brucine in boiling methylene chloride. Under these circumstances the brucine is converted to the insoluble brucine-N-oxide in quantitative yield.

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RECEIVED OCTOBER 29, 1956

ENZYMATIC PHOSPHORYLATION OF D-XYLULOSE IN LIVER

Sir:

L-Xylulose, a naturally occurring sugar found in urine, has been shown to be glucogenic in diabetic dogs although the mechanism has been hitherto unknown.[1] Recently Touster, *et al.*,[2,3] have reported that L-xylulose may be converted to D-xylulose in guinea pig liver. Since D-Xu5P[4] is readily converted to G 6P,[5,6] it is clear that the phosphorylation of D-xylose in mammalian tissue is the only missing step in the formation of glucose from L-xylulose. The present finding of D-xylulokinase in liver completes this reaction sequence and supports the hypothesis of Touster which may be formulated as

$$\text{L-xylulose} \xrightarrow[\text{TPNH}]{} \text{xylitol} \xrightarrow[\text{DPN}]{}$$

$$\text{D-xyluose} \xrightarrow{\text{ATP}} \text{D-Xu5P} \underset{\text{cycle}}{\overset{\text{Pentose}}{\rightleftharpoons}} \text{G 6P} \longrightarrow \text{glucose}$$

TABLE I

ASSAY OF THE D-XYLULOKINASE REACTION PRODUCTS

Component	Phosphate ester formed[a] μmoles	Dephosphorylated sugar recovered[e] μmoles
Xylulose	150[b]	100[f]
Ribulose	100[c]	67[g]
Ribose	80[d]	55[g]
Total	330	222

[a] The reaction mixture containing 1000 μmoles each of D-xylulose, ATP and $MgCl_2$ was incubated with 100 mg. of the enzyme preparation in a total volume of 150 ml. of 0.02*M* triethanolamine buffer, *p*H 7.6, for 30 minutes at 37°. The reaction was stopped with perchloric acid and the supernatant treated with Norite to remove the adenine nucleotides. The filtrate was neutralized and the sugar phosphates precipitated by the addition of barium acetate and ethanol. The dry barium salt contained 360 μmoles of total phosphorus and 30 μmoles of inorganic phosphorus. [b] Xu5P was determined by a specific enzymatic assay employing rat liver transketolase and triosephosphate dehydrogenase.[7] [c] Ru5P was measured by the cysteine–carbazole method,[8] allowance being made for the Xu5P present. [d] R5P was assayed by the phloroglucinol reaction of Dische.[9] [e] Further confirmation of the identity of the reaction products was obtained by enzymatic dephosphorylation and isolation of the free sugars by Dowex-1 borate chromatography as previously described.[10] [f] Xylulose was identified by its characteristic reaction in the cysteine-carbazole and orcinol reaction, its optical rotation, $\alpha^{24}D$ −33° (H_2O, *c* 1.35), its behavior on paper chromatography in saturated phenol–water, and by the formation of a crystalline *p*-bromophenylhydrazone, the melting point of which remained unchanged at 126–128° when mixed with a similar derivative prepared from authentic D-xylulose. [g] The ribulose fraction exhibited an optical rotation of $\alpha^{24}D$ −15° (H_2O, *c* 0.90) and the ribose a rotation of $\alpha^{24}D$ −22° (H_2O, *c* 0.45). The identity of both sugars was further checked by paper chromatography and colorimetric analysis as above.

D-Xylulokinase has been purified about 10-fold from a water extract of calf liver acetone powder and shown to be specific for D-xylulose. The preparation was inactive toward D-xylose, L-xylulose, D-ribulose, D-ribose and D-fructose. The partially purified enzyme was free from transketolase but was contaminated with phosphoketopentoepimerase and phosphoribose isomerase. Consequently the over-all reaction observed was

$$\text{D-xylulose} + \text{ATP} \xrightarrow[\text{kinase}]{\text{xylulo-}} \text{D-Xu5P} \underset{}{\overset{\text{epimerase}}{\rightleftharpoons}}$$

$$\text{D-Ru5P} \underset{}{\overset{\text{isomerase}}{\rightleftharpoons}} \text{D-R5P}$$

Following incubation of ATP and D-xylulose with the enzyme preparation, the reaction mixture was found to contain all three phosphate esters in the quantities shown in Table I.

The inability of the enzyme preparation to react with either D-ribose or D-ribulose eliminated the possibility that either of these sugars might have served as substrate with subsequent conversion to D-Xu5P, It is therefore clear that mammalian tissue does possess the complete enzymatic structure necessary to carry out the conversion of L-xylulose to D-glucose as postulated by Touster. The identity of the enzymatic step, or steps, which are lacking in essential pentosuria remains to be determined.

(1) H. W. Larson, W. H. Chambers, N. R. Blatherwick, M. E. Ewing and S. D. Sawyer, *J. Biol. Chem.*, **129**, 701 (1939).

(2) O. Touster, V. H. Reynolds and R. M. Hutcheson, *ibid.*, **221**, 697 (1956).

(3) S. Hollmann and O. Touster, THIS JOURNAL, **78**, 3544 (1956).

(4) These abbreviations are used: D-Xu5P, D-xylulose 5-phosphate; G 6P, glucose 6-phosphate; D-Ru5P, D-ribulose 5-phosphate; D-R5P, D-ribose 5-phosphate; ATP, adenosinetriphosphate; TPNH, reduced triphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide.

(5) P. A. Srere, J. R. Cooper, V. Klybas and E. Racker, *Arch. Biochem. Biophys.*, **59**, 535 (1955).

(6) B. L. Horecker, J. Hurwitz and P. Z. Smyrniotis, THIS JOURNAL, **78**, 692 (1956).

(7) Method to be published elsewhere.

(8) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

(9) The authors are grateful to Dr. Dische for kindly making the details of his method available in advance of publication.

(10) G. Ashwell and J. Hickman, THIS JOURNAL, **76**, 5889 (1954).

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RECEIVED OCTOBER 17, 1956

THERMODYNAMIC CALCULATIONS FOR THE Mo–C–H SYSTEM

Sir:

Browning and Emmett[1] have reported on "Equilibrium Measurements in the Mo–C–H System," the equilibria investigated being

$$2\,Mo + CH_4 = Mo_2C + 2H_2 \qquad K_1 = (H_2)^2/CH_4 \quad (1)$$

and

$$Mo_2C + CH_4 = 2MoC + 2H_2 \qquad K_2 = (H_2)^2/CH_4 \quad (2)$$

From a plot of log K_p *vs.* $10^3/T$, °K. for reactions (1) and (2), Browning and Emmett calculated the

(1) L. C. Browning and P. H. Emmett, THIS JOURNAL, **74**, 4773 (1952).

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.

MERCK SHARP & DOHME
RESEARCH LABORATORIES PETER A. TAVORMINA
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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).

(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.