Found: (I) (for corresponding iodide): Os, 30.3; N, 13.55; I, 41.0. Calcd. for $(Os(en\text{-}H)_2en)I_2$, (en-H=$NH_2CH_2CH_2NH$[1]): Os, 30.56; N, 13.52; I, 40.87. Found (II): Os, 28.9; N, 12.64; Br, 36.3. Calcd. for $(Os(en\text{-}H)en_2)Br_3 \cdot 3H_2O$: Os, 28.69; N, 12.68; Br, 36.20.

In anhydrous ethylenediamine at 100° in the absence of air, (I) dissolved to a red solution which became intense green in color. The green solid (IV) precipitated by alcohol lost ethylenediamine very easily with reversion to (I) and could not be obtained pure. It appeared to be $(Os(en\text{-}H)_2\text{-}en_2)I_2$. In such an 8-covalent complex, (d^4sp^3 bonds) two electrons must be promoted to the 7s or 6d orbitals—presumably the former, since (IV) was diamagnetic. In the air (IV) rapidly oxidized to yield two greenish brown substances (V), (VI) separated by crystallization from methanol and ether. The less soluble (V) was paramagnetic, (1.78 B.M.) and is probably a pentavalent osmium compound—the first recorded. Found: Os, 25.4; N, 14.7; I, 33.6; H_2O, 9.6. Calcd. for $(Os(en\text{-}H)_3\text{-}en)I_2 \cdot 4H_2O$: Os, 25.25; N, 14.87; I, 33.74; H_2O, 9.57. (VI) was diamagnetic (no unpaired electrons), and appears to be an 8-covalent hexavalent osmium compound. Found: Os, 25.8; N, 15.3; I, 34.2. Calcd. for $(Os(en\text{-}H)_4)I_2 \cdot 3H_2O$: Os, 25.91; N, 15.25; I, 34.60. Dilute aqueous solutions of (V) and (VI) were brown and green, respectively, reacted alkaline and accepted up to two equivalents of acid. They were interconvertible by oxidizing and reducing agents.

DEPARTMENT OF CHEMISTRY
SYDNEY UNIVERSITY F. P. DWYER
SYDNEY, AUSTRALIA J. W. HOGARTH

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THE COENZYME FUNCTION OF THIAMINE PYROPHOSPHATE IN PENTOSE PHOSPHATE METABOLISM

Sir:

One of the products of pentose phosphate cleavage by enzymes from red cells,[1] bacteria and yeast,[2] liver[3] and plant sources[4] has been identified as triose phosphate. The two-carbon fragment, however, has not been characterized; glycolaldehyde, the expected product, does not accumulate or react in the presence of such enzyme preparations.[2,5]

In a previous report[3] we have described the formation of sedoheptulose phosphate from pentose phosphate with an enzyme preparation from rat liver. A similar conversion has now been observed with a highly purified spinach enzyme preparation. This preparation contains substantial amounts of thiamin pyrophosphate (ThPP) in a bound form which can be separated by precipitation of the protein with ammonium sulfate at low *p*H. The inactive enzyme obtained in this manner can be almost completely reactivated by the addition of ThPP.

The ThPP content of a purified enzyme preparation, determined manometrically with the carboxylase assay of Lohmann and Schuster[6] was found to be 0.8×10^{-2} micromole per mg. of protein. On the assumption that the molecular weight is about 100,000, each mole of protein contained about 0.8 mole of ThPP. Since the enzyme preparation had been purified about 100-fold from spinach leaf extracts by a procedure which included several ammonium sulfate fractionations, dialysis, fractionation with acetone and absorption on calcium phosphate gel and elution, it is evident that the coenzyme is not readily dissociated from the protein. Essentially complete separation of the coenzyme was obtained at *p*H 3 in the presence of ammonium sulfate (45% saturated). With 10^{-4} *M* ThPP (Table I) the activity obtained represented

TABLE I

THE THPP REQUIREMENT OF THE ACID-PRECIPITATED ENZYME

In Experiment 1 pentose phosphate splitting activity was followed spectrophotometrically by measuring triose phosphate formation according to Racker.[7] The absorption cell contained 3.4×10^{-4} *M* ribulose-5-phosphate, 5.8×10^{-6} *M* reduced DPN, 0.05 mg. of crude rabbit muscle fraction containing α-glycerophosphate dehydrogenase and triose phosphate isomerase and 0.004 mg. of spinach enzyme. The total volume was 1.06 ml. In Experiment 2 sedoheptulose phosphate formation was followed with the orcinol reaction.[3] The incubation mixture contained 5×10^{-3} *M* ribulose-5-phosphate and 0.5 mg. of acid-precipitated enzyme. The total volume was 0.5 ml. Glycylglycine, buffer, 0.01 *M*, *p*H 7.4, was present in both experiments. The temperature was 25°. $MgCl_2$ and ThPP, when added, were 2×10^{-3} and 10^{-4} *M*, respectively.

Enzyme	ThPP	$MgCl_2$	Experiment 1 Triose P[a]	Experiment 2 Sedoheptulose P[b]
Original	−	−	8.1×10^{-3}	
Acid treated	+	+	6.0×10^{-3}	
Acid treated	−	+	0.8×10^{-3}	
Acid treated	+	−	1.7×10^{-3}	
Acid treated	−	−		0
Acid treated	+	+		1.1

[a] Micromoles formed per minute. [b] Micromoles formed in thirty minutes. The reaction at this time was essentially complete.

TABLE II

THE FORMATION OF PENTOSE PHOSPHATE AND HEPTULOSE PHOSPHATE FROM L-ERYTHRULOSE AND TRIOSE PHOSPHATE

The reaction mixture contained 0.02 *M* L-erythrulose,[8] 0.004 M hexosediphosphate as a source of triose phosphate, 0.047 mg. of crystalline muscle aldolase and 0.6 unit of resolved enzyme in a total volume of 0.79 ml. In the complete system 10^{-4} *M* ThPP and 10^{-3} *M* $MgCl_2$ were added. Pentose and heptulose were determined in the orcinol reaction. Amounts are in micromoles.

Time, minutes	No ThPP		Complete system	
	Pentose	Heptulose	Pentose	Heptulose
30	0	0	1.2	1.2
60	0	0	1.8	1.6
120	0	0	2.3	2.4
180	0	0	2.5	2.9

(1) Z. Dische, *Naturwiss.*, **26**, 252 (1938).
(2) E. Racker, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 147.
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(5) Z. Dische, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 195.
(6) K. Lohmann and P. Schuster, *Biochem. Z.*, **294**, 188 (1937).
(7) E. Racker, *J. Biol. Chem.*, **167**, 843 (1947).
(8) Kindly furnished by Dr. G. C. Mueller of the McArdle Memorial Laboratory, Madison, Wis.

71% of the original activity. Only partial reactivation occurred in the absence of Mg^{++} ions.

The spinach enzyme also catalyzes the reaction of L-erythrulose and D-glyceraldehyde-3-phosphate to form a mixture of pentose phosphate and heptulose phosphate. Neither product is formed in the absence of ThPP (Table II).

While the mechanism of sedoheptulose phosphate formation is not yet known, the reactivity of erythrulose in this system suggests that it may be formed by the reactions

2 D-ribulose-5-phosphate $\rightleftharpoons$ L-erythrulose + 2 D-glyceraldehyde-3-phosphate (1)

L-erythrulose + D-glyceraldehyde-3-phosphate $\longrightarrow$ sedoheptulose-7-phosphate (2)

An alternative mechanism, supported by the requirement for ThPP for sedoheptulose phosphate synthesis from erythrulose, would be a condensation of ribose phosphate with an active two-carbon fragment. Pentose phosphate isomerase is still present in the enzyme preparation and the participation of ribose phosphate has not been excluded. In either case an activated form of glycolaldehyde, formed in the cleavage of pentose phosphate, would undergo an acyloin condensation. The synthesis of acetoin in such reactions is known to require ThPP.[9,10] The name transketolase, suggested by Racker, de la Haba and Leder[11] is consistent with this formulation. In the presence of spinach enzyme pentose formation from erythrulose is observed with other aldehydes, such as D-glyceraldehyde and L-glyceraldehyde-3-phosphate.

(9) M. Silverman and C. H. Werkman, *J. Biol. Chem.*, **138**, 35 (1941).

(10) D. E. Green, W. W. Westerfeld, B. Vennesland and W. E. Knox, *J. Biol. Chem.*, **145**, 69 (1942).

(11) E. Racker, G. de la Haba and I. G. Leder, THIS JOURNAL, **75**, 1010 (1953).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY
BETHESDA 14, MARYLAND
B. L. HORECKER
P. Z. SMYRNIOTIS

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THIAMINE PYROPHOSPHATE, A COENZYME OF TRANSKETOLASE

Sir:

In a note by Horecker and Smyrniotis[1] previous work on enzymes concerned in the breakdown of pentose phosphate is quoted. We have isolated from baker's yeast a crystalline enzyme which catalyses the cleavage of ribulose-5-phosphate with the formation of D-glyceraldehyde-3-phosphate, identified by means of glyceraldehyde-3-phosphate dehydrogenase free of triose isomerase. The cleavage of ribulose-5-phosphate[2] occurs only on the addition of an "acceptor aldehyde" such as ribose-5-phosphate, glycolaldehyde, or glyceraldehyde. The enzyme was also found to decarboxylate hydroxypyruvate in the presence of an "acceptor aldehyde." With D-glyceraldehyde-3-phosphate (formed from fructose-1,6-diphosphate by aldolase) as acceptor aldehyde, the decarboxylation of hydroxypyruvate led to the formation of ribulose-5-phosphate.[3] The pentose phosphate was isolated as an alcohol insoluble barium salt and determined by two independent tests as shown in Table I. Similar results were obtained when DL-glyceraldehyde-3-phosphate (Concord Laboratories) was used instead of fructose-1,6-diphosphate and aldolase.

TABLE I

ENZYMATIC FORMATION OF RIBULOSE-5-PHOSPHATE FROM HYDROXYPYRUVATE AND TRIOSE PHOSPHATE

0.5 mg. of purified yeast transketolase (22,000 units per mg. protein) was used in these experiments. Carbon dioxide was measured manometrically. In Expt. 1, the reaction mixture (2 ml.) contained 100 micromoles of potassium phosphate (*p*H 6.5), 5 micromoles of fructose-1,6-diphosphate, 20 micrograms of aldolase, 12 micromoles of $MgCl_2$, 20 micrograms of ThPP and about 30 micromoles of sodium hydroxypyruvate. In Expt. 2, 100 micromoles of tris-(hydroxymethyl)-aminomethane (*p*H 6.9) was used instead of potassium phosphate and the concentration of fructose-1,6-diphosphate was increased to 10 micromoles. The vessels were incubated at 37° for 75 minutes in Expt. 1 and 175 minutes in Expt. 2. Deproteinization with 5% trichloroacetic acid was followed by the isolation of an alcohol-insoluble barium salt which was analyzed colorimetrically as well as spectrophotometrically. In the latter test transketolase free of pentose isomerase was used and triose phosphate formation was measured with either glycolaldehyde, glyceraldehyde, or ribose-5-phosphate as "acceptor aldehydes."

Expt.	CO_2 liberation, micromoles	Isolated ribulose-5-phosphate, micromoles	
		Orcinol reaction	Spectrophotometric
1	4.9	1.8	1.6
2	4.0	3.1	2.9

TABLE II

THIAMINE PYROPHOSPHATE REQUIREMENT OF TRANSKETOLASE

The enzyme preparation was dialyzed against 1000 volumes of 0.6% Versene in 0.02 *M* potassium phosphate of *p*H 7.4 for 20 hours and then against 1000 volumes of 0.6% Versene in 0.9% KCl for another 20 hours. The enzyme was assayed by measuring triose phosphate formation from ribulose-5-phosphate in the presence of ribose-5-phosphate as "acceptor aldehyde."

Enzyme preparation	Additions to test system	Activity (units per ml.)
Undialyzed	.....	50,000
Dialyzed for 40 hours	.....	2,000
	3 μM. $MgCl_2$	5,000
	50 μg. ThPP and 3 μM. $MgCl_2$	43,000
Dialyzed for 40 hours then left in icebox for 24 hours	.....	500
	3 μM. $MgCl_2$	1,000
	50 μg. of ThPP	7,000
	50 μg. of ThPP and 3 μM. of $MgCl_2$	40,000

Since the formation of ribulose-5-phosphate represents a ketol condensation, and no free glycolaldehyde is formed, one must assume the formation of an "active glycolaldehyde" which condenses with the "acceptor aldehyde" to form a ketosugar. The enzyme may therefore be termed a transketolase.

(1) B. L. Horecker and P. Z. Smyrniotis, THIS JOURNAL, **75**, 1009 (1953).

(2) We wish to thank Dr. B. L. Horecker for a gift of ribulose-5-phosphate.

(3) A similar reaction catalyzed by rabbit muscle mince has been described by S. Akabori, Kihachiro Uehara and I. Muramatsu, *Proc. Japan Academy*, **28**, 39 (1952).