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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RECEIVED MARCH 12, 1954

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

(1) Aided by grants from the National Institutes of Health, United States Public Health Service, the American Cancer Society (recommended by the Committee on Growth of the National Research Council), and by a contract (N6onr279, T.O. 6) between the Office of Naval Research and New York University College of Medicine.

(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).

(6) Recently the GSH-synthesizing enzyme has been reported to catalyze this reaction. J. E. Snoke, THIS JOURNAL, **75**, 4872 (1953).

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

TABLE I

EXCHANGE REACTIONS CATALYZED BY THE P ENZYME

In experiment A, the complete system contained 1.3 μM. succinyl CoA, 50 μM. TRIS *p*H 7.4, 5 μM. C^{14}-succinate, 0.17 mg. enzyme protein (5.2 units), 5 μM. PO_4 and 5 μM. $MgCl_2$; 2.5 μM. ADP added when indicated; final volume 1.0 ml.; incubated 6 minutes at 20°. After incubation, NH_2OH was added and the succinohydroxamic acid separated from succinate by chromatography on Dowex 1. About 0.90–1.17 μM. of succinohydroxamic acid was isolated from the column. The specific radioactivity of the succinate after incubation varied between 11800 and 12200 c.p.m./μM. In experiment B, the complete system contained 1.5 μM. P^{32}-labeled ADP, 4.5 μM. ATP, 50 μM. TRIS *p*H 7.4, 5 μM. $MgCl_2$, 0.012 mg. enzyme protein (0.34 unit), 10 μM. of succinate and 0.7 μM. of CoA-SH or derivatives; 5 μM. GSH added when indicated; final vol. 1.0 ml.; incubated 10 minutes at 20°. The specific radioactivity of the ADP after incubation varied between 11,000 and 12,000 c.p.m./μM.

A			B		
Components	Succinohydroxamic acid, c.p.m./μM.	Exchange, %	Components	ATP, c.p.m./μM.	Exchange, %
Complete	1663	14.4	Complete	760	22.8
Complete + ADP	1208	10.4	+ Succinate	790	23.8
No phosphate	629	5.4	+ CoA	360	10.9
No Mg^{++}	45	0.4	+ Acetyl CoA	770	23.3
			+ Oxidized CoA	710	21.4
			+ GSH	750	22.6
			+ GSH + Pantethine[a]	780	23.4
			No enzyme	84	

[a] Kindly supplied by Dr. E. E. Snell.

which could lead to labeling of ATP in the presence of P_{32}-ADP.[8] That the exchange is indeed catalyzed by the P enzyme is further supported by the finding that a loss in the ADP–ATP reaction parallels the loss in the over-all activity in aged enzyme preparations.

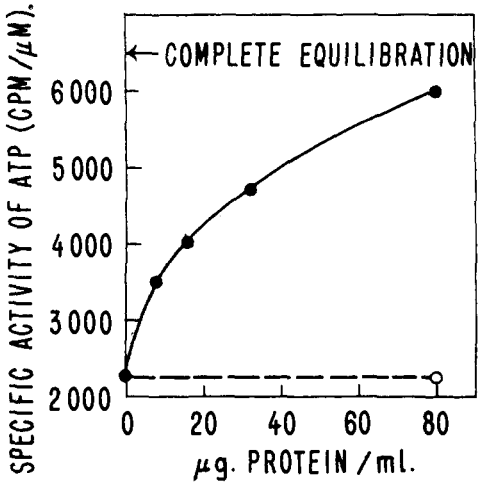

Fig. 1.—Rate of ADP–ATP exchange as a function of P enzyme concentration. The complete system contained 1.0 μM. P^{32}-ADP, 1.0 μM. ATP, 50 μM. TRIS *p*H 7.4, 5.0 μM. $MgCl_2$; final volume 1.0 ml.; incubated 10 minutes at 20° (open circle, system without $MgCl_2$). The enzyme solution had 44 units/ml., and 3.2 mg. protein/ml.; it was dialyzed free of phosphate prior to the experiment. The specific radioactivity of ADP at the end of incubation varied between 6000 and 8400 c.p.m./μM. in the individual experiments.

(8) The best preparations of the spinach enzyme contain traces of ATPase. It has been shown that myosin ATPase does not catalyze this exchange at a significant rate.

The results of the isotope experiments and the failure to obtain indications for the formation of a free intermediate are consistent with the scheme

$$\text{Enz} + \text{ATP} \overset{(Mg^{++})}{\rightleftharpoons} \text{enz-P} + \text{ADP} \quad (2)$$

$$\text{Enz-P} + \text{CoA} \rightleftharpoons \text{enz (P-CoA)} \quad (3)$$

$$\text{Enz (P-CoA)} + \text{succinate} \overset{(Mg^{++})}{\rightleftharpoons} \text{enz} + \text{succinyl-S-CoA} + \text{P} \quad (4)$$

The occurrence of succinyl P as a bound intermediate (as represented by the reaction sequence 2, 3a, 4a) cannot be ruled out even though the enzyme fails to catalyze an exchange of C^{14}-succinate with synthetic succinyl P.[4,9]

$$\text{Enz-P} + \text{succinate} \rightleftharpoons \text{enz (succinyl P)} \quad (3a)$$

$$\text{Enz (succinyl P)} + \text{CoA} \rightleftharpoons \text{enz} + \text{succinyl-S-CoA} + \text{P} \quad (4a)$$

In a further attempt to decide between the two alternate schemes, the effect of added substrates on the ADP-ATP exchange was investigated. As shown in Table I, succinate has no significant effect on the rate of exchange. In contrast, CoA markedly inhibits the reaction. That the CoA effect is specific, is shown by the absence of inhibition by equivalent amounts of oxidized CoA, acetyl CoA or pantetheine. The results would seem to favor the reaction sequence 2 to 4. The mechanism of reaction 1 is basically different from that of the ATP-CoA-acetate reaction which involves an enzyme-AMP intermediate.[10]

We are indebted to Mrs. Suzanne Loebl for technical assistance.

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RECEIVED MARCH 15, 1954

(9) S. Kaufman, synthesis of succinyl monophosphate, to be published.

(10) M. E. Jones, F. Lipmann, H. Hilz and F. Lynen, THIS JOURNAL, **75**, 3285 (1953).

(11) National Research Council (Canada) Fellow, now at Rockefeller Institute for Medical Research, New York, N. Y.

RELATIVE IMPORTANCE OF STERIC AND INDUCTIVE EFFECTS IN S_N2 DISPLACEMENT REACTIONS

Sir:

It is well known that the reactivity of saturated isomeric alkyl halides in typical SN2 type reactions varies in the order primary > secondary> tertiary. The decrease in reactivity as alkyl groups are added to the carbon atom containing the halogen atom has been explained as being due to (a) the inductive effect of the alkyl groups and/or (b) a steric effect whereby the extra alkyl groups hinder the entering group from attacking from the rear. There has been no way of determining the relative importance of inductive and steric effects in this type of reaction.[1]

We have now obtained evidence which shows the inductive effects to be of little, if any, importance

(1) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 409–410.