In a similar manner we have derived the following equation for the ratio ($C^{14}O_2$ from 6-C^{14}-glucose/$C^{14}O_2$ from 1-C^{14}-glucose), which we shall designate W

$$W = \frac{(E/6R)}{(E/6R) + (1 - E)}, \text{ whence}$$

$$E = \frac{6RW}{1 + W(6R - 1)} \qquad (2)$$

Again, using the experimental values of Bloom, *et al.*,[2,3] for R and W, namely, 2 and 0.3, respectively, we calculate that 84% of the CO_2 derived from glucose was formed glycolytically, a value in good agreement with that obtained from our equation (1).

In our experiments with rat liver slices, the observed ratio $U = a/b$ was higher than that reported by Bloom, *et al.*,[2] and varied between 1 and 1.8. However, these workers added lactate, acetate and gluconate, in high concentrations, to their media. Under such conditions, as shown in Table I, $C^{14}O_2$ formation from glucose is depressed, and the relative importance of the hexose monophosphate shunt is increased.

TABLE I

250-mg. rat liver slices incubated with 2.5 ml. of Krebs–Ringer bicarbonate buffer containing 55 μmoles of labeled glucose. The addition was 50 mm. each of lactate, acetate and gluconate. Gas phase 95% O_2 + 5% CO_2; incubated for 3 hours at 37°.

Label in glucose	Addition		% of C^{14} in $C^{14}O_2$	$U = a/b$	$E^a \times 100\%$
even-C^{14}	None	a	2.8		
				1.2	94
1-C^{14}	None	b	2.3		
even-C^{14}	+	a	0.8		
				0.6	79
1-C^{14}	+	b	1.4		

[a] These values of E are calculated from our equation (1) using the value of $R = 2$ determined experimentally by Bloom, *et al.*[2]

Thus, in rat liver slices, over 90% of the CO_2 is derived from glucose *via* glycolysis, and even under the special conditions of Bloom, *et al.*,[2,3] about 80% of the CO_2 is formed glycolytically.

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RECEIVED JANUARY 25, 1954

IDENTIFICATION OF A FOURTH ABNORMAL HUMAN HEMOGLOBIN

Sir:

In addition to normal adult (A) and fetal (F) hemoglobins three abnormal forms (S, C and D) of human hemoglobin have been described.[1,2] We wish to report the identification of a fourth abnormal form in the erythrocytes of a child (M. M.) with an atypical anemia. Filter paper electrophoresis of hemoglobin from this individual in 0.01 M sodium barbital, pH 9.2, at room temperature revealed two components, one with the mobility of hemoglobin F and the other with a mobility very nearly that of hemoglobin C. The same result was obtained by moving boundary electrophoresis in 0.01 M Na_2HPO_4, pH 8.8, at 1.4°. Moving boundary electrophoresis in cacodylate buffer of ionic strength 0.1 and pH 6.5 at 1.4° showed a component with the mobility of hemoglobin F and a component with a mobility greater than that of hemoglobin A but slightly less than that of sickle cell (S) hemoglobin. The mobilities of hemoglobins A, S and C in this buffer are, respectively, 2.4, 2.9 and 3.2 × 10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}.[1] The component having the mobility of hemoglobin F comprised 41% of the total by electrophoretic analysis. This component was isolated from the ascending limb of a moving boundary experiment in 0.01 M Na_2HPO_4; its ultraviolet absorption spectrum was found to be that of hemoglobin F. The ultraviolet spectrum of the original specimen did not differ significantly from that of a comparable mixture of hemoglobins A and F.[3] When the original specimen was partially denatured with dilute sodium hydroxide, the amount of alkali-resistant hemoglobin recovered corresponded to the amount of the fetal electrophoretic component in the specimen. The alkali-resistant component was found to have the ultraviolet spectrum of hemoglobin F. The non-fetal component, isolated from the ascending limb of the pH 6.5 moving boundary experiment, had the color and visible spectrum of hemoglobin. The solubility of the original specimen as amorphous ferrohemoglobin in 2.58 M phosphate buffer of pH 6.8 at 25° was 1.94 g. per liter, a value similar to those of mixtures of hemoglobins A and F under the same conditions.[4] We conclude from these results that the hemoglobin specimen examined consisted of a mixture of hemoglobin F and a hitherto undescribed abnormal human hemoglobin, which we shall call hemoglobin E. It differs from all of the previously described forms in its electrophoretic behavior. Its absorption spectrum, solubility and lability to alkali denaturation are similar to those of normal adult hemoglobin. A detailed account of this work will be published later.

(1) H. A. Itano, *Science*, **117**, 89 (1953).
(2) J. V. Neel, *et al.*, *ibid.*, **118**, 116 (1953).
(3) G. H. Beaven, H. Hoch and E. R. Holiday, *Biochem. J.*, **49**, 374 (1951).
(4) H. A. Itano, *Arch. Biochem. Biophys.*, **47**, 148 (1953).

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RECEIVED MARCH 22, 1954

REACTION OF BIS-(CYCLOPENTADIENYL)-TITANIUM DICHLORIDE WITH ARYLLITHIUM COMPOUNDS[1]

Sir:

Bis-(cyclopentadienyl)-iron was first described[2] in 1951. Since then, analogous complexes of a number of other transition elements have been prepared.[3] The structure of the iron complex has

(1) This work was carried out under Contract Nonr-582(00) with the Office of Naval Research.
(2) T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951).
(3) (a) G. Wilkinson, THIS JOURNAL, **74**, 6146, 6148 (1952); **76**, 209 (1954); (b) G. Wilkinson, P. L. Pauson, J. M. Birmingham and F. A. Cotton, *ibid.*, **75**, 1011 (1953).

been determined,[4] and the nature of the bonding has been discussed.[4,5] This complex may be regarded as a new type of aromatic system.[6] From another point of view, it may be considered that the laminate complex itself partakes of the nature of a transition metal. The halides of such complexes might then be expected to react with Grignard reagents or with organolithium compounds to produce organometallic derivatives of the complex.

We have carried out a number of such reactions with titanium compounds. Bis-(cyclopentadienyl)-titanium dichloride, dark red crystals (decomposed on heating; calcd. for $C_{10}H_{10}TiCl_2$: C, 48.23; H, 4.05; Cl, 28.48; Ti, 19.24. Found: C, 48.24; H, 4.10; Cl, 28.68; Ti, 19.1), was prepared in 72% yield from cyclopentadienyllithium and titanium tetrachloride.[7] Reaction of this dichloride with two equivalents of phenyllithium gave diphenyl-bis-(cyclopentadienyl)-titanium, in yields up to 81%, as orange-yellow crystals which could be recrystallized from methylene chloride–pentane mixtures (decomposed on heating; calcd. for $C_{22}H_{20}Ti$: C, 79.52; H, 6.07; Ti, 14.42; mol. wt., 332. Found[8]: C, 79.35, 79.12; H, 6.22, 6.19; Ti, 14.62, 14.59; mol. wt. cryoscopic in benzene, 317). Similarly, di-*p*-tolyl bis-(cyclopentadienyl)-titanium (orange-yellow; calcd. for $C_{24}H_{24}Ti$: Ti, 13.29. Found: Ti, 13.02, 13.07) and di-*p*-dimethylaminophenyl-bis-(cyclopentadienyl)-titanium (maroon; calcd. for $C_{26}H_{30}N_2Ti$: Ti, 11.45. Found: Ti, 11.31, 11.25) were prepared.

The thermal stability of these products varied with the nature of the R group in the $R_2[(C_5H_5)_2Ti]$ molecule. The diphenyl and di-*p*-tolyl compounds could be stored for some days at room temperature, although there was apparently slow decomposition. Pyrolysis of the dry diphenyl compound at temperatures above 105°, under nitrogen, gave benzene, plus other products. The di-*p*-dimethylaminophenyl compound was less stable thermally, although it could be preserved in crystalline form in a cold chest. Pyrolysis of this substance gave N,N-dimethylaniline and traces of cyclopentadiene, plus residues. Attempts to carry out analogous preparations from α-naphthyl- or *o*-tolyllithium gave crystalline crude products, but these decomposed during attempts at recrystallization. With care, these products could probably be purified.

The diphenyl-bis-(cyclopentadienyl)-titanium, treated with phenyllithium in ether, dissolved in part to give a dark orange-brown or nearly black solution, and phenyllithium was apparently used up in the process. By hydrolysis of the ether solution, the diphenyl compound could be recovered. Formation of the laminate complex presumably requires two of the d orbitals of titanium.[4,5] The bonds to the two phenyl groups would require two more, and there would then remain one empty d orbital, plus the 4s orbital. The reaction with phenyllithium may involve the establishment of bonds with phenyl anions, using these orbitals, to form a complex anion in equilibrium with the neutral compound. The situation is somewhat

$$(C_6H_5)_2[(C_5H_5)_2Ti] + C_6H_5Li \rightleftharpoons (C_6H_5)_3[(C_5H_5)_2Ti]^- Li^+$$

analogous to the case of diphenyllead, which is believed[9] to enter into such an equilibrium involving phenyllithium and the triphenyllead anion. These reactions are being investigated further.

(4) E. O. Fischer and W. Pfab, *Z. Naturforsch.*, **7b**, 377 (1952); P. F. Eiland and R. Pepinsky, THIS JOURNAL, **74**, 4971 (1952); J. D. Dunitz and L. F. Orgel, *Nature*, **171**, 121 (1953).

(5) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2125 (1952); H. H. Jaffé, *J. Chem. Phys.*, **21**, 156 (1953).

(6) R. B. Woodward, M. Rosenblum and M. C. Whiting, THIS JOURNAL, **74**, 3458 (1952).

(7) The dibromide has been described previously, ref. 3b.

(8) Carbon-hydrogen analyses by Clark Microanalytical Laboratory, Urbana, Illinois. The analyses were complicated by a tendency of the samples to explode when heated in oxygen.

(9) H. Gilman, L. Summers and R. W. Leeper, *J. Org. Chem.*, **17**, 630 (1952).

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ON THE MECHANISM OF THE ENZYMATIC SYNTHESIS OF SUCCINYL CoA[1]

Sir:

The purification and properties of the P enzyme[2] from heart muscle have been reported.[3] The enzyme, which catalyzes the reversible reaction 1,

$$ATP + CoA\text{-}SH + Succinate \overset{(Mg^{++})}{\rightleftharpoons} ADP + P + Succinyl\text{-}S\text{-}CoA \quad (1)$$

has now been purified extensively from spinach[4] and the reaction mechanism studied with the aid of isotopes.

In agreement with previous results with the heart enzyme,[3] the incorporation of P in ATP requires the presence of both succinate and CoA. The enzyme also catalyzes the exchange of C^{14}-succinate with succinyl CoA. This reaction (*cf.* Table I) is stimulated by P and requires Mg^{++}. Addition of ADP, which completes the system, causes some inhibition. The enzyme is free of CoA transferase[5] which catalyzes a similar exchange in the absence of P and Mg^{++}.

Further light was shed by studying the exchange of P^{32}-ADP with ATP.[6] The purified spinach enzyme catalyzes this exchange at very low protein concentrations (Fig. 1). This exchange is also Mg^{++}-dependent. The enzyme is free of myokinase and the ITP-ADP transphosphorylase[7] either of

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(2) Abbreviations: phosphorylating enzyme, P enzyme; adenosine di- and triphosphate, ADP and ATP; inosine triphosphate, ITP; adenosine-5-phosphate, AMP; orthophosphate, P; coenzyme A (reduced), CoA or CoA-SH; acyl coenzyme A derivatives, acyl CoA or acyl-S-CoA; succinyl phosphate, succinyl P; reduced glutathione, GSH; tris-(hydroxymethyl)-aminomethane-HCl buffer, TRIS; micromoles, μM.; counts per minute, c.p.m.

(3) S. Kaufman, C. Gilvarg, O. Cori and S. Ochoa, *J. Biol. Chem.*, **203**, 869 (1953).

(4) S. Kaufman and S. G. A. Alivisatos, to be published.

(5) F. Lynen and S. Ochoa, *Biochim. et biophys. Acta*, **12**, 299 (1953).

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(7) H. A. Krebs and R. Hems, *Biochim et biophys. Acta*, **12**, 172 (1953); P. Berg and W. K. Joklik, *Nature*, **172**, 1008 (1953).