25} +33.9^\circ$ (chloroform).

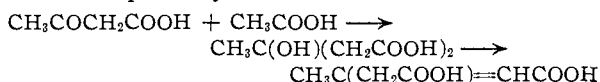
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The Biosynthesis of Radioactive Cholesterol, β -Methylglutaconic Acid and β -Methylcrotonic Acid by Aqueous Extracts of Liver¹

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It has been previously reported that aqueous particle-free extracts of rat liver which possess the ability to incorporate C¹⁴-labeled acetate or pyruvate into cholesterol² can also synthesize β -hydroxy- β -methylglutaric³ and β -methylcrotonic acids.⁴ Further investigation revealed the presence of another radioactive acid which has now been found to be *trans*- β -methylglutaconic acid (Table I). It is probable that this acid is derived from β -hydroxy- β -methylglutaric acid (HMG) by an enzymatically catalyzed dehydration. The formation of these two acids is consistent with the metabolic pathway



Evidence for the mechanism of formation of the Coenzyme A derivative of HMG has recently been obtained by Robinson, Bachhawat and Coon.⁵ The above postulated pathway undoubtedly also involves the participation of Coenzyme A.

C¹⁴-Methyl-labeled HMG is incorporated into cholesterol by surviving liver slices, homogenates, or particle-free extracts of rat liver (Table II). When compared with 2-C¹⁴-acetate it was found to be equally well incorporated. Similarly, the incorporation of HMG into β -methylglutaconic and β -methylcrotonic acids was equal to that observed with 2-C¹⁴-acetate (Table III).

The role of these substances as precursors of cholesterol is under further investigation.

Experimental

Particle-free extracts (7 ml.) of rat liver,³ homogenates (5 ml.), or tissue slices (1 g.) were incubated with 1 mg. each of adenosinetriphosphate, diphosphopyridine nucleotide and with 2-C¹⁴-potassium acetate (1 mc./mmole) or C¹⁴-methyl-HMG (0.08 mc./mmole). The labeled HMG was prepared from 2-C¹⁴-acetate by the method of Klosterman.⁶ The gas phase was 95% O₂-5% CO₂, and the time of incubation was 3 hours at 37°. The conditions used were those found to be optimum for cholesterol biosynthesis.³ Following the incubation period, 34 mg. of carrier methylglutaconic acid, 20 mg. of β -methylcrotonic acid or 1 mg. of carrier cholesterol were added to separate incubation flasks. Each specimen was saturated with KCl, acidified to pH 2 with metaphosphoric acid, and extracted continuously with

(1) Supported by grants of the Heart Institute of the National Institutes of Health and the American Cancer Society administered by the Committee on Growth.

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TABLE I
INCORPORATION OF 2-C¹⁴-ACETATE INTO β -METHYLGLUTA-
CONIC ACID BY AQUEOUS EXTRACTS OF RAT LIVER

Experiment	Radioactivity recovered in		
	β -Methyl- glutaconic acid, c.p.m./mg. C	β -Bromo- β -methyl- glutaric acid, c.p.m./mg. C	α,β -Dibromo- β -methyl- glutaric acid, c.p.m./mg. C
1	354	302	..
2	791
3	269	..	234

TABLE II
INCORPORATION OF 2-C¹⁴-ACETATE AND METHYL-C¹⁴-HMG
INTO CHOLESTEROL

Precursor	Radioactivity recovered ^a		
	Aqueous extract	Homog- enate	Slices
2-C ¹⁴ -Acetate	0.0073	0.0081	0.0095
3'-C ¹⁴ - β -Hydroxy- β -methylglu- taric acid	.0086	.0093	.0071

^a Recovered radioactivity expressed as μ moles of precursor incorporated = total recovered counts/counts per μ mole of precursor. Aliquots of same extract, homogenate and slices obtained from same liver were employed.

TABLE III
INCORPORATION OF C¹⁴-LABELED HMG AND 2-C¹⁴-ACETATE
INTO CHOLESTEROL, β -METHYLGLUTACONIC AND β -METHYL-
CROTONIC ACIDS BY PARTICLE-FREE EXTRACTS OF RAT LIVER

Precursor	Radioactivity recovered ^a		
	Cholesterol	β -Methyl- glutaconic acid	β -Methyl- crotonic acid
2-C ¹⁴ -Acetate	0.003	0.183	0.095
3'-C ¹⁴ - β -Hydroxy- β -methyl- glutaric acid	.005	.134	.094

^a Radioactivity expressed as μ moles of precursor incorporated in recovered product (see Table II).

ether for 24–48 hours. The ether solution was evaporated to dryness and kept in a desiccator over KOH for 24 hours. The residue was extracted with 10% acetic acid and again extracted continuously with ether. The ether extract was afterward evaporated to dryness. For the separation of either β -methylcrotonic acid or β -methylglutaconic acid, the oily residue was transferred to a vacuum micro sublimation apparatus and heated with a small micro burner at 1 millimeter of mercury until sublimation ceased. A pale yellow crystalline sublimate was obtained. This material was dissolved in a few milliliters of absolute ether, decolorized by warming with charcoal and petroleum ether added to the filtrate to incipient turbidity. When β -methylglutaconic acid was isolated, the material obtained (4–5 mg.) melted at 114–115°. A mixed melting point with an authentic sample⁷ showed no depression. The sublimed acid was plated and assayed for radioactivity; the counts were corrected to infinite thinness. The acid was diluted with carrier and subsequently converted to α,β -dibromo- β -methylglutaric acid⁸ which was recrystallized (m.p. 145°). A mixed melting point with authentic dibromo- β -methylglutaric acid showed no change. The β -bromo- β -methylglutaric derivative⁹ was also prepared (m.p. 129°); a mixed melting point with an authentic sample was unchanged. Subsequent radio-assay yielded the expected activity. Cholesterol was isolated as the digitonide and plated.³ Incubations with inactive preparations of particle-free extracts of rat liver yielded no radioactive β -methylglutaconic acid. Variations in the activity of different derivatives of the same sample were within counting errors.

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