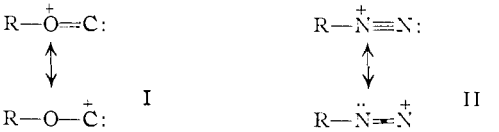
effective than chloroform, but $CHClF_2$ and $CHCl_2F$ fail to affect dehydrations.

The dehydration of neopentyl, *i*-butyl and *n*-butyl alcohols reported here produces essentially the same products as those obtained by nitrous acid deamination of neopentyl,[5] isobutyl[6] and *n*-butyl[7] amines. The ratio *cis/trans* olefins is 0.54, 0.60, 0.55 for *n*-, *s*- and *i*-butyl alcohols, respectively, and the ratio 2-methyl-1-butene/2-methyl-2-butene is 4.0 and 2.1 for *t*- and neopentyl alcohols. Thus we are led to the postulate that these reactions proceed through *s*-butyl and *t*-amyl carbonium ions, respectively.

An isostere of a diazonium cation, RN_2^+, is the ion $(ROC)^+$



I II

Loss of the very stable nitrogen molecule provides the driving force for decomposition of alkyldiazonium cations to produce carbonium ions. Loss of carbon monoxide from (I) should provide a similar driving force.

Evidence will be presented later for the mechanisms.

$$RO^- + CX_2 \longrightarrow X^- + R{-}O{-}C{-}X \longrightarrow R^- + CO + X^-, \text{ or}$$

$$RO^- + CX_2 \longrightarrow 2X^- + R{-}OC^+ \longrightarrow R^- + CO.$$

If X is fluorine, the ROCF intermediate does not release F^- and CO, but reacts instead by addition of alcohol.[8]

The over-all reaction is the removal of oxide ion, $O^=$, from alkoxide ion. Numerous applications involving *de-oxideations* of oxygen anions by CX_2 suggest themselves and are under active investigation.

$$JO^- + CX_2 \longrightarrow J^- + CO + 2X^-$$

(5) M. Freund and F. Lenze, *Ber.*, **24**, 2150 (1891).

(6) L. Cannell and R. W. Taft, Jr., THIS JOURNAL, **78**, 5812 (1956).

(7) F. C. Whitmore and D. P. Langlois, *ibid.*, **54**, 3441 (1932).

(8) J. Hine and K. Tanabe, *ibid.*, **79**, 2654 (1957); **80**, 3002 (1958).

DEPARTMENT OF CHEMISTRY P. S. SKELL
THE PENNSYLVANIA STATE UNIVERSITY
UNIVERSITY PARK, PENNSYLVANIA I. STARER

RECEIVED JUNE 15, 1959

A MAMMALIAN SYSTEM FOR THE INCORPORATION OF CYTIDINE TRIPHOSPHATE INTO RIBONUCLEIC ACID[1]

Sir:

A mammalian preparation that incorporates cytidylate from cytidine-P^{32}-P-P into RNA[2] and is markedly stimulated by ATP, UTP, and GTP, is described in this report.

CMP-5′-P^{32} was prepared by a modified procedure for the phosphorylation of 2′,3′-benzylidene-O-cytidine.[3] Labeled CTP was prepared from P^{32}-CMP by a cytidylate kinase isolated from brewers' yeast. Twice washed nuclei were prepared from a 20% rat liver homogenate in 0.25 molar sucrose and centrifuged for six minutes at $600 \times g$.

When P^{32}-CTP was incubated with the $600 \times g$ preparation, in the presence of all the ribonucleoside triphosphates, a significant amount of label was incorporated into the RNA fraction. Omission of any one of the triphosphates resulted in a reduction of 85% or more of P^{32}-CTP incorporation (Table I). Desoxyribonuclease depresses the incorporation slightly, whereas ribonuclease causes a marked reduction. In other experiments, a requirement for Mg^{++} was demonstrated and a fivefold excess of deoxy-CTP did not reduce the incorporation of P^{32}-CTP.

When P^{32}-labeled RNA (70,000 total counts), formed by this system, was isolated and hydrolyzed with alkali, the mononucleotides separated on Dowex-1-Cl contained these counts: 2′(3′)-CMP, 22,890; 2′(3′)-AMP, 8,680; 2′(3′)-GMP, 8,800; 2′(3′)-UMP, 21,940.

TABLE I

REQUIREMENTS FOR P^{32}-CYTIDINE TRIPHOSPHATE INCORPORATION INTO RNA

The complete system contained in 10 μmole $MgCl_2$, 100 μmole TRIS·HCl, *p*H 8.0, 0.1 μmole P^{32}-CTP (16.8×10^6 counts/μmole), 0.1 μmole ATP, 0.1 μmole UTP, 0.1 μmole GTP, 100 μmole KCl, 40 μmole NaF, 10 μmole cysteine, and 10–12 mg. of twice washed nuclei (dry weight), in a total volume of 2.0 ml. After incubation at 37° for 12 minutes 5 ml. of cold 5% TCA was added. The acid insoluble material was washed 3 times with 5% TCA, 2 times with ethanol–ether (3:1), and extracted 3 times with 2 ml. of 10% NaCl at 100°, *p*H 8.0, with 2 mg. of yeast RNA added. The combined extracts were precipitated twice with 2 volumes of ethanol. The residue was dissolved in 4 ml. of water and 1.0 ml. was dried and assayed in a windowless flow counter.

Reaction mixture	Radioactivity RNA (total counts)
Complete	3872
Omit ATP	636
Omit UTP	280
Omit GTP	104
Omit ATP, UTP and GTP	60
Complete + 20γ ribonuclease	828
Complete + 20 γ desoxyribonuclease	2940
Complete + 10 μmole inorganic pyrophosphate	100
Complete in 100 μmole Pi buffer, *p*H 7.5 (no TRIS)	4030
Complete: ADP, UDP, GDP in place of ATP, UTP, GTP	1620
Complete: AMP, UMP, GMP in place of ATP, UTP, GTP	128

The appearance of label in all the mononucleotides, after hydrolysis, suggests strongly that P^{32}-CTP is incorporated into the interpolynucleotide linkages of RNA rather than terminally.[4] The

(1) Supported by funds from the Argonne Cancer Research Hospital, operated by the University of Chicago, for the United States Atomic Energy Commission.

(2) Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CTP, ATP, UTP and GTP for the tri- and ADP, UDP and GDP for the di- and CMP, AMP, UMP and GMP for the monophosphates of cytidine, adenosine, uridine and guanosine; TRIS, tris-(hydroxymethyl)-aminomethane; TCA, trichloroacetic acid; Pi, inorganic phosphate.

(3) J. Baddiley, J. G. Buchanan and A. R. Sanderson, *J. Chem. Soc.*, 3107 (1958).

(4) (a) E. S. Canellakis, *Biochim. Biophys. Acta*, **25**, 271 (1957); (b) M. Edmonds and R. Abrams, *ibid.*, **26**, 226 (1957); (c) L. I. Hecht, P. C. Zamecnik, M. L. Stephenson and J. F. Scott, *J. Biol. Chem.*, **233**, 954 (1958).

requirement for the four ribonucleoside triphosphates, which are more than twice as effective as the corresponding diphosphates, as well as the inhibition by pyrophosphate but not by inorganic phosphate, suggests that the reaction mechanism resembles that described for DNA formation[5] rather than RNA synthesis by polynucleotide phosphorylase.[6,7]

(5) M. Bessman, I. R. Lehman, E. S. Simms and A. Kornberg, *Fed. Proc.*, 16, 153 (1957).

(6) M. Grunberg-Manago, P. J. Oritz and S. Ochoa, *Science*, **122**, 907 (1955).

(7) R. J. Hilmoe and L. A. Heppel, THIS JOURNAL, **79**, 4810 (1957)

ARGONNE CANCER RESEARCH HOSPITAL AND
DEPARTMENT OF BIOCHEMISTRY SAMUEL B. WEISS
UNIVERSITY OF CHICAGO LEONARD GLADSTONE
CHICAGO, ILLINOIS

RECEIVED JUNE 11, 1959

CAPROYL COENZYME A DEPENDENT MALONYL COENZYME A—BICARBONATE EXCHANGE REACTION

Sir:

Enzyme preparations of *Clostridium kluyveri* catalyze an exchange reaction between malonyl CoA and $HC^{14}O_3^-$. Experiment I (Table I) indicates complete dependence of the reaction upon enzyme, malonyl CoA and boiled extract, and partial dependence upon acetyl CoA and glutathione.

Labeled malonyl CoA was identified by (1) conversion to the hydroxamate derivative which chromatographed with authentic malonyl monohydroxamate[1]; and by (2) alkaline hydrolysis and chromatography with authentic malonate.[2]

Malonyl CoA is not decarboxylated by this enzyme preparation and C^{14}-acetyl CoA is not incorporated into malonyl CoA. This plus the dependence of the reaction upon malonyl CoA indicate an exchange phenomenon rather than a net carboxylation of acetyl CoA.

The factor in boiled extract has been isolated by steam distillation and chromatography on a silica gel column and identified as caproic acid by Duclaux distillation.[3] Experiment II (Table I) indicates that 10^{-4} *M* sodium caproate replaces boiled extract. Experiment III (Table I) shows that caproyl CoA at levels of 10^{-4} *M* replaces boiled extract and acetyl CoA, suggesting that acetyl CoA functions by transfer of its CoA to caproate.

TABLE I

FIXATION OF $HC^{14}O_3^-$ INTO MALONYL CoA

Complete systems contained: (I) 100 μmoles of potassium phosphate buffer, *p*H 6.7, 25 μmoles of $KHC^{14}O_3$ (2.5 μc.), 0.2 μmole of malonyl CoA, 0.4 μmole of acetyl CoA, 6 μmoles of glutathione, 0.1 ml. of boiled extract, and 1.5 mg. of enzyme in a final volume of 1.0 ml.; (II) same as (I) except 0.1 μmole of sodium caproate replaced the boiled extract; (III) same as (I) except 0.1 μmole of caproyl CoA replaced both boiled extract and acetyl CoA. Experiments were incubated at 30° for 2 hours. Reactions were stopped with perchloric acid. Aliquots of the supernatant solutions were counted directly.

Experiment	Component omitted	C.p.m.
I	None	4,550
	None (boiled control)	44
	Malonyl CoA	90
	Boiled cell extract	350
	Acetyl CoA	2,930
	Glutathione	3,250
II	None	4,460
	Sodium caproate	242
	Acetyl CoA	1,300
III	None	4,200
	Caproyl CoA	370
	Acetyl CoA	4,300

Valerate is as effective as caproate, whereas butyrate, caprylate and caprate are less effective in this reaction.

The observed exchange reaction is consistent with a reaction mechanism involving a reversible condensation between malonyl CoA and caproyl CoA coupled with a decarboxylation

$$HOOC^{14}—CH_2—COSCoA + CH_3—(CH_2)_4—COSCoA \rightleftharpoons CH_3—(CH_2)_4—CO—CH_2—COSCoA + C^{14}O_2 + CoASH$$

or

$$CH_3—CO—(CH_2)_5—COSCoA + C^{14}O_2 + CoASH$$

With the recent implications of malonyl CoA in fatty acid biosynthesis,[4,5] such a reaction sequence could be involved in the pathway of long chain fatty acid synthesis.

(1) R_f 0.5 in pyridine:2-butanol:water (1:1:1); R_f 0.27 in pyridine:isoamyl alcohol:water (3:4:1.9).

(2) F. W. Denison, Jr., and E. F. Phares, *Anal. Chem.*, **24**, 1628 (1952).

(3) H. A. Barker, in S. P. Colowick and N. O. Kaplan (Editors), "Methods in Enzymology," Vol. III, Academic Press, Inc., New York, N. Y., 1957, p. 372.

NATIONAL HEART INSTITUTE
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE P. R. VAGELOS
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
BETHESDA 14, MARYLAND

RECEIVED JUNE 8, 1959

(4) R. O. Brady, *Proc. U. S. Nat. Acad. Sci.*, **44**, 993 (1958).

(5) S. J. Wakil, THIS JOURNAL, **80**, 6465 (1958).