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

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

$$2Mo + C = Mo_2C \quad \Delta F^\circ_{950^\circ K.} = -8{,}862\ \text{cal./mole} \quad (4)$$

$$MoC + Mo = Mo_2C \quad \Delta F^\circ_{950^\circ K.} = -5{,}651\ \text{cal./mole} \quad (5)$$

and

$$2MoC = Mo_2C + C \quad \Delta F^\circ_{950^\circ K.} = -2{,}441\ \text{cal./mole} \quad (6)$$

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 $\Delta F^\circ_{950^\circ K.}$ 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.

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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).

(1) This investigation was supported in part by American Cancer Society Institutional Grant No. 22 C and by the Michigan Memorial-Phoenix Project No. 45.

may be involved in the uptake of fructose by Ehrlich ascites tumor cells. Washed, intact tumor cells suspended in a Krebs–Ringer bicarbonate buffer at *p*H 7.45 were incubated anaerobically at 37° in a Warburg apparatus. The rate of anaerobic glycolysis[2] was followed after D-fructose and D-galactose were added singly as substrates. Table I shows that D-galactose was not glycolyzed by the tumor cells, whereas D-fructose served as an excellent glycolytic substrate. In confirmation, D-galactose, determined by the Somogyi method,[3] was not consumed significantly upon incubation with the tumor cells. When increasing quantities of galactose were added with fructose, however, a progressive inhibition in the rate of anaerobic glycolysis was observed. This inhibition was fully reversed by additional fructose. The utilization of glucose, however, was not affected by galactose.

TABLE I

EFFECT OF D-GALACTOSE ON D-FRUCTOSE UTILIZATION IN EHRLICH ASCITES TUMOR

System	Substrate present per vessel: D-Fructose μmoles	D-Galactose μmoles	μl./mg./hr.	% Change with galactose
Intact	16.7	...	25.3	...
Cells	16.7	222.0	8.7	−65
	16.7	444.0	4.8	−80
	39.0	222.0	15.9	−37
	140.0	222.0	25.7	0
	...	278.0	0	...
Tumor	16.7	...	21.0	...
Homogenate[a]	16.7	222.0	22.4	+ 5
	16.7	444.0	21.0	0
	...	222.0	1.5	...
Fructose consumption			μM./mg./hr.	
Intact	16.7	...	− 0.67	...
Cells	16.7	139.0	− 0.41	−40
Tumor	11.1	...	− 0.99	...
Homogenate[a]	11.1	167.0	− 0.99	0

[a] Tumor homogenates were prepared by the application and rapid release of a pressure of 1200 lb./sq. in. of nitrogen or by 100 passes in a Potter homogenizer. Each preparation yielded like results.

Measurements of anaerobic glycolysis with tumor homogenates were performed in a medium modified from that of LePage.[4] In sharp contrast to the results obtained with the cellular system, the experiments with tumor homogenates (Table I) demonstrated no effect whatsoever of galactose on fructose utilization. These results with intact cells and homogenates were confirmed by determining the disappearance of fructose from the medium by the method of Roe[5] (Table I). Therefore it is clear that high concentrations of galactose have no effect upon fructose utilization when cellular integrity has been destroyed.

Particular attention has been paid to the hexokinase step in fructose utilization since it has been reported in abstract form[6] that galactose can inhibit the hexokinase of Ehrlich ascites tumor. This has been critically reexamined by two independent techniques other than the manometric measurements: namely, disappearance of fructose and appearance of fructose-6-phosphate. The latter technique employed a direct spectrophotometric assay.[7] The data of Fig. 1 for TPN.H formation show that levels of galactose high enough to give 80–90% inhibition of fructose utilization in a cellular system had no effect upon the hexokinase of Ehrlich ascites tumor.

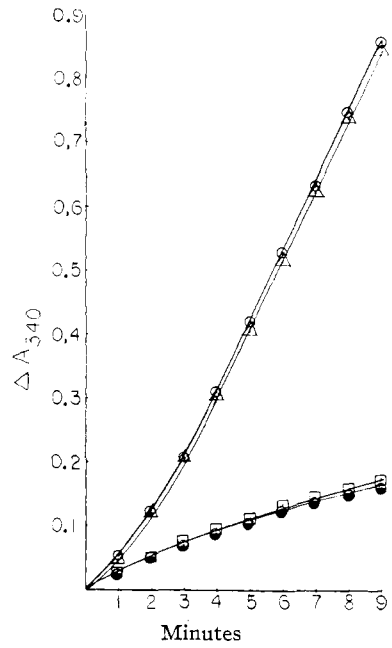

Fig. 1.—The effect of galactose on the hexokinase of Ehrlich ascites tumor: ⊙ 11 μmoles D-fructose, △ 11 μmoles D-fructose + 355 μmoles D-galactose, ◒ 355 μmoles D-galactose, ⊡ no substrate. These amounts of substrates were put into 3.0 ml. of a reaction mixture which contained: 100 μmoles of tris-(hydroxymethyl)-aminomethane buffer (*p*H 8.0), 5 μmoles of adenosine triphosphate, 10 μmoles of $MgCl_2$, 1.2-μmoles of triphosphopyridine nucleotide (TPN), 5 μmoles of KF, 0.2 K units of glucose-6-phosphate dehydrogenase, 2.5 mg. of crude phosphohexoisomerase and sufficient tumor hexokinase preparation (0.05–0.10 ml.) to give the indicated reaction rate. The soluble tumor hexokinase preparation was obtained by centrifuging a 33% homogenate at 20,000 × *g* for 30 min. at 5°.

The competition between D-galactose and D-fructose in cell suspensions requires a specific reaction step as the site of competition. Fructose reversal of the galactose inhibition and the lack of inhibition of glucolysis serve also to rule out an interference with diffusion. The complete absence of effect of galactose in homogenates and extracts leads to the conclusion that there must be a reaction step in fructose utilization by the cells which is prior to the hexokinase reaction. This prior step, possibly

(2) W. W. Umbreit, R. H. Burris and J. F. Stauffer, "Manometric Techniques and Tissue Metabolism," Burgess Publishing Co., Minneapolis, Minn., 1949.

(3) M. Somogyi, *J. Biol. Chem.*, **195**, 19 (1952).

(4) G. A. LePage, *ibid.*, **176**, 1009 (1948).

(5) J. H. Roe, *ibid.*, **107**, 15 (1934).

(6) R. K. Crane, 3rd Int. Cong. Biochem., Abstracts of Comm., Bruxelles, p. 108 (1955).

(7) M. W. Slein, G. T. Cori and C. F. Cori, *J. Biol. Chem.*, **186**, 763 (1950).

non-phosphorylative, may well effect the transfer of fructose into the cell.

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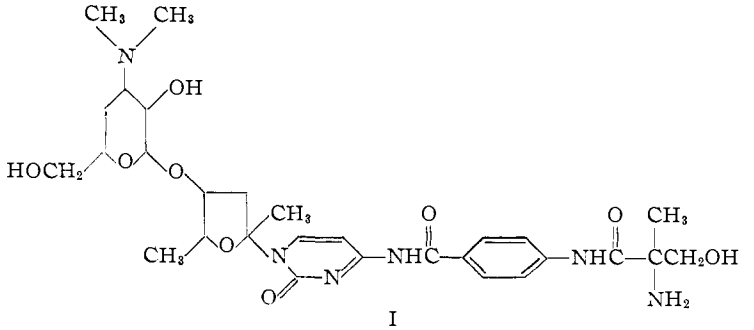
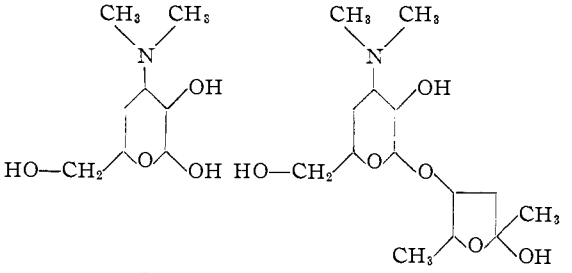
THE STRUCTURE OF AMICETIN. A NEW DIMETHYLAMINO SUGAR

Sir:

Structure I, which incorporates the new dimethylamino sugar *amosamine* (II), is presented for the antibiotic amicetin. In 1953, Flynn, Hinman, Caron and Woolf[1] published evidence in support of a partial structure for amicetin which included the α-methylserine, *p*-aminobenzoic acid, and cytosine moieties of structure I with an unknown C_{14} fragment attached to the 1-position of cytosine.

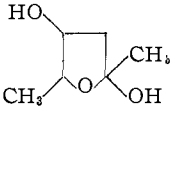


I



II

III



IV

From the acid hydrolysis of amicetin the C_{14} fragment was isolated as the crystalline hydrochloride, m.p. 170.5–171.5° (Calcd. for $C_{14}H_{27}NO_6 \cdot HCl$: C, 49.19; H. 8.26; N, 4.10; Cl, 10.37. Found: C, 49.14; H, 8.40; N, 4.12; Cl, 10.45), for which the name *amicetamine* and structure III[2] are proposed. Analysis of the amorphous free base indicated the presence of two N-methyl and two C-methyl but no O-methyl groups. The glycoside did not reduce Fehling or Benedict solution but possessed a potential carbonyl group as indicated by hydroxylamine titration although the infrared spectrum of the hydrochloride, when determined as a Nujol mull, did not show carbonyl absorption. The fact that the glycoside gave a positive iodoform test while cytosamine (the C_{14}-cytosine[1] moiety) did not indicated that the cytosine moiety was attached to C_{14} moiety III *via* a potential methyl ketone.

(1) E. H. Flynn, J. W. Hinman, E. L. Caron and D. O. Woolf, Jr., THIS JOURNAL, **75**, 5867 (1953).

(2) Reference 1 incorrectly reported the empirical formula of amicetin as $C_{29}H_{44}N_6O_9$ rather than $C_{29}H_{42}N_6O_9$ and consequently of the C_{14} portion as $C_{14}H_{28}NO_5$ rather than $C_{14}H_{26}NO_5$.

Aqueous sodium metaperiodate oxidized the glycoside III with the consumption of 2.7 to 2.9 moles in 24 hours. From these oxidations, dimethylamine was isolated as the *p*-hydroxyazobenzene *p'*-sulfonic acid salt, formic acid as the barium salt, glyoxal as the phenylosazone, formaldehyde as the dimedone derivative, and a small amount of acetaldehyde as the 2,4-dinitrophenylhydrazone derivative.

Glycoside III was hydrolyzed with the aid of a sulfonic acid resin (Dowex-50), and from the hydrolysis the dimethylamino sugar, amosamine (II), was isolated as the crystalline hydrochloride, m.p. 192–193° (calcd. for $C_8H_{17}NO_4 \cdot HCl$: C, 42.30; H, 7.97; N, 6.15; Cl, 15.57; mol. wt., 227.7. Found: C, 42.09; H, 8.13; N, 6.13; Cl, 15.18; mol. wt., 222), $[\alpha]^{25}D$ + 45.5 (1% in water). II reduced Fehling solution and consumed three moles of periodate from which one equivalent of formaldehyde could be isolated as the dimedone derivative and two equivalents of the volatile organic acid (formic) shown by titration. These data allow only a 2- or 4-deoxyaldohexose; the isolation of glyoxal from the C_{14} oxidation provides evidence for the 4-deoxy structure II. The dimethylamino group was shown to be in the 3-position (β-dimethylaminoaldehyde) by application of the procedure of Hochstein and Regna,[3] which compares the instability of various amino sugars in alkali; under the conditions they describe II lost 43% of one equivalent of dimethylamine in two hours.

The structure of the neutral moiety IV was deduced from the following data. The stable hemiketal structure for III demands a pyranose or furanose ring; the fact that the two C-methyl groups must reside in IV indicates the furanose form of IV in the glycoside III as well as in the antibiotic I. The position of the remaining methyl and hydroxyl groups in IV was determined by periodate oxidations. Cleavage of amicetin with methanolic hydrogen chloride followed by methanolysis (Dowex-50) of the resulting crude methyl glycoside of III gave a neutral fragment (IV methyl glycoside) which did not reduce periodate. The corresponding ketose IV consumed one equivalent of periodate from which 52% of acetaldehyde 2,4-dinitrophenylhydrazone could be isolated. These data allow only structure IV for the neutral moiety.

The cytosine was shown to be attached through the ketal carbon of IV by the iodoform tests. The reducing aminosugar must be attached, therefore, to the only remaining hydroxyl in IV *via* the aldehyde carbon to form the non-reducing glycoside III and the antibiotic amicetin I.

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RECEIVED OCTOBER 26, 1956

(3) F. A. Hochstein and P. P. Regna, THIS JOURNAL, **77**, 3354 (1955).