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

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

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

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.

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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	Isolated ribulose-5-phosphate, micromoles 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.

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[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).

The activity of a partially purified transketolase from *E. coli* was doubled by the addition of thiamine pyrophosphate (ThPP). No requirement for ThPP was found with a twice recrystallized preparation of the yeast enzyme, but extensive dialysis against a Versene–KCl solution caused nearly complete inactivation. Addition of magnesium chloride and of ThPP to the dialyzed enzyme restored the activity, as shown in Table II. The crystalline yeast enzyme shows no aldolase, triose isomerase or pentose isomerase activity.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY E. RACKER
YALE UNIVERSITY G. DE LA HABA
NEW HAVEN, CONNECTICUT I. G. LEDER

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BIS-CYCLOPENTADIENYL DERIVATIVES OF SOME TRANSITION ELEMENTS

Sir:

The structure suggested[1] for bis-cyclopentadienyliron(II)[2] (ferrocene), in which the iron atom is symmetrically placed between two cyclopentadienyl rings, has been confirmed by X-ray crystal structure measurements.[1b,3] The original proposals[1] of this formulation were coupled with the suggestion that the electronic structure of the iron atom attains an inert gas configuration, and this idea could also be extended to the ruthenium analog $C_{10}H_{10}Ru$[4] and to the bis-cyclopentadienylcobalt(III) (cobalticinium) ion $[C_{10}H_{10}Co]^+$,[1b,5] which is isoelectronic with ferrocene.

In addition to the objection that a high negative charge would be placed on the central metal atom, the aromatic properties[6] of ferrocene make it seem most unlikely that all the π electrons of the cyclopentadienyl rings can be involved in the filling of the orbitals of the metal atom. More definite evidence against this view has now been obtained.

Bis-cyclopentadienylnickel(II) has been prepared by the reaction of cyclopentadienylmagnesium bromide with nickel(II) acetylacetonate. (*Anal.* Calcd. for $C_{10}H_{10}Ni$: C, 63.6; H, 5.3; Ni, 31.0. Found: C, 63.7; H, 5.5; Ni, 31.0). It forms dark green crystals from ligroin which decompose slowly even in absence of air and light; it sublimes above 130° but decomposes below the melting point. Solutions in an alcoholic supporting electrolyte show a polarographic anodic wave at −0.08 volt *versus* the saturated calomel electrode. The yellow solutions obtained by oxidation contain the bis-cyclopentadienylnickel(III) ion, $[C_{10}H_{10}Ni]^+$, and give precipitates with silicotungstic acid, Reinecke's salt, potassium triiodide, etc., as do the ferricinium,[1] ruthenicinium[4] and cobalticinium[5] ions. Aqueous solutions of the bis-cyclopentadienylnickel(III) ion are rather unstable, and decompose in a few minutes. An unstable dark brown crystalline picrate is obtained by mixing ether solutions of bis-cyclopentadienylnickel (II) and picric acid in presence of air (*Anal.* Calcd. for $C_{16}H_{12}N_3O_7Ni$: Ni, 14.1. Found Ni, 14.0).

On the above view, the nickel atom in bis-cyclopentadienylnickel(II) should have two electrons in excess of the krypton structure, which would be expected to occupy the 5s orbital. Magnetic susceptibility measurements show, however, that bis-cyclopentadienylnickel(II) has two unpaired electrons ($\chi^{25°}_{\text{mol}}$. = $+3440 \times 10^{-6}$ c.g.s.u. corrected for diamagnetic contribution, $\mu_{\text{eff}} = 2.88$ B.M.). In our view, this fact may be best accommodated by assuming that in these bis-cyclopentadienyl compounds, the metal ion utilizes three of the electrons from each of the two cyclopentadienyl anions, forming bonds involving the *s* and two *d* orbitals of the metal. In the case of Ni(II), the formation of $C_{10}H_{10}Ni$ would involve promotion of two electrons to the 4p orbitals, which must be singly occupied. Further, we have been unable to obtain a bis-cyclopentadienyl derivative of Cu(II), which has only one *d* orbital available, and cyclopentadienyl derivatives of the zinc group show properties of typical organo-metallic compounds. We have, however, been able to prepare bis-cyclopentadienyl compounds of titanium, zirconium and vanadium, where sufficient electrons are not available for completion of an inert gas configuration of the metal atom.

Bis-cyclopentadienyltitanium(IV) dibromide has been prepared by the reaction of excess cyclopentadienylmagnesium bromide with titanium tetrachloride in toluene solution. It forms dark red crystals, m.p. 240–243°, from toluene (*Anal.* Calcd. for $C_{10}H_{10}TiBr_2$: C, 35.6; H, 3.0; Ti, 14.2; Br, 47.3. Found: C, 36.0; H, 3.1; Ti, 14.3; Br, 47.3) and is diamagnetic ($\chi^{25°}_{\text{mol}} = -145 \times 10^{-6}$ c.g.s.u.). It is to some extent hydrolyzed by water, giving a yellow solution, which gives precipitation reactions similar to those of other bis-cyclopentadienyl metal ions. A crystalline picrate (m.p. 139–141°, explodes) has been isolated (*Anal.* Calcd. for $C_{22}H_{14}N_6O_{14}Ti$: Ti, 7.55. Found: Ti, 7.48). Aqueous perchlorate solutions show a polarographic cathodic wave at −0.44 volt *versus* the saturated calomel electrode. Controlled potential reduction, or reduction using a Jones reductor, produces a green solution containing the bis-cyclopentadienyltitanium(III) ion which shows a polarographic anodic wave at −0.44 volt.

The almost colorless bis-cyclopentadienylzirconium(IV) dibromide (*Anal.* Calcd. for $C_{10}H_{10}ZrBr_2$, C, 31.5; H, 2.7; Zr, 24.0; Br, 41.9. Found: C, 31.4; H, 2.7; Zr, 23.9; Br, 42.0) (m.p. 260° C. dec.) was prepared from zirconium tetrachloride and cyclopentadienylmagnesium bromide. Aqueous solutions of this compound show no polarographic reduction wave.

Vanadium tetrachloride reacts similarly, forming a dark green, rather unstable, ligroin soluble, bromide (*Anal.* Calcd. for $C_{10}H_{10}VBr_2$: V, 14.9; Br, 46.9. Found: V, 14.8; Br, 47.1) and a pale green, ligroin insoluble chloride (*Anal.* Calcd. for $C_{10}H_{10}VCl_2$: C, 47.6; H, 4.0; V, 20.2; Cl, 28.2. Found:

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[2] (a) T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951); (b) S. A. Miller, J. A. Tebboth and J. F. Tremayne, *J. Chem. Soc.*, 632 (1952).

[3] (a) P. F. Eiland and R. Pepinsky, THIS JOURNAL, **74**, 4971 (1952); (b) J. D. Dunitz and L. E. Orgel, *Nature*, **171**, 121 (1953).

[4] G. Wilkinson, THIS JOURNAL, **74**, 6146 (1952).

[5] G. Wilkinson, *ibid.*, **74**, 6148 (1952).

[6] R. B. Woodward, M. Rosenblum and M. C. Whiting, *ibid.*, **74**, 3458 (1952).