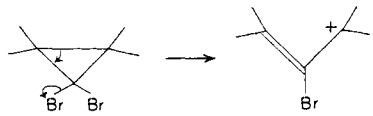
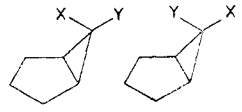
from the relief of strain in opening the cyclopropane ring.



A striking consequence of the stereochemistry of the bicyclic systems is indicated by the isolation of two isomers (α and β) of 2-bromo-2-chlorobicyclo-[3,1,0]hexane, and *the stereospecificity of their reactions with* Ag^+. The α-isomer loses Cl^- at the same



α- and β- isomers

rate as III to produce II, while the β-isomer loses Br^- at the same rate as I to produce IV. Analogous results are obtained with the two isomeric 2-bromo-2-chlorobicyclo[4,1,0]heptanes.

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BIOSYNTHESIS OF METHYL GROUPS OF CHOLINE FROM FORMALDEHYDE BY LIVER PREPARATIONS

Sir:

A previous communication[1] reported no choline formation when rat liver homogenates were incubated with aminoethanol and methionine-C^{14}-methyl. Phospholipid choline became labeled when glycine-2-C^{14} was used as substrate.[2] Stekol, *et al.*,[3] concluded from feeding experiments that, in the methylation of aminoethanol to choline, the first two methyl groups are derived by reduction of a one carbon entity, while the third methyl group is transferred to dimethylaminoethanol from methionine.

We have now determined that there is present an active soluble enzyme system in liver which synthesizes choline methyl groups from formaldehyde with either aminoethanol or dimethylaminoethanol as acceptors, the yield of choline being higher with dimethylaminoethanol (Table I). Tetrahydrofolic acid is a cofactor of the reaction. In confirmation of previous work, no choline synthesis was obtained with methionine. With aminoethanol as methyl group acceptor, paper chromatography revealed evidence of formation of methylated intermediates.

The significant role of tetrahydrofolic acid in the reaction is demonstrated in Fig. 1. At low concentrations of the coenzyme, but not at higher concentrations, methyl group synthesis is enhanced in the crude system by the presence of either reduced or oxidized diphosphopyridine nucleotide. In part, this results from a protection of tetrahydrofolic acid against decomposition.

(1) L. O. Pilgeram, R. E. Hamilton and D. M. Greenberg, *J. Biol. Chem.*, **227**, 107 (1957).

(2) P. Vohra, F. H. Lantz and F. H. Kratzer, *ibid.*, **221**, 501 (1956).

(3) J. A. Stekol, S. Weiss and E. I. Anderson, THIS JOURNAL, **77**, 5192 (1955).

TABLE I

CHOLINE METHYL GROUP BIOSYNTHESIS[a]

No.	Substrates	C^{14}-Choline formed,[b] μmoles	% of total isotope incorporated
1	Aminoethanol, $H_2C^{14}O$	0.45	9.0
2	Dimethylaminoethanol, $H_2C^{14}O$	.77	15.3
3	No enzyme (control for No. 1 and 2)	.12	...
4	Methionine-$C^{14}H_3$[c]	.01	0.13
5	No enzyme (control for No. 4)	.006	...

[a] Incubation media contained 0.5 ml. of enzyme preparation (centrifuged homogenate of rat liver, treated with Dowex-1-Cl^- and dialyzed against 0.05 *M* Tris buffer, *p*H 7.0, for 6 hr.), 1.0 ml. of 0.1 *M* Tris buffer, *p*H 7.0, $H_2C^{14}O$ (5 μmoles, 4.4×10^4 c.p.m.), tetrahydrofolic acid (100 μg.) DPN (10 μmoles) and aminoethanol or dimethylaminoethanol (8 μmoles) in total volume of 3.3 ml. Incubated in Dubnoff apparatus for 90 minutes at 37°. [b] After incubation 100 mg. of carrier choline was added, and precipitated as the reineckate complex by the addition of 5 vol. of 2% reineckate salt in methanol, then recrystallized from propanol to constant radioactivity. [c] Medium contained 8 μmoles (3.25×10^5 c.p.m.) methionine-$C^{14}H_3$ in place of formaldehyde, dimethylaminoethanol (8 μmoles), ATP (5 μmoles) DPN (10 μmoles).

Proof of the presence of the label in the methyl groups of choline was obtained by separation of choline on a Dowex-50 column[4] from the incubation mixture and by cleaving the trimethylamine moiety of choline isolated as the reineckate, and determining its radioactivity after conversion to the chloroplatinate complex.

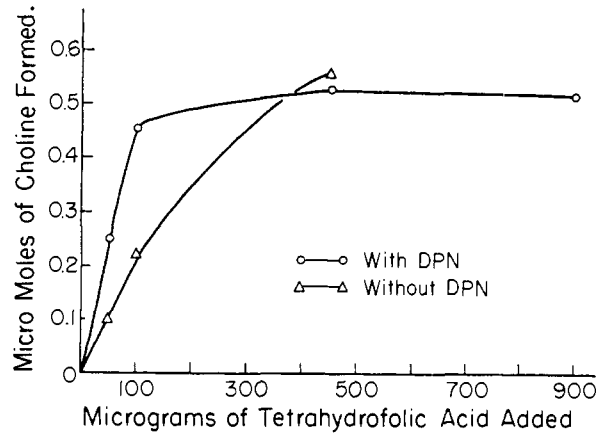

Fig. 1.—Effect of concentration of tetrahydrofolic acid and diphosphopyridine nucleotide on choline methyl group synthesis. Conditions of incubation same as in Table I, dimethylaminoethanol being used as the substrate.

Our results are at variance with the conclusion of Stekol, *et al.*, that the third methyl group of choline is secured by transmethylation from methionine, although it is quite possible that the enzyme system responsible for transmethylation does not withstand homogenization.

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(4) L. O. Pilgeram, E. M. Gal, E. N. Sassenrath and D. M. Greenberg, *J. Biol. Chem.*, **204**, 367 (1953).