would expect the multiple bond frequency to remain near where it is observed in benzene. The ring breathing and the in-plane ring distortion frequencies should appear lower because of their dependence upon the size of the ring. In agreement with this the multiple bond frequency of the tropylium ion occurs at 1594 cm.$^{-1}$ as compared to 1596 cm.$^{-1}$ for benzene. The ring breathing mode dropped from a value of 992 cm.$^{-1}$ in benzene to a value of 868 cm.$^{-1}$ in the tropylium ion. The 606 cm.$^{-1}$ in-plane bending frequency of benzene drops to 433 cm.$^{-1}$ in the tropylium ion. Similar considerations apply to a comparison of their infrared spectra. It is significant that there are no Raman frequencies observed from 1600–1700 cm.$^{-1}$ A conjugated non-aromatic system of double bonds gives one or more very strong Raman lines in this region. The failure to observe such lines can be taken as further evidence for the aromatic nature of the tropylium ion.

TABLE I

THE VIBRATIONAL SPECTRUM OF TROPYLIUM ION COMPARED WITH THAT OF BENZENE

Key: vs = very strong; s = strong; m = medium; mw = medium weak; w = weak.

Raman				Infrared			
$C_7H_7^+$		C_6H_6[a]		$C_7H_7^+$		C_6H_6[a]	
cm.$^{-1}$	I	cm.$^{-1}$	I	cm.$^{-1}$	I	cm.$^{-1}$	I
433	m	605	m	633	s	671	s
		849	mw	658	m		
868	vs	992	vw	992	mw	1037	s
925[b]	w						
1210	mw	1178	m	1222	w		
				1278	w		
1594	m	1585	m	1477	vs	1485	s
		1606					
				2060	w	1807	m
						1964	m
		3047	s	3020	s	3405	s
3045–3085[c]	s	3062	vs	3080	w	3099	s

[a] G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules" D. Van Nostrand Company, Inc., New York, 1945, p. 364–365. [b] This line was obtained on only two plates. [c] The intensity distribution of this broad band is asymmetrical and represents two incompletely resolved lines.

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CYTIDINE DIPHOSPHATE CHOLINE: A NEW INTERMEDIATE IN LECITHIN BIOSYNTHESIS[1]

Sir:

Two pathways for the biosynthesis of lecithin occur in mammalian liver preparations. Pathway I incorporates free choline into the lecithin molecule by a reaction sequence which does not involve the intermediary formation of phosphorylcholine[2] but does require ATP[3] and CoA.[4] A thiophosphate ester of CoA, phosphatidyl CoA, has been suggested as an intermediate.[4]

In pathway II, discovered by Kornberg and Pricer,[5] phosphorylcholine is incorporated as a unit into the lecithin structure. We have now found that the washed rat liver mitochondrial preparations already shown to catalyze the reactions of pathway I also catalyze the reactions of pathway II, but the conditions required are very different (Table I).

TABLE I

PROPERTIES OF ENZYME SYSTEMS FOR LECITHIN BIOSYNTHESIS

	Pathway I	Pathway II
Active precursor	Choline	Phosphorylcholine
*p*H optimum	9.4	7.0
Fluoride inhibition	0	+
Needed cofactors: ATP	+	0
CoA	+	0
CTP	0	+

Relatively large amounts of ATP (Pabst lot 116, *ca.* 95% pure) were at first found to support the reactions of pathway II, but when crystallized ATP (Pabst lot 122) was substituted no activity was observed. Further investigation led to the discovery of a highly specific requirement for CTP as seen in Table II. The activity of the less pure ATP lot 116 is explained by its content (<1%) of CTP. The addition of inorganic pyrophosphate markedly reverses the stimulation observed with added CTP.

TABLE II

COFACTOR REQUIREMENT FOR PATHWAY II

	Added cofactors	Lecithin synthesized (total counts)
1	5 μM of ATP lot 116	590
2	5 μM of ATP lot 122	20
3	5 μM of ATP lot 122 + 0.5 μM ITP	0
4	5 μM of ATP lot 122 + 0.5 μM UTP	50
5	5 μM of ATP lot 122 + 0.5 μM GDP	57
6	5 μM of ATP lot 122 + 0.5 μM CTP	1677
7	5 μM of ATP lot 122 + 0.5 μM CTP + 2.5 μM inorganic pyrophosphate	750

Each tube contained 10 μM $MgCl_2$, 50 μM of phosphate buffer, *p*H 7.4, 3 μM of phosphorylcholine-P^{32} (113,000 cts./μM) and 25 mg. of lyophilized mitochondria in a total volume of 1.0 ml. Incubated at 37° for 1 hour. Phospholipide counted as previously described.[2]

The following abbreviated reaction scheme was postulated to explain the activation of this system by CTP and its reversal by inorganic pyrophosphate:

(1) CTP + phosphorylcholine $\rightleftharpoons$ cytidine diphosphate choline + pyrophosphate

(2) Cytidine diphosphate choline + acceptor $\longrightarrow$ lecithin + CMP

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(2) E. P. Kennedy, *J. Biol. Chem.*, **209**, 525 (1954).

(3) Abbreviations: ATP, adenosine-5'-triphosphate; CoA, coenzyme A; CTP, cytidine-5'-triphosphate; UTP, uridine-5'-triphosphate; ITP, inosine-5'-triphosphate; GDP, guanosine-5'-pyrophosphate.

(4) E. P. Kennedy, *Federation Proc.*, **13**, 241 (1954).

(5) A. Kornberg and W. E. Pricer, Jr., *ibid.*, **13**, 241 (1952).

Direct proof of this formulation has been obtained by the synthesis of cytidine diphosphate choline (P^1-cytidine-5′-P^2-choline-pyrophosphate) from cytidine-5′-phosphate and phosphorycholine-1,2-C^{14} using N,N′-dicyclohexylcarbodiimide as condensing agent in the elegant procedure of Khorana.[6] The synthetic labeled cytidine diphosphate choline, isolated and purified by ion exchange chromatography, has been found to be converted to lecithin at rates far exceeding those observed with CTP + phosphorylcholine and in yields approaching one mole of lecithin for each mole of cytidine diphosphate chloline added. The nature of the lipide acceptor compound involved in reaction (2) is not known with certainty but is presumed to be an α,β-diglyceride.

These results are the first demonstration of cytidine-containing coenzymes in a specific role of major metabolic importance. Presumably compounds such as cytidine diphosphate ethanolamine may participate in cephalin synthesis. Further, reaction (2) involves a novel type of group transfer reaction in which the entire phosphorylcholine moiety is transferred, with the formation of a monophosphorus diester linkage. Reactions of this type may perhaps serve as models not only for synthesis of glycerophosphatides, but also of nucleic acids, where similar linkages are present.

(6) H. G. Khorana, THIS JOURNAL, **76**, 3517 (1954).

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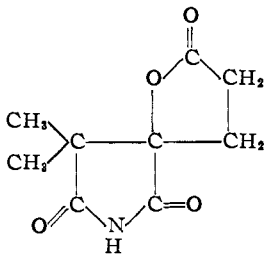
VITAMIN B_{12}. XXIV. DL-3,3-DIMETHYL-2,5-DIOXO-4-HYDROXYPYRROLIDINE-4-PROPIONIC ACID LACTONE AND DL-3,3-DIMETHYL-2,5-DIOXOPYRROLIDINE-4-PROPIONIC ACID, NEW DEGRADATION PRODUCTS

Sir:

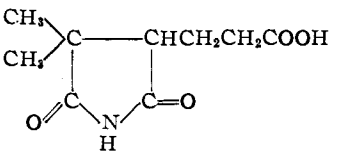
The oxidation of acid-hydrolyzed vitamin B_{12} with sodium chromate in acetic acid has given new pyrrole-like degradation products which have been identified by degradation and synthesis. These pyrrole-like products constitute the first detailed organic structural evidence concerning the heterocyclic nature of the red cobalt complex which remains after hydrolysis.

The chloroform-soluble portion of the oxidation reaction mixture was subjected to countercurrent distribution between chloroform and water. Material with a distribution coefficient near unity yielded a crystalline, weakly acidic substance (I) of m.p. 151–152°, of the composition $C_9H_{11}NO_4$. The compound showed infrared absorption bands at 3.02, 5.65, and 5.80 μ, in agreement with an imide structure containing a γ-lactone group. Hydrolysis converted the imide to a lactone dicarboxylic acid, m.p. 167–168°. The material from the chloroform–water distribution which was excessively soluble in the aqueous phase was redistributed between water and ether to yield a second imide (II), m.p. 143–144°, of the composition $C_9H_{13}NO_4$. Hydrolysis of the imide yielded a tricarboxylic acid, m.p. 152–153°.

Fusion of the imide (I) with potassium hydroxide gave succinic and isobutyric acids. When this imide was distilled with zinc dust, a basic product was obtained which gave a positive Ehrlich pyrrole test. Of two possible structures for the imide (I), DL-3,3-dimethyl-2,5-dioxo-4-hydroxypyrrolidine-4-propionic acid lactone was found to be correct by synthesis.



I

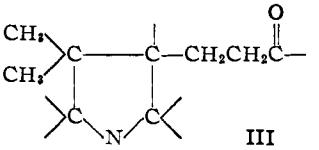


II

The sodium enolate of ethyl isobutyrate was treated with β-carbethoxypropionyl chloride. The resulting diethyl 2,2-dimethyl-3-oxo-adipate, on addition of hydrogen cyanide and hydrolysis of the cyanohydrin diethyl ester so produced, gave a compound identical with the lactone dicarboxylic acid. Pyrolysis of the ammonium salt of this synthetic lactone acid yielded the imide (I).

The composition and melting point of the tricarboxylic acid were found to correspond closely to those properties of 2-methyl-pentane-2,3,5-tricarboxylic acid.[1] A synthetic specimen of this acid was prepared[1] and found to be identical to that obtained by degradation. This synthetic acid on treatment with one mole of ammonia yielded, after pyrolysis, DL-3,3-dimethyl-2,5-dioxo-4-hydroxypyrrolidine-4-propionic acid, which was found to be identical to the imide (II).

The imide grouping of the degradation products I and II is most probably the result of the oxidation reaction. The imides are probably derived from a moiety or moieties in the vitamin B_{12} molecule having a pyrrolidine, pyrroline or pyrrolenine nucleus as represented by the partial structure III. Imides I and II are optically inactive. In view of the



III

polyamide character of vitamin B_{12},[2] and our observation that the yield of acidic oxidation products is increased by prior hydrolysis, the presence of the free carboxyl group of partial structure III

(1) Perkin and Thorpe, *J. Chem. Soc.*, **85**, 128 (1904).

(2) J. B. Armitage, J. R. Cannon, A. W. Johnson, S. F. J. Parker, E. S. Smith, W. H. Stafford and A. R. Todd, *ibid.*, 3849 (1953); H. Diehl and J. L. Ellingboe, *Iowa State College Journal of Science*, **27**, 421 (1953).