fed to rats the choline from the internal organs[2] was extensively labeled in the β- and methyl carbon atoms $[(CH_3)_3{}^+N\text{-}{}^{\alpha}CH_2\text{-}{}^{\beta}CH_2OH]$ and in the nitrogen.[3] From Table I it can be seen that the isotope dilutions for all three positions are approximately the same. A smaller incorporation of C^{14} into the α-carbon of choline also took place. It would seem that β-labeled serine can give rise to α,β-labeled serine which is then converted to ethanolamine labeled in both carbon atoms.[4] Further investigation is continuing to explain this finding.

TABLE I

ISOTOPE DISTRIBUTION IN CHOLINE FOLLOWING ADMINISTRATION OF L-SERINE AND GLYCINE

	Isotope concentration in precursor		Isotope concentration in choline			
Compound administered	N^{15} concn. atom % excess	Activity in labeled carbon c. p. m.[a] $\times 10^{-3}$	Methyl c. p. m.[a] $\times 10^{-3}$	Nitrogen atom % excess	α-Carbon c. p. m.[a] $\times 10^{-3}$	β-Carbon c. p. m.[a] $\times 10^{-3}$
3-C^{14}, N^{15}-L-serine[b]	25.8	1,060	10.1	0.277	2.3	10.8
2-C^{14}, N^{15}-glycine[c]	21	13,000	4.0	.041	17.5	4.2
1-C^{14}, N^{15}-glycine[d]	27.6	389	0	.430	0	0

[a] Counts per minute per dish (2.5 sq. cm.) of carbon at infinite thickness. [b] Fed 0.47 mM. per 100 g. of body weight per day for two days. [c] Fed 0.40 mM. per 100 g. for one day. [d] Fed 1.45 mM. per 100 g. per day for two days.

The α-carbon and nitrogen[5] of glycine are also available for the formation of the methyl groups and ethanolamine moiety of choline, while the carboxyl carbon of glycine is lost in this process. It is apparent that the utilization of glycine does not involve the reduction of the carboxyl group.[5] The isotope dilutions per millimole of precursor fed being greater in the glycine feeding, it is reasonable to conclude that glycine is converted to serine[6] prior to its use for the synthesis of ethanolamine. A point of interest is the nearly equal activity in the β-carbon atom and methyl groups of choline following the administration of either glycine or serine.[7]

The ability of the rat to synthesize methyl groups from the β-carbon atom of serine is in agreement with the recent report[8] that diets devoid of methyl group donors will support growth when folic acid and vitamin B_{12} are present.

DEPARTMENT OF BIOCHEMISTRY ARTHUR WEISSBACH
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY DAVID ELWYN[9]
NEW YORK, N. Y. DAVID B. SPRINSON

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(2) Details of the isolation and degradation procedures will be published elsewhere.

(3) Stetten, *J. Biol. Chem.*, **144**, 501 (1942).

(4) Elwyn and Sprinson, *ibid.*, **184**, 465 (1950).

(5) Stetten, *ibid.*, **140**, 143 (1941).

(6) Sakami, *ibid.*, **178**, 519 (1949).

(7) Following the completion of these experiments it was reported by Dr. H. G. Wood (Harvey Lecture, N. Y., February, 1950) that the synthesis of labile methyl groups in the rat from the methyl carbon of acetone and from formate has been observed by Dr. W. Sakami.

(8) Bennett, *Science*, **110**, 589 (1949).

(9) Life Insurance Medical Research Student Fellow, 1949-1950.

THE EXTENSIVE SYNTHESIS OF THE METHYL GROUP OF THYMINE IN THE ADULT RAT[1]

Sir:

The utilization of a one-carbon fragment derived from the β-carbon atom of L-serine or the α-carbon atom of glycine for the N-methylation of ethanolamine[2] suggested the possibility that the synthesis of thymine proceeded *via* C-methylation of the number 5 carbon atom of a pyrimidine nucleus. In the experiments previously described[2] nucleic acids were extracted with sodium chloride solution and separated into ribonucleic acids (RNA) and desoxyribonucleic acids (DNA).[3] Purines from 2*N* hydrochloric acid hydrolysates of RNA and purines and pyrimidines from formic acid hydrolysates of DNA[4] were isolated following separation by chromatography[5] on Dowex 50. Thymine was degraded by conversion to 5-bromo-4-hydroxyhydrothymine,[6] hydrolysis of the latter with warm *M* sodium bicarbonate solution to acetol, and treatment of the reaction mixture with hypoiodite. The resulting iodoform represents the methyl group of the original thymine. The results are shown in Table I.

TABLE I

ACTIVITIES OF PURINES AND PYRIMIDINES OF NUCLEIC ACIDS FOLLOWING ADMINISTRATION OF L-SERINE AND GLYCINE[a]

Purines and pyrimidines isolated[b]		Activity[c] following administration of 3-C^{14}-L-serine[d] c. p. m.[c]	Activity[c] following administration of 2-C^{14}-glycine[e] c. p. m.[c]
DNA	Thymine	5,610	3,070
	methyl	25,000	13,760
	ring[f]	748	405
	Cytosine	272	697
	Adenine	17,800	27,300
	Guanine	13,800	26,000
RNA	Adenine	18,300	27,100
	Guanine	17,800	32,500

[a] Rats weighing 250 g. were used. [b] Adenine and cytosine were isolated and counted as picrates, guanine as sulfate, and thymine as free base. [c] Counts per minute per dish (2.5 sq. cm.) of carbon at infinite thickness. [d] Fed 0.47 mM. per 100 g. body weight per day for two days; activity of β-carbon 1.06×10^6 c. p. m. [e] Fed 0.40 mM. per 100 g. for one day; activity of α-carbon 1.30×10^7 c. p. m. [f] Calculated values.

The activity of the methyl carbon atom of thymine following the administration of β-labeled serine accounts for 90% of the total activity of the molecule, and is about 2.5 times that of the methyl groups of choline.[2] A comparison of the activities of thymine and the DNA and RNA

(1) This work was supported by a grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

(2) Weissbach, Elwyn and Sprinson, THIS JOURNAL, **72**, 3317 (1950).

(3) Hammarsten, *Acta Med. Scand.*, suppl. **196**, 634 (1947).

(4) We are grateful to Dr. E. Chargaff for informing us of this modification of a previously published method of hydrolysis; *cf.* Chargaff, *et al.*, *J. Biol. Chem.*, **177**, 405 (1949).

(5) Cohn, *Science*, **109**, 377 (1949).

(6) Baudisch and Davidson, *J. Biol. Chem.*, **66**, 283 (1925).

purines in this experiment indicates that the activity of the thymine methyl carbon is close to the expected activities of the 2- and 8-positions of the purines.[7]

When α-labeled glycine was fed 90% of the activity was again present in the methyl group. This finding makes it unlikely that the methyl carbon was introduced together with carbon 5 of the pyrimidine ring, since α-labeled glycine gives rise to α,β-labeled serine[8] or to equally labeled acetate.[9] It appears that the methyl group of thymine is derived from a one-carbon intermediate[7,8] by methylation of a pyrimidine nucleus. This is in accord with the suggestion that the conversion of N^{15}-cytidine to thymine in the rat proceeds by a direct conversion to thymidine.[10]

It is difficult to reconcile the activities of thymine and DNA purines reported here with the concept of "biochemical stability" of DNA proposed on the basis of experiments with N^{15}-labeled adenine.[11]

The reported role of folic acid and vitamin B_{12} in the synthesis of labile methyl groups,[12] and the common origin of the methyl groups of choline[2] and thymine and the 2- and 8-positions of uric acid,[7] suggest an explanation for the known replacibility of folic acid[13] or vitamin B_{12}[14] by thymidine, and of *p*-aminobenzoic acid by thymine, purines and methionine, in certain deficient microörganisms.[15]

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF PHYSICIANS AND SURGEONS
COLUMBIA UNIVERSITY DAVID ELWYN[16]
NEW YORK, N. Y. DAVID B. SPRINSON

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(7) This is based on the finding that following administration of serine to pigeons the activity of the 2- and 8-positions accounted for 90% of the total activity of excreted uric acid (Elwyn and Sprinson, *J. Biol. Chem.*, **184**, 465 (1950)).

(8) Sakami, *ibid.*, **178**, 519 (1949).

(9) Sprinson, *ibid.*, **178**, 529 (1949).

(10) Hammarsten, Reichard and Saluste, *ibid.*, **183**, 251 (1950).

(11) Furst, Roll and Brown, *ibid.*, **183**, 251 (1950).

(12) Bennett, *Science*, **110**, 589 (1949).

(13) Shive, *et al.*, THIS JOURNAL, **70**, 2299 (1948).

(14) Snell, *et al.*, *J. Biol. Chem.*, **175**, 473 (1948).

(15) Lampen, Jones and Roepke, *ibid.*, **180**, 423 (1949).

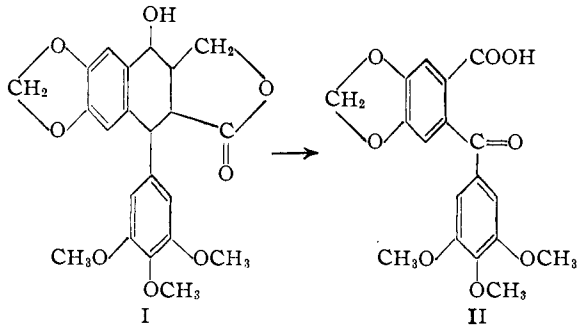
(16) Life Insurance Medical Research Student Fellow, 1949-1950.

SYNTHESIS OF A DEGRADATION PRODUCT FROM PICROPODOPHYLLIN[1]

Sir:

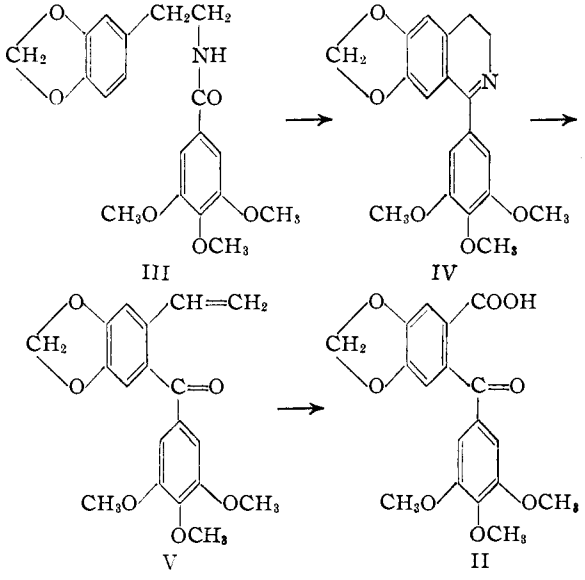
One of the oxidation products of picropodophyllin (I[2]) is considered, on the basis of degradative evidence, to have the structure of trimethoxybenzoylpiperonylic acid (II).[3] We wish to report the synthesis of this compound and thereby confirmation of the assigned structure.

Reaction of homopiperonylamine (prepared by reduction of piperonylidenenitromethane with



I II

lithium aluminum hydride) with trimethoxybenzoyl chloride leads to amide III, m. p. 135.2–135.7°, in 80% yield. (*Anal.* Calcd. for $C_{19}H_{21}$-



III IV

V II

O_6N: N, 3.9. Found: N, 3.9, 3.9.). Heating the amide with phosphorus oxychloride in toluene results in cyclization (93%) to 1-trimethoxyphenyl - 6,7 - methylenedioxy - 3,4 - dihydroisoquinoline (IV), m. p. 160.2–160.6°. (*Anal.* Calcd. for $C_{19}H_{19}O_5N$: N, 4.1. Found: N, 4.1, 4.2). This compound, on treatment with excess methyl sulfate and aqueous alcoholic alkali, is transformed to the vinylbenzophenone derivative (V), m. p. 139.2–139.8°, in 80% yield. (*Anal.* Calcd. for $C_{19}H_{18}O_6$: C, 66.68; H, 5.26. Found: C, 66.5, 66.6; H, 5.3, 5.4). Finally, oxidation of V with permanganate furnishes the desired keto-acid (II), m. p. 215.2–215.7°, in 50–70% yield (*Anal.* Calcd. for $C_{18}H_{16}O_8$: C, 60.02, H, 4.44. Found: C, 59.8, 60.0; H, 4.5, 4.6). The mixed melting point with the keto-acid obtained from picropodophyllin[3] shows no depression.

The novel one-step conversion of isoquinoline IV to the vinyl compound V must proceed through several intermediate stages. It is likely that the first step involves formation of a dihydroisoquinolinium cation (VI). On treatment with alkali, such compounds are known to yield the

(1) This work has been supported by American Cancer Society Grant-in-aid No. CBC-6 as recommended by the Committee on Growth of the National Research Council.

(2) Borsche and Niemann, *Ann.*, **499**, 59 (1932).

(3) Späth, Wesely and Nadler, *Ber.*, **66**, 125 (1933).