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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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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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).