β-carbon of serine underwent approximately the same dilution.

The methyl group of the thymine, isolated in experiment 1, had an activity of 7360 c.p.m.[3] and 0.0126 atom D. This would indicate a dilution of 43 for the carbon and 57 for the D.

A contribution of D to the methyl groups from the α position of serine (via α-deuterioglycine[6]), which would significantly change these ratios, is unlikely, since glycine is a poor source of methyl groups,[2,3] and the α-hydrogen atoms of glycine[7] and serine[8] undergo extensive labilization *in vivo*.

These findings impose certain restrictions on hypotheses concerning the mechanism of transport of one-carbon units. In the synthesis of methyl groups from serine the β-carbon does not appear to go through the oxidation level of formate since that would result in loss of at least half of its D. It should be noted that the DL-serine, from which the L-serine was obtained, was synthesized by reduction of ethyl formylhippurate with Al–Hg in the presence of D_2O. This makes it likely that the D is predominantly attached to the β-carbon atom of L-serine in only one of two possible configurations. If the unlabeled hydrogen is selectively eliminated[9] by enzymatic oxidation, the C^{14}/D ratio would remain unchanged even if conversion to formate had occurred.

Exclusion of formate would also exclude formyl derivatives of folic acid, such as N^{10}-formylfolic[10] or N^5-formyl-5,6,7,8-tetrahydrofolic acid[11–13] ("citrovorum factor," folinic acid-SF, leucovorin) as actual carriers of a one-carbon fragment in this process, unless they also serve as specific carriers of the β-hydrogens of serine. There is considerable evidence to show that folic acid is linked to the metabolic reactions of one-carbon units, such as the synthesis of the methyl groups of choline and thymine and the various reactions of formate. Subject to the indicated limitations, our results suggest, however, that if leucovorin is the biological form of folic acid, its function is other than that of carrier of these units. These considerations may be limited to the reactions studied. In the utilization of other precursors, and in the synthesis of other products (*e.g.*, purines) a different mechanism may be involved.

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(6) D. Shemin, *J. Biol. Chem.*, **162**, 297 (1946).

(7) D. B. Sprinson and D. Rittenberg, *ibid.*, **184**, 405 (1950).

(8) D analyses on the administered serine and formaldehyde dimedon derivative obtained from carbon-3 (following oxidation of serine with $NaIO_4$) showed that the D was equally distributed between carbons 2 and 3. A similar degradation of serine isolated from the internal organ proteins in exp. 1 showed the β-C^{14}/β-D/N^{15} ratios to be the same as in the compound fed. The α-D was labilized, being only $^1/_3$ as high as the β-D (*cf.* ref. 7).

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(10) M. Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).

(11) J. A. Brockman, Jr., *et al.*, *ibid.*, **72**, 4325 (1950).

(12) (a) M. May, *et al.*, *ibid.*, **73**, 3067 (1951); (b) A. Pohland, *et al.*, *ibid.*, **73**, 3247 (1951).

(13) H. P. Broquist, *et al.*, *ibid.*, **73**, 3538 (1951).

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CRYSTALLINE CITROVORUM FACTOR FROM LIVER

Sir:

Subsequent to our observation that the citrovorum factor in liver (I) differed from a synthetic compound[1] resulting from the formylation and reduction of petroylglutamic acid[2] (II), we have been able to isolate citrovorun factor as its crystalline barium salt from horse liver.

The method used involved the following fractionation steps: (1) autolysis of the ground liver, (2) adsorption on charcoal and elution therefrom, (3) precipitation and removal of water-acid insoluble materials, (4) extraction into butanol at *p*H 3, (5) precipitation of impurities in the aqueous ammoniacal extract of the butanol extract with methanol, (6) adsorption on Dowex 1 column and subsequent elution, (7) adsorption of active fraction on charcoal and subsequent elution, (8) adsorption on Al_2O_3 column from aqueous alcohol solution and elution therefrom, (9) fractional crystallization of the barium salt.

When assayed with *Leuconostoc citrovorum* the isolated crystalline barium salt of citrovorum factor (I) was found to contain 237 units[3] per γ. However, the barium salt of the synthetic compound (II) was calculated to contain 115 units per γ based on the reported activity of the free acid of II.[1] Thus, the product which we have obtained from horse liver is approximately twice as active for *L. citrovorum* as is the synthetic compound II.

The crystalline barium salt (I) at a concentration of 10 mg./l. in 30% ethanol containing 0.03% NH_3 showed a maximum at 286 mμ (T = 35.3%) and a minimum at 243 mμ (T = 77.9%). The X-ray powder diffraction data (obtained by William C. White) are given in Table I.

TABLE I

INTERPLANAR SPACINGS OF CRYSTALLINE BARIUM SALT (I)

Å.					
	8.11	7.51[a]	7.31[a]	6.52[a]	5.35
	5.06	4.70	4.45	4.01	3.50[a]

[a] Denotes most intense lines.

(1) M. Silverman and J. C. Keresztesy, THIS JOURNAL, **73**, 1897 (1951).

(2) J. A. Brockman, B. Roth, H. P. Broquist, M. E. Hultquist, J. M. Smith, M. J. Fahrenbach, D. B. Cosulich, R. P. Parker, E. L. R. Stokstad and T. H. Jukes, *ibid.*, **72**, 4325 (1950).

(3) J. C. Keresztesy and M. Silverman, *J. Biol. Chem.*, **183**, 473 (1950).

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MANY-MEMBERED CARBON RINGS. IV. SYNTHESIS OF CYCLONONYNE AND CYCLODECYNE

Sir:

We have found that the synthesis of many-membered carbon rings containing an acetylenic group using the methods employed by Ruzicka in preparing cyclopentadecyne and cycloheptadecyne,[1] by Stoll in obtaining cycloheptadecyne-10-one,[2] and

(1) L. Ruzicka, M. Hürbin and H. A. Boekenoogen, *Helv. Chim. Acta*, **16**, 498 (1933).

(2) M. Stoll, J. Hultskamp and A. Rouve, *ibid.*, **31**, 543 (1948).