scribed. A further study of transmethylation in the ergosterol-synthesizing system was desirable to determine whether the sulfonium derivative is of importance there also.

Methods developed in this Laboratory were used for the preparation of S-adenosylmethionine in high purity.[8] S-Methylmethionine was prepared according to the procedure of Toennies and Kolb.[9] Methionine and $C^{14}H_3$-methionine were obtained from commercial sources. A cell-free ergosterol-synthesizing system was prepared from bakers yeast.[10] Cells were disrupted by sonic treatment. Methyl donors were allowed to react in the enzyme system for 5 hours at 30°. Reaction vessel contents were then saponified with KOH and the non-saponifiable fraction extracted with petroleum ether. A Packard Tri-Carb liquid scintillation counter was used for radioactivity measurements. The results are shown in the Table I.

TABLE I

C-14 INCORPORATED INTO ERGOSTEROL FROM VARIOUS METHYL DONORS

C-14 methyl donor[a]	Non-radioactive substrate[b]	C-14 in ergosterol, c.p.m.
S-Adenosylmethionine	None	3795
	Methionine	3400
	Serine	3345
Control[c]	None	15
Methionine	None	1670
	S-Adenosylmethionine	90
	Serine	1235
Control	None	50
S-Methylmethionine	None	60
	S-Adenosylmethionine	25
	Methionine	10
	Serine	30
Control	None	40

[a] Each complete reaction mixture in a total volume of 4.5 ml. contained 3 ml. of enzyme representing 37.5 mg. of protein; 100 μmoles of potassium acetate; 20 μmoles of ATP; and 0.2 μc. C-14 representing 7.5 μmoles of the substrate. [b] 7.5μ moles of the non-radioactive substrate was added. [c] Control flasks were stopped at 0 time with KOH.

In these experiments S-adenosylmethionine is more efficient as a methyl group donor than is methionine. Incorporation of the labeled methionine does occur however, probably because of adenosylmethionine synthesis in the extracts since *Saccharomyces* extracts possess a methionine-activating system operable under the conditions of these experiments. Added non-radioactive adenosylmethionine greatly suppresses the methyl incorporation from free methionine, demonstrating preferential incorporation from the adenosyl sulfonium derivative. While methylmethionine has been found under certain conditions to be an excellent methyl source in the synthesis of methionine[11] it is virtually inactive in ergosterol synthesis.

(8) F. Schlenk and R. DePalma, *J. Biol. Chem.*, **229**, 1037 (1957).

(9) G. Toennies and J. J. Kolb, THIS JOURNAL, **67**, 849 (1945).

(10) H. P. Klein and Z. K. Booher, *Proc. Soc. Exptl. Biol. Med.*, **89**, 43 (1955).

(11) S. K. Shapiro, *J. Bacteriol.*, **72**, 730 (1956).

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RECEIVED MARCH 8, 1958

REACTIONS OF 1,1-DIHALOCYCLOPROPANES WITH ELECTROPHILIC REAGENTS. SYNTHETIC ROUTE FOR INSERTING A CARBON ATOM BETWEEN THE ATOMS OF A DOUBLE BOND

Sir:

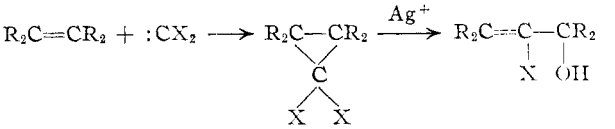
The chance observation that 2,2-dibromobicyclo-[3,1,0]hexane (I) reacted rapidly with alcoholic silver nitrate[1] indicated the desirability of further examination of this reaction.

Shaking I with aqueous or alcoholic silver nitrate results in the rapid precipitation of 1 mole of silver bromide per mole of I, the second bromine being unreactive. In aqueous alcoholic media a mixture of a bromoalcohol and a bromoether is obtained. In the aqueous media I is cleanly converted to the bromoalcohol, to which the structure 2-bromo-2-cyclohexen-1-ol (II) is assigned. Infrared (3.05, 6.12 μ) and proton magnetic resonance spectra are

I II

consistent with this assignment. The analogous 1,1-dichlorobicyclo[3,1,0]hexane (III) reacts similarly to produce the known 2-chloro-2-cyclohexen-1-ol (IV),[2] compared as the alcohol and the acetate.

This reaction has been extended to other systems and appears to be general for electrophilic attack on 1,1-dihalocyclopropanes. Thus a sequence of reactions of general character is available for extending a carbon chain[3] through insertion of a carbon atom *between* the double-bonded atoms. Isobutylene, styrene, *cis*- and *trans*-2-butene have been studied and the reaction is being extended to other olefins.



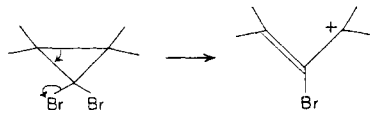
The strain in the 5–3 ring system of I results in greatly enhanced reactivity, I being 200 times as reactive as the analogous 2,2-dihalobicyclo[4,1,0]-heptane (V) which has a 6–3 ring system. Monocyclic dihalocyclopropanes have reactivities similar to V. The driving force for this reaction, in addition to the formation of an allylic cation, is derived

(1) A. Y. Garner, Pennsylvania State University Thesis, 1956. This compound is not dehydrohalogenated by alcoholic alkoxides at room temperature.

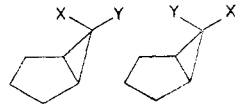
(2) M. Mousseron and R. Jacquier, *Bull. soc. chim. France*, 648 (1950).

(3) Specific applications of this reaction are known. (a) Indenes to β-halonaphthalenes: W. E. Parham and H. E. Reiff, THIS JOURNAL, **77**, 1177 (1955); W. E. Parham, H. E. Reiff and P. Swartzentruber, *ibid.*, **78**, 1437 (1956); W. E. Parham and R. R. Twelves, *J. Org. Chem.*, **22**, 730 (1957); W. E. Parham and C. D. Wright, *ibid.*, **22**, 1473 (1957). (b) Pyrroles to β-substituted pyridines: J. Hine, THIS JOURNAL, **72**, 2444 (1950); E. R. Alexander, A. B. Herrick and T. M. Roder, *ibid.*, **72**, 2760 (1950); G. L. Ciamician, *Ber.*, **37**, 4234 (1904); G. L. Ciamician and M. Dennstedt, *ibid.*, **14**, 1153 (1881); **15**, 1172 (1882); G. L. Ciamician and P. Silber, *ibid.*, **20**, 191 (1887); M. Dennstedt and J. Zimmermann, *ibid.*, **18**, 3316 (1885); G. Plancher and U. Ponti, *Atti. accad. naz. Lincei*, [5] **18**, II, 469 (1909); O. Bocchi, *Gazz. chim. ital.*, **30**, [I] 89 (1900). (c) Indoles to β-haloquinolines: G. Magnanini, *Ber.*, **20**, 2608 (1887); A. Ellinger, *ibid.*, **39**, 2517 (1906); G. Plancher and O. Carrasco, *Atti. accad. naz. Lincei.* [5] **13**, I, 573, 632 (1904).

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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RECEIVED FEBRUARY 20, 1958

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.

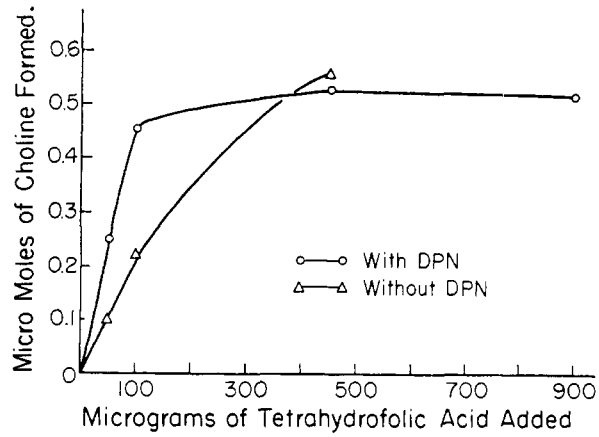

Fig. 1.—Effect of concentration of tetrahydrofolic acid and diphosphopyridine nucleotide on choline methyl group synthesis. Conditions of incubation same as in Table I, dimethylaminoethanol being used as the substrate.

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).