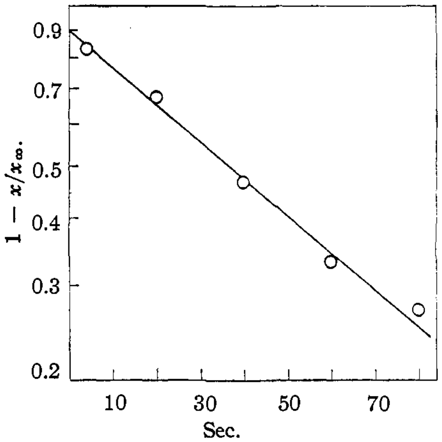

Fig. 1.—Ferrous–ferric exchange: x, radioactivity of ferrous fraction; x_∞, equilibrium value; $T_{1/2}$ = 44 ± 4 sec.

mary of the data obtained is given in Table I. All runs were made at room temperature 23 ± 2°. The results indicate that catalysis by traces of chloride was not an important factor in determining the rate; possible effects of other undetected impurities are, of course, not eliminated. The change of half-time with iron concentration shows the reaction to be second order, presumably first order in ferric and in ferrous iron, in which case the rate constant in 0.4 *f* perchloric acid is 16 mole^{-1}-l.-sec.$^{-1}$ at 23°.

Experiments on the various factors which affect the rate are being continued.

CHEMISTRY DEPARTMENT
BROOKHAVEN NATIONAL LABORATORY
UPTON, N. Y. RICHARD W. DODSON

RECEIVED MAY 19, 1950

THE *IN VIVO* SYNTHESIS OF LABILE METHYL GROUPS

Sir:

In the course of an investigation into the metabolism of amino acids, evidence has been obtained which can best be explained on the assumption of an *in vivo* synthesis of labile methyl groups in the rat. These methyl groups may be derived from the α-carbon of glycine, directly, or indirectly through the β-carbon atom of serine, or both, and therefore from serine itself. This is contrary to the belief that labile methyl groups cannot be synthesized in the animal organism but must be provided in the diet.[1,2]

Serine labeled in the β-position with C^{14} was synthesized according to the method of King.[3] A total of 20.1 mg. of DL-serine-β-C^{14} in 10 ml. of water was given intraperitoneally to a 150-g. male rat in divided doses twice daily over a period of five days. The rat was kept on the ordinary stock diet. The serine contained a total radioactivity of 2.8 microcuries. The rat was sacrificed and the choline in the liver isolated as the reineckate, then purified through the chloroplatinate, degraded to trimethylamine, which was precipitated as the chloroplatinate and recrystallized, all according to the method of du Vigneaud, *et al.*[1] Radioactivity was found in the methyl groups from the choline.

SPECIFIC ACTIVITY PER MILLIMOLE IN COUNTS PER MIN.

		Platinum, %	
		Calcd.	Found
DL-Serine	2.2×10^7		
Choline chloroplatinate	2.3×10^4	31.7	31.5
Trimethylamine chloroplatinate	1.3×10^4	37.0	37.0

Confirmation of these findings was obtained with another animal.

That all of the choline radioactivity is not in the trimethylamine fraction indicates that serine, at least in part, has been converted to ethanolamine; this is additional support for the findings of Stetten.[4]

There is reason to believe that pteroylglutamic acid and possibly vitamin B_{12} may be involved in these transformations.

The finding of radioactivity in the choline methyl group demonstrates the *in vivo* synthesis of labile methyl groups from the β-carbon of serine and hence[5] from the α-carbon of glycine.

The authors wish to thank Dr. Louis DeSpain Smith for his valuable advice.

After this work had been completed we became aware of a report by Sakami[6] and Welch and Sakami[7] on the incorporation of the methyl group of acetone and of formate into methionine and choline methyl groups. We have obtained similar results with radioactive formaldehyde.

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(1) du Vigneaud, Cohn, Chandler, Schenck and Simmonds, *J. Biol. Chem.*, **140**, 625 (1941).

(2) du Vigneaud, THIS JOURNAL, **72**, 1049 (1950), did demonstrate the incorporation of methyl groups from methanol into choline; methanol, however, is not a normal dietary constituent.

(3) King, *ibid.*, **69**, 2738 (1947).

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

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

(6) Sakami, *Federation Proc.*, **9**, 222 (1950), abstract.

(7) Welch and Sakami, *ibid.*, **9**, 245 (1950), abstract.

BIOCHEMICAL RESEARCH FOUNDATION
SIGURDUR JONSSON
NEWARK, DELAWARE WILLIAM A. MOSHER

RECEIVED MAY 15, 1950

THE SYNTHESIS OF THE METHYL GROUPS AND ETHANOLAMINE MOIETY OF CHOLINE FROM SERINE AND GLYCINE IN THE RAT[1]

Sir:

In an investigation of the mechanism of formation of the phospholipid bases it was found that L-serine is a source not only of the ethanolamine portion of choline, but also of its methyl carbon atoms. When L-serine labeled with N^{15} in the amino group and C^{14} in the β-carbon atom was

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

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] × 10^{-3}	Methyl c. p. m.[a] × 10^{-3}	Nitrogen atom % excess	α-Carbon c. p. m.[a] × 10^{-3}	β-Carbon c. p. m.[a] × 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

RECEIVED MAY 15, 1950

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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]	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

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(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.*, **64**, 233 (1925).