free energy change at 950°K. to be −12,172 cal./mole for reaction (1) and −869 cal./mole for reaction (2). From these values, they further calculated free energies at 950 °K. for the reactions

$$\text{Mo} + \text{C}\ (\beta\text{-graphite}) = \text{MoC} \quad \Delta F^\circ_{950^\circ\text{K.}} = -3{,}211\ \text{cal./mole} \quad (3)$$

$$2\text{Mo} + \text{C} = \text{Mo}_2\text{C} \quad \Delta F^\circ_{950^\circ\text{K.}} = -8{,}862\ \text{cal./mole} \quad (4)$$

$$\text{MoC} + \text{Mo} = \text{Mo}_2\text{C} \quad \Delta F^\circ_{950^\circ\text{K.}} = -5{,}651\ \text{cal./mole} \quad (5)$$

and

$$2\text{MoC} = \text{Mo}_2\text{C} + \text{C} \quad \Delta F^\circ_{950^\circ\text{K.}} = -2{,}441\ \text{cal./mole} \quad (6)$$

The authors used 3310 cal./mole[2] for the free energy of formation of methane from β-graphite and hydrogen at 950°K.

From the log K_p *vs.* $10^3/T$ plot at $10^3/T = 1.0526$ ($T = 950$°K.), log $K_1 = 2.80$ and $\Delta F^\circ = -RT \ln K_p = -12{,}172$ cal./mole in agreement with the above calculation; however, log $K_2 = 0.47$ and $\Delta F^\circ = -2{,}043$ cal./mole for reaction (2).

Therefore, the free energy values calculated by Browning and Emmett for reactions (1) and (4) are correct, but the values for $\Delta F^\circ_{950^\circ\text{K.}}$ for reactions (2), (3), (5), and (6) should be −2,043, −3,798, −5,064, and −1,267 cal./mole.

(2) F. D. Rossini, *et al.*, Circular of the National Bureau of Standards C461 (1946).

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RECEIVED SEPTEMBER 24, 1956

THE METABOLISM OF β,γ-DIHYDROXY-β-METHYLVALERIC ACID BY LIVER HOMOGENATES

Sir:

The utilization of DL-β,δ-dihydroxy-β-methylvaleric acid (mevalonic acid, MVA)[1] in the biogenesis of cholesterol and, presumably, of other compounds that arise *via* the polymerization of isoprenoid units has been reported.[2]

This observation led us to consider the metabolic behavior of mevalonic acid with regard to certain aspects of the condensation process that occurs in the course of the reaction sequence leading to cholesterol. The high degree of incorporation of mevalonic acid into cholesterol (43% of the isotope of DL-2-C^{14}-MVA)[2] suggests that the molecule may be utilized without suffering the loss of more than one carbon. Squalene[3] (or a compound having the same carbon skeleton)[4,5] has been shown to be a precursor of cholesterol. Comparison of the structures of mevalonic acid and squalene offers the possibility that at some stage in the biosynthesis of cholesterol all of the carbons that originate from the carboxyl group of mevalonic acid may be lost.

In order to test this hypothesis, mevalonic acid labeled with C^{14} in the carboxyl group was prepared[6] and incubated with cell-free homogenates of rat liver.[7]

In each of the three experiments reported in Table I, we incubated, separately, 2-C^{14}-MVA and 1-C^{14}-MVA. A third and fourth series of flasks were incubated in experiment 3. These contained as substrate, 1-C^{14}-NaOAc alone, or together with 1-C^{14}-MVA.

TABLE I

CHOLESTEROL SYNTHESIS FROM MVA

Each flask contained 5 ml. of liver homogenate, 1 mg. each of ATP and DPN, and substrate as indicated. Final volume was 9.5 ml. Gas phase was 95% O_2–5% CO_2 except in experiment 2, where 100% O_2 was used. In a given numbered experiment all flasks contained aliquots of the same liver preparation. Incubation with agitation was carried out at 37° for 4.5 hours. Cholesterol was isolated and counted as the digitonide.

Expt.	Substrate added: Compound	μM	c.p.m. × 10^{-3}	Recovered cholesterol, c.p.m./mg. C
1a	2-C^{14}-MVA	0.6	11.5	4650
1b	1-C^{14}-MVA	6.0	124.5	8
2a	2-C^{14}-MVA	0.6	11.5	4500
2b	1-C^{14}-MVA	6.0	124.5	2
3a	2-C^{14}-MVA	0.6	11.5	4470
3b	1-C^{14}-MVA	6.0	124.5	2
3c	1-C^{14}-NaOAc	1.2	114.5	1670
3d	1-C^{14}-NaOAc + 1-C^{14}-MVA	1.2 + 6.0	114.5 + 124.5	1230

In every experiment the 2-C^{14}-MVA exhibits the high degree of incorporation into cholesterol previously encountered. On the other hand, carboxyl-labeled mevalonic acid contributes no isotope to the sterol.

When both carboxyl-labeled acetate and carboxyl-labeled mevalonic acid are incubated together (expt. 3d) the cholesterol that is produced has a specific activity lower than that observed when carboxyl-labeled acetate is incubated alone. The decrease is in the same order of magnitude as we experience when incubating equivalent amounts of *non-labeled* mevalonic acid with equivalent quantities of 1-C^{14}-NaOAc as the sole source of isotope.[7] This indicates that 1-C^{14}-MVA is utilized for cholesterol synthesis, but without inclusion of the carboxyl carbon of the mevalonic acid.

In experiment 2 we collected the CO_2 produced. All of the radioactivity of the 1-C^{14}-MVA could be accounted for in the barium carbonate that was isolated.

The data presented permit the conclusion that at some stage in the biosynthesis of cholesterol the carboxyl carbon of β,δ-dihydroxy-β-methylvaleric is lost.

(6) Kindly synthesized for us by Dr. C. S. Miller of this laboratory.

(7) Details of our procedure together with supplementary data will be presented in a future publication.

MERCK SHARP & DOHME
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WEST POINT, PA.

RECEIVED OCTOBER 29, 1956

ON THE MODE OF HEXOSE UPTAKE BY ASCITES TUMOR CELLS[1]

Sir:

The purpose of this communication is to present evidence for a step prior to hexokinase action which

(1) The letters MVA, rather than the previously used DVA, will serve to designate DL-β,δ-dihydroxy-β-methylvaleric acid, which has been renamed "mevalonic acid"; D. E. Wolf, C. H. Hoffman, P. E. Aldrich, H. R. Skeggs, L. D. Wright and K. Folkers, THIS JOURNAL, in press.

(2) P. A. Tavormina, M. H. Gibbs and J. W. Huff, *ibid.*, **78**, 4498 (1956).

(3) R. G. Langdon and K. Bloch, *J. Biol. Chem.*, **200**, 129 (1953).

(4) G. Popják, *Arch. Biochem. and Biophys.*, **48**, 102 (1954).

(5) F. Dituri, F. A. Cobey, J. V. B. Warms and S. Gurin, *J. Biol. Chem.*, **221**, 181 (1956).

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