of methionine by transmethylation *in vivo* is not ethanolamine, and that in the *in vivo* synthesis of this acceptor, folic acid or its derivative is a co-factor. The data summarized briefly in Table I show that the extent of incorporation of choline-CH_3-C^{14} into the phospholipid choline is not inhibited by folic acid deficiency, whereas the incorporation of the carbon of the methyl group of methionine or of aminoethanol into choline was markedly reduced. The administration of amino-

TABLE I

THE UTILIZATION OF AMINOETHANOL-1,2-C^{14} AND THE EFFECT OF AMINOETHANOL, MONOMETHYLAMINOETHANOL, DIMETHYLAMINOETHANOL, AND *Citrovorum* FACTOR ON THE UTILIZATION OF METHIONINE-CH_3-C^{14} FOR THE SYNTHESIS OF PHOSPHOLIPID CHOLINE IN FOLIC ACID-DEFICIENT RATS[a]

Isotope injected	Supplement injected with the isotope	Per cent. of total activity in phospholipid choline: Normal	Per cent. of total activity in phospholipid choline: Folic acid-deficient
Choline-CH_3-C^{14}	None	73.0	76.0
Methionine-CH_3-C^{14}	None	30.0	18.4
Methionine-CH_3-C^{14}	Aminoethanol		17.8
Methionine-CH_3-C^{14}	Monomethylaminoethanol		20.0
Methionine-CH_3-C^{14}	Dimethylaminoethanol		38.8
Methionine-CH_3-C^{14}	*Citrovorum* factor	29.8	34.8
Aminoethanol-1,2-C^{14}	None	3.0	0.7

[a] Thirty-days old female rats were maintained for two months on a folic acid-free diet or on the same diet supplemented with folic acid. 1×10^{-3} mM. of the isotope per 100 g. wt. alone or together with 1×10^{-1} mM. of the non-isotopic amines was injected intraperitoneally in a single dose. *Citrovorum* factor (Leucovorin, Lederle), 0.1 mg., was injected two hours before the radiomethionine. All animals were sacrificed one hour after the injection of the isotopes. Phospholipid choline was isolated from the entire pooled carcasses (2–3 animals per pool).

ethanol or of monomethylaminoethanol together with radiomethionine did not improve the utilization of methionine methyl group for choline formation in the deficient animals. However, the administration of either the *Citrovorum* factor or dimethylaminoethanol together with radiomethionine promptly enhanced the incorporation of the methyl group of methionine into choline. Injection of the *Citrovorum* factor into normal animals did not increase the extent of transfer of the methyl group of methionine to choline. Increasing the period of the *in vivo* reaction to 20 or 48 hours did not improve the utilization of the methyl group of methionine on administration of aminoethanol or monoaminoethanol in folic acid-deficient rats. These data strongly suggest that choline is synthesized in the rat not by transfer of three methyl groups of methionine to aminoethanol, but by transfer of one methyl group of methionine to dimethylaminoethanol as the direct acceptor. The data further indicate that the *de novo* synthesis of the two methyl groups of dimethylaminoethanol is mediated by a folic acid derivative, and that folic acid or its derivatives are not involved in the transfer of the methyl group of methionine to dimethylaminoethanol.

The composition of the diets, the procedures for the isolation of choline, and the radio assay procedure employed were the same as previously reported.[2]

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STEREOISOMERIC TETRA-*o*-TOLYLSILANES[1]

Sir:

In connection with an investigation of highly-substituted aromatic organosilicon compounds,[2] stereoisomers of a novel type have been isolated.[3] The aromatic groups of tetra-*o*-tolylsilane are not free to rotate about the carbon–silicon bonds and it is possible to construct no fewer than eight models of the molecule, representing four *meso* compounds and two racemic pairs.

Using as starting materials SiX_4 or $(o\text{-}CH_3C_6H_4)_3$-SiX (where —X is —Cl, —OCH_3, or —OC_2H_5) and $o\text{-}CH_3C_6H_4Li$, four different compounds have been isolated which appear to be tetra-*o*-tolylsilane stereoisomers. These melt at 145°, 228°, 300° and 344°. A fifth material which melts at 270° may also be a stereoisomer.[4] We wish to draw particular attention to the 145° and 228° isomers.

145° Isomer.—*o*-Tolyllithium (1.16 moles) in ether was slowly added to methyl silicate (0.20 mole). After 117 hours at room temperature and 35 hours of reflux (all under nitrogen), the reaction mixture was hydrolyzed.[2a] Crystalline products could be isolated only after distillation of the crude syrups. The fraction boiling 200–210° (1 mm.) (50% yield if tetra-*o*-tolylsilane) was extracted repeatedly with hot methanol. The cooled extracts slowly deposited solids. The solids from the first extracts melted as low as 45°, but those from later extracts 110–120°.[5] Six recrystallizations of the latter material raised the melting point to 143–145°, and five additional recrystallizations to 144.8–145.7° (2 g., 3%). The recrystallization solvents were methanol–benzene (9:1), ligroin, methanol, ethanol, propanol-2 and acetic acid. Considerable amounts of low melting solids and syrups also were obtained. These appeared to be mixtures of tetra-*o*-tolylsilanes. *Anal.* Calcd. for $C_{28}H_{28}Si$: C, 85.66; H, 7.19; Si, 7.15. Found: C, 85.18; H, 7.05; Si, 7.15. *Ultraviolet data.*[6] $\lambda_{max.}$ in mμ (and ϵ) for cyclo-

(1) The authors gratefully acknowledge the financial support of the Research Corporation.

(2) (a) H. Gilman and G. N. R. Smart, *J. Org. Chem.*, **15**, 720 (1950); (b) **16**, 424 (1951); **19**, 441 (1954).

(3) The absorption spectra of triphenylmethyl radicals, Crystal Violet ions, and related types have been interpreted in terms of the existence of stereoisomeric forms (G. N. Lewis, T. T. Magel, and D. Lipkin, THIS JOURNAL, **64**, 1774 (1942)). The polymorphism of certain tri-1-naphthylboron–amine addition compounds has been attributed to the restricted rotation of the naphthyl groups (H. C. Brown and S. Sujishi, *ibid.*, **70**, 2793 (1948)).

(4) The principal position isomers have been prepared and are different from the tetra-*o*-tolylsilanes.

(5) Melting points are uncorrected.

(6) The absorption curves for the 145° and 228° isomers are experimentally indistinguishable in the region 290–212 mμ. Above 290 mμ, the solutions are transparent. Below 246 mμ, there is a rapid rise in absorption continuing into the vacuum ultraviolet (ϵ at 212 mμ, 60,000). These spectra are related to those of tetraphenylsilane and tetra-*p*-tolylsilane.

hexane solution: 277.7 (2370) and 270.5 (2440); $\lambda_{min.}$ (and ϵ): 275.0 (1590) and 246.5 (470).

228° Isomer.—This isomer has been prepared from ethyl silicate, tri-*o*-tolylcholorosilane, and tri-*o*-tolylmethoxysilane. Tri-*o*-tolylchlorosilane (0.175 mole), for example, was refluxed under nitrogen for 48 hours with *o*-tolyllithium (0.25 mole) in ether. The ether was distilled and the residue heated at 140–180° for 5 hours. After hydrolysis,[2a] the ether soluble material was separated and distilled. The fraction boiling 215–220° (1 mm.) and the adjoining fractions gave 11 g. (16%) tetra-*o*-tolylsilane melting 215–225° after treatment with ligroin. Recrystallizations from ethanol–benzene (9:1) and from ligroin raised the melting point to 227.5–228.0°. Additional recrystallizations from these solvents and from acetic acid did not change the melting point. *Anal.* Calcd. for $C_{28}H_{28}Si$: C, 85.66; H, 7.19; Si, 7.15. Found: C, 85.63; H, 7.03; Si, 7.11. *Ultraviolet data.*[6] $\lambda_{max.}$ in mμ (and ϵ) for cyclohexane solution: 277.8 (2520) and 270.5 (2470); $\lambda_{min.}$ (and ϵ): 275.0 (1640) and 246.5 (440).

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AN ALTERNATE FATTY ACID CYCLE INVOLVING THIOESTERS OF PANTETHEINE[1]

Sir:

It is now well established[2-4] that fatty acid oxidation and synthesis can proceed via a reversible cycle of enzymes utilizing thioesters of CoA.[5] Recent experiments demonstrate that the same or similar enzymes catalyze an analogous sequence of reactions involving thioesters of pantetheine:[6]

BUT-S-pantetheine + indophenol $\rightleftharpoons$ CROT-S-pantetheine + indophenol-H_2 (1)

CROT-S-pantetheine + H_2O $\rightleftharpoons$ *d*-BOH-S-pantetheine (2)

d-BOH-S-panthetheine + DPN^+ $\rightleftharpoons$ AcAc-S-pantetheine + DPNH + H^+ (3)

AcAc-S-pantetheine + pantetheine-SH $\rightleftharpoons$ 2Ac-S-pantetheine (4)

Enzymes catalyzing reaction 1 have been found in soluble extracts of pigeon and ox liver (Table I). Using a modified indophenol assay,[7] the enzymatic oxidation of BUT-S-pantetheine was measured (a) as the decrease in light absorption at λ 600 mμ due to dye reduction and (b) by demonstrating a concomitant increase in absorption at λ 240 mμ due to formation of CROT-S-pantetheine. This reaction proceeds to an equilibrium, at which point addition of *trans*-CROT-S-pantetheine causes reoxidation of dye and attainment of a new equilibrium. The *cis*-isomer also is reduced, but less rapidly. Crystalline liver crotonase[8] was found to hydrate both *trans*- and *cis*-isomers of CROT-S-pantetheine. Although the rates were only 0.013% and 0.0023% of those for the respective CoA esters, they are significant in view of the very high turnover number (730,000) of crystalline crotonase. A partially purified preparation of crotonase-free heart *d*-BOH-S-CoA dehydrogenase[9] reacts almost equally well with the pantetheine as with the CoA thioesters of AcAc and *d*-BOH.

TABLE I

REACTIVITY OF PANTETHEINE AND COA ESTERS

Reactants as indicated: 0.01 *M* TRIS–HCl buffer, 1–2 × 10^{-4} *M* thioester, 8.2 × 10^{-5} *M* dye, 3 × 10^{-4} *M* DPN^+, 1.7 × 10^{-4} DPNH. Specific activity = μM. thioester reacting per minute per mg. protein at 25°.

	Reaction	*p*H	Specific activity R = Pantetheine	R = CoA
1	BUT–S–R + dye	7.5	0.0039[a]	0.0055
	BUT–S–R + dye	7.5	.0018[b]	.0025
2	*trans*-CROT–S–R + H_2O	7.5	.220[c]	1700
	cis-CROT–S–R + H_2O	7.5	.012	510
3	*d*-BOH–S–R + DPN^+	9.1	.52[d]	0.68
	AcAc–S–R + DPNH	7.0	3.1	3.1

[a] Dialyzed pigeon liver extract. [b] Ox liver mitochondrial extract after salt fractionation. [c] Crystalline liver crotonase[10] (representing 800-fold purification). [d] Partially purified pig heart *d*-BOH-S-CoA dehydrogenase.

A highly purified preparation of heart thiolase[9,10] (Table II) was found to catalyze (a) the thiolysis of AcAc-S-pantetheine[11] by pantetheine, (b) the condensation of two molecules of Ac-S-pantetheine to AcAc-S-pantetheine, and (c) the transfer of the acetyl group from Ac-S-pantetheine (but not Ac-S-Ac-N-βMEA) to CoA-SH (reaction 5). The rate of this thioltransacetylation reaction is rather

Ac-S-pantetheine + CoA-SH $\rightleftharpoons$ Ac-S-CoA + pantetheine-SH (5)

more rapid than the rate of the condensation reaction and is further evidence for the hypothesis of Lynen that reaction (6) (R = CoA or pantetheine) is a partial reaction of the overall thiolase reaction (4).

Ac-S-R + *thiolase*-SH $\rightleftharpoons$ Ac-S-*thiolase* + HS-R (6)

Ac-S-pantetheine was inactive as substrate for the crystalline citrate condensing enzyme.[12] Ac-Ac- and succinyl-S-pantetheine compounds did not react with highly purified CoA transferase.[9,10]

(1) Supported by grants from the U. S. Public Health Service and the Williams-Waterman Fund of Research Corporation.

(2) F. Lynen and S. Ochoa, *Biochim. Biophys. Acta*, **12**, 299 (1953).

(3) H. R. Mahler, *Federation Proc.*, **12**, 694 (1953).

(4) G. D. Greville and H. B. Stewart, *Chem. Soc. Ann. Reports*, **50**, 301 (1954).

(5) Abbreviations: Coenzyme A (reduced), CoA-SH; thioesters, acyl-S-R; acids: acetic, Ac; butyric, BUT; crotonic, CROT; β-hydroxybutyric, BOH; acetoacetic, AcAc; *d* refers to direction of rotation; DPN^+ and DPNH, oxidized and reduced diphosphopyridine nucleotide; TRIS, tris-(hydroxymethyl)-aminomethane; β-MEA, β-mercaptoethylamine; ATP, adenosine triphosphate; 5-AMP, adenosine-5′-phosphate; PP, pyrophosphate.

(6) Pantetheine was kindly supplied by Dr. O. D. Bird, Parke, Davis and Co.

(7) D. E. Green, S. Mii, H. R. Mahler and R. M. Bock, *J. Biol. Chem.*, **206**, 1 (1954).

(8) J. R. Stern and A. del Campillo, THIS JOURNAL, **75**, 2277 (1953).

(9) J. R. Stern, M. J. Coon and A. del Campillo, *ibid.*, **75**, 1517 (1953).

(10) J. R. Stern, in S. P. Colowick and N. O. Kaplan, "Methods in Enzymology," New York **1**, 559 and 573 (1955).

(11) See reference 2 for preparation of thioesters and optical methods of assay.

(12) S. Ochoa, J. R. Stern and M. C. Schneider, *J. Biol. Chem.*, **193**, 691 (1951).