COMMUNICATIONS TO THE EDITOR

THE ENZYMATIC CARBOXYLATION OF ACETYL COENZYME A

Sir:

Recent experiments indicate that the enzymatic synthesis of fatty acids probably occurs via an aldol condensation of the Knoevenagel type between aliphatic aldehydes and malonyl coenzyme A[1,2] (malonyl CoA). The carboxylation of propionyl CoA and β-methyl-crotonyl-CoA in the presence of CO_2 and adenosine triphosphate (ATP) has been demonstrated.[3,4] Experiments were undertaken to determine if such a reaction could be observed between acetyl CoA and HCO_3^- to form malonyl CoA.

Extracts of pig heart tissue were prepared[5] and dialyzed overnight against 100 volumes of 0.02 *M* Tris buffer, *p*H 7.4. Extracts of pigeon liver tissue were prepared[6] and the fraction precipitating between 0 and 30% saturation with ammonium sulfate was taken up in 0.04 *M* $KHCO_3$ and dialyzed against the same solution for four hours. Enzymes present in these extracts catalyzed the fixation of $HC^{14}O_3^-$ (Table I). The reaction catalyzed by extracts of pig heart tissue was found to be dependent upon supplemental ATP, acetyl CoA, and Mg^{++} ions. The fixation of $C^{14}O_2$ catalyzed by pigeon liver preparations was dependent upon supplemental ATP and $MgCl_2$ and was markedly enhanced by the addition of acetyl CoA.

TABLE I

FIXATION OF $C^{14}O_2$ BY ACETYL COENZYME A

The reaction mixtures contained 80 μmoles of imidazole hydrochloride buffer, *p*H 7.0, 3 μmoles of $MgCl_2$, 3 μmoles of ATP, 1 μmole of $KHC^{14}O_3$ (9×10^5 c.p.m.), 1 μmole of acetyl CoA, extract of pig heart tissue (3 mg. of protein, Experiment 1) or pigeon liver (5 mg. of protein, Experiment 2) in 1.0 ml. After incubating for 60 minutes at 30°, 0.2 ml. of 2 *N* perchloric acid was added and the amount of fixed radioactivity was determined as described by M. Flavin, H. Castro-Mendoza and S. Ochoa, *J. Biol. Chem.*, **229**, 981 (1957).

Experiment no.	Reactant omitted	$C^{14}O_2$ fixed, c.p.m.
1. Pig heart extract	None	2050
	ATP	20
	Acetyl CoA	60
	$MgCl_2$	50
2. Pigeon liver extract	None	763
	ATP	48
	Acetyl CoA	380
	$MgCl_2$	40

To identify the product of the reaction, an aliquot from an acid-deproteinized sample of the pig heart preparation was neutralized and incubated with 500 μmoles of hydroxylamine hydrochloride for ten minutes at 23°. Hydroxamic acids were extracted and chromatographed on Whatman No. 3 filter paper in water-saturated butanol.[7] Monomalonyl hydroxamic acid exhibited an R_f of 0.36 in this system. The chromatogram was examined with a strip counter, and the major portion of the radioactivity was localized in the region between R_f 0.33 and 0.41. For additional identification, the supernatant solution from an acid-deproteinized incubation mixture was extracted for 18 hours with ethyl ether. The radioactivity remained in the aqueous phase which was therefore adjusted to *p*H 9.0 with 2 *N* potassium hydroxide and heated for 90 minutes at 50°. The solution was re-acidified and extracted with ether. The radioactivity was now present in the ether phase. The ether extract was dried over sodium sulfate and the solvent was removed. The residue was taken up in 25% methanol and chromatographed on paper according to Flavin and Ochoa.[3] Malonic acid exhibited an R_f of 0.53 in this system. Nearly all of the radioactivity was confined to this region of the chromatogram.

(1) R. O. Brady, *Proc. U. S. Nat. Acad. Sci.*, **44**, 993 (1958).
(2) D. M. Gibson, E. B. Titchener and S. J. Wakil, *Biochim. et Biophys. Acta*, **30**, 376 (1958).
(3) M. Flavin and S. Ochoa, *J. Biol. Chem.*, **229**, 965 (1957).
(4) J. Knappe and F. Lynen, "Abstr. IV International Congress of Biochemistry," Vienna, Austria, 1958, p. 49.
(5) B. K. Bachhawat and M. J. Coon, *J. Biol. Chem.*, **231**, 625 (1958).
(6) S. J. Wakil, J. W. Porter and D. M. Gibson, *Biochim. et Biophys. Acta*, **24**, 453 (1957).
(7) O. Hayaishi, *J. Biol. Chem.*, **215**, 125 (1955).

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A METAL CARBONYL COMPOUND OF TITANIUM

Sir:

Metal carbonyl compounds of the Group IV-B transition metals have not been reported previously.[1] The titanium carbonyl derivative, bis-(cyclopentadienyl)-titanium dicarbonyl (I) has now been prepared by the reaction of bis-(cyclopentadienyl)-titanium dichloride (II) in benzene with two equivalents of cyclopentadienyl sodium, and then treatment of the intermediate product with carbon monoxide at 135 atm. and 100° for eight hours. Analysis of the crude reaction product by measurement of the carbon monoxide evolved on treatment with iodine[2] indicates that a 50% over-all yield of the carbonyl is present in the reaction product. The extremely air sensitive carbonyl can be isolated in pure form in an over-all yield of 18% by removal of the benzene from the reaction product and then recrystallization from air-free hexane (all operations under nitrogen). Dark reddish brown crystals of I are obtained which decompose above 90° under nitrogen.

Anal. Calcd. for $C_{12}H_{10}TiO_2$: C, 61.56; H, 4.31; Ti, 20.46. Found: C, 61.56; H, 4.55; Ti, 20.38.

(1) The possibility of preparing metal carbonyl compounds of the Group IV-B metals has been anticipated by J. E. Brown and H. Shapiro in U. S. Patent 2,818,416 (to Ethyl Corp.) December 31, 1957.
(2) H. W. Sternberg, I. Wender and M. Orchin, *Anal. Chem.*, **24**, 174 (1952).

The infrared spectrum of I shows two strong bands in the metal carbonyl stretching region at 5.09 and 5.31 microns.

The carbonyl (I) also can be prepared by treatment of II with two equivalents of butyllithium and then reaction with carbon monoxide, or by reaction of titanium tetrachloride in benzene with four equivalents of cyclopentadienyl sodium with subsequent reaction with carbon monoxide. Heating bis-(cyclopentadienyl)-titanium dichloride or diphenyl bis-(cyclopentadienyl)-titanium with carbon monoxide resulted in the formation of little or no I.

Details of this work and some reactions of I will be reported at a later date.

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COUNTERCURRENT DISTRIBUTION OF AN ACTIVE RIBONUCLEIC ACID

Sir:

The enzymatic transfer of activated amino acids to ribonucleic acid (RNA), suggested by the discovery of an alanine-dependent, ribonuclease-inhibited incorporation of AMP into ATP,[1] and subsequently established by the work of Hoagland, *et al.*,[2] Ogata and Nohara,[3] Berg and Ofengand,[4] Lipmann, *et al.*,[5] Schweet, *et al.*,[6] and Zamecnik, *et al.*,[7] is of interest as a possible intermediate reaction in protein synthesis.

This communication presents the results of countercurrent distribution of RNA isolated from the "soluble" fraction of rat liver homogenate.[8] The solvent system described by Warner and Vaimberg[9] was used with slight modification, and the distribution was carried out in the 100-tube countercurrent apparatus described by Raymond.[10]

As shown in Fig. 1, countercurrent distribution of the RNA gives a broad distribution pattern, measured by absorption at 260 mμ. Redistribution (Fig. 2) of material from different parts of the first distribution pattern establishes that there has been actual fractionation of the RNA.

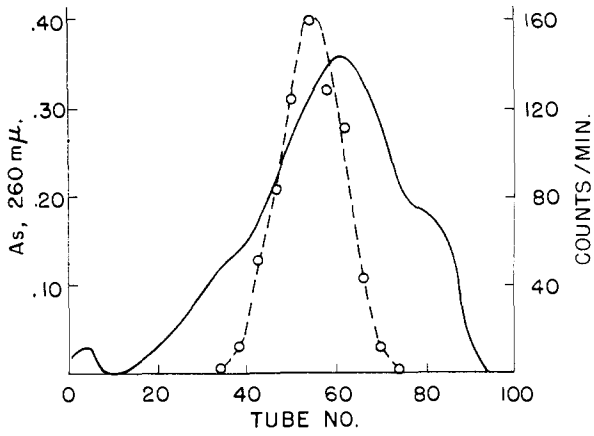

Fig. 1.—Countercurrent distribution of 20 mg. of RNA isolated from the "soluble" fraction of rat liver homogenate: ———, absorbance of fractions, measured in the Beckman DU spectrophotometer; —O—, activity of fractions, after dialysis and lyophilization, given as counts/min. observed in Ba_2ATP obtained from assay for activity in the alanine-dependent incorporation of radioactive AMP into ATP (blank value of 5 counts/min. subtracted).

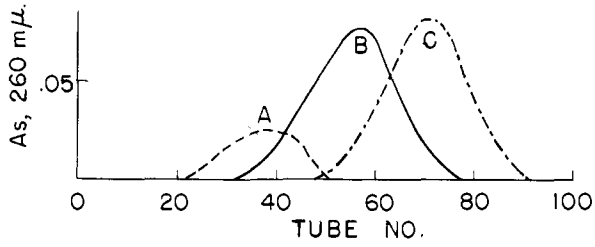

Fig. 2.—Redistribution of fractions of the RNA: the contents of these tubes from the distribution shown in Fig. 1 were placed in tubes 1–6 or 7 of the apparatus at the start of the redistribution: A, tubes 25, 27, 29, 31, 33, 35, 37; B, tubes 45, 47, 49, 51, 53, 55; C, tubes 65, 67, 69, 71, 73, 75.

Using alanine-dependent AMP incorporation[1,8] to assay for the alanine-active RNA, activity is found significantly displaced from the peak of ultraviolet absorption and separated from much of the original RNA (Fig. 1). The location of activity for amino acids other than alanine remains to be determined.

Because of the possibility that different molecular species of "soluble" fraction RNA act in protein synthesis by transferring specific amino acids to the "template," and the possibility that amino acid specificity is carried in nucleotide sequences which may be related to nucleotide sequences in DNA, the isolation of these different molecular species of RNA is a problem of great significance. The above results indicate that countercurrent distribution furnishes a promising approach to this problem.

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(9) R. C. Warner and P. Vaimberg, *Fed. Proc.*, **17**, 331 (1958). The amount of formamide was increased 10% in these experiments. By varying the concentrations of 2-propanol and formamide, the gross partition coefficient of the rat liver RNA can be varied at least from 0.1 to 1.5.

(10) S. Raymond, *Anal. Chem.*, **30**, 1214 (1958).