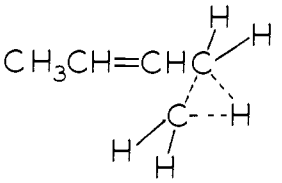
butenes has been used to detect other carbene reaction intermediates. For example, photolyses of ethyl diazoacetate in the presence of these olefin produces carbethoxycarbene, $:CHCOOC_2H_5$, since the ethyl 2,3-dimethylcyclopropanecarboxylates are obtained in stereospecific reactions. Copper-bronze catalysis produces the same results as photolysis.

Conversion of C–H bonds to C–CH_3 bonds has been described.[8] The failure to observe isomerization in the products *cis*- and *trans*-2-pentene from $:CH_2$ and *cis*- and *trans*-2-butene, respectively, suggests that the intermediate in this substitution reaction has structure (V). This intermediate for



V

the substitution reactions is similar to that proposed for the $CH_2 + H_2$ reaction.[9]

(8) A. Meerwein, H. Rathjen and H. Werner, *Ber.*, **75**, 1610 (1942); W. von E. Doering, R. G. Buttery, R. G. Laughlin and N. Chandhuri, THIS JOURNAL, **78**, 3224 (1956).

(9) J. Chanmugam and M. Burton, *ibid.*, **78**, 509 (1956). See also for references to earlier discussions of this mechanism.

WHITMORE LABORATORY
THE PENNSYLVANIA STATE UNIVERSITY PHILIP S. SKELL
UNIVERSITY PARK, PENNSYLVANIA
ROBERT C. WOODWORTH

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GLUTAMINE REQUIREMENT FOR THE INOSINIC ACID TRANSFORMYLASE REACTION

Sir:

Flaks and Buchanan[1] described the purification of pigeon liver inosinic acid transformylase which catalyzed the following reaction: IMP-5'[2] + glycine $\rightleftharpoons$ serine + AICR. These investigators demonstrated that leucovorin was a required co-factor. It is the purpose of this communication to present data to show that L-glutamine is also required in this reaction.

The pigeon liver extracts were prepared according to the method of Greenberg[3] except that bicarbonate was omitted from the buffer and the homogenization performed at 4° in a blender for 15 seconds. The supernate after centrifugation at 36,000 × *g* was absorbed twice with norite (20 mg./ml.) at 0°. This extract required either ATP and CF or anhydroleucovorin *per se* as co-factors for the transformylase reaction. The reaction was followed by aryl amine liberation.[4] Greenberg[5,6] has shown that CF must be activated by ATP before it functioned as a transformylation co-factor. Precipitation of the norite-treated extract with 50% acetone at 0° resolved the enzymatic activity into two fractions. Both the acetone precipitate and the acetone supernate were necessary for transformylation activity. The activity of the latter was replaced by L-glutamine. Although glutamic acid and NH_4^+ ions had a slight effect, asparagine, NH_4^+ or glutamic acid were ineffective. Vitamins, purines, pyrimidines, nucleotides, nucleosides, DPNH, TPNH, DPN and TPN were incapable of replacing glutamine. The activity of the apo-enzyme was limited by the concentration of L-glutamine in the reaction mixture. The results are shown in the table.

TABLE I

THE EFFECT OF GLUTAMINE ON THE FORMATION OF ARYL AMINE BY INOSINIC ACID TRANSFORMYLASE OF PIGEON LIVER

The reaction vessels were incubated for 30 minutes at 37° and the reaction stopped by adding 2 ml. of 0.2 *N* HCl and 0.2 ml. of 20% trichloroacetic acid. The acetone precipitate of the extract represented 1 ml. of the original cell-free extract; total volume 1.0 ml. at *p*H 7.5. The substrates were: Na inosinate, 0.6 μM.; glycine, 100 μM.; leucovorin, 0.1 μM.; $CuSO_4 \cdot 5H_2O$, 0.1 μM.; ATP, 0.2 μM.

		μmoles aryl amine (AIC) I	II[b]	III[c]
1	Acetone precipitate + CF[a] + ATP + glycine + inosinate	0.011	0.007	0.007
2	1 + 0.08 μM. L-glutamine	.027	...	...
3	1 + 0.2 μM. L-glutamine	.068	...	...
4	1 + 0.4 μM. L-glutamine	.104	...	...
5	1 + 0.8 μM. L-glutamine	.104	.054	.077
6	1 + 0.8 μM. L-asparagine	.011	...	...
7	1 + 0.8 μM. DL-glutamic acid	.011	...	...
8	1 + 2 μM. NH_4Cl	.016	...	...
9	1 + 0.8 μM. DL-glutamic acid + 2μM. NH_4Cl	.035	...	...
10	5 minus ATP	.038	.025	.045
11	5 minus CF	.026	.022	...
12	5 minus glycine	.045	.018	.028
13	5 minus inosinate	.050	.017	.028
14	5 + 15 μM. DL-serine	.053	.007	.007
15	5 minus acetone ppt.	.005	...	.001

[a] Anhydroleucovorin can replace CF. [b] Assay contained 14 μM. glycine. [c] Acetone reprecipitated enzyme; 14 μM. glycine in reaction vessel.

The following criteria were used to show that the liberation of aryl amine was due to the inosinic acid transformylase reaction: (a) serine specifically inhibited aryl amine liberation; (b) both glycine and inosinic acid were required for optimal activity (some enzyme preparations contained small amounts of these substrates); (c) the reaction required both ATP and CF[7]; (d) the liberated aryl amine could not be acetylated by acetic anhydride[8]; and (e) the acid-hydrolysis product of the liberated aryl amine was identified as AIC when chromatographed according to the method of Greenberg and Spilman.[9]

(1) J. G. Flaks and J. M. Buchanan, THIS JOURNAL, **76**, 2275 (1954).

(2) Abbreviations: ATP, adenosine triphosphate; CF, leucovorin; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; AIC, 4-amino-5-imidazolecarboxamide; IMP-5', inosinic acid; AICR, 4-amino-5-imidazolecarboxamide ribotide.

(3) G. R. Greenberg, *J. Biol. Chem.*, **190**, 611 (1951).

(4) A. C. Bratton and E. K. Marshall, Jr. *ibid.*, **128**, 537 (1939).

(5) G. R. Greenberg, THIS JOURNAL, **76**, 1458 (1954).

(6) G. R. Greenberg, *Fed. Proc.*, **13**, 221 (1954).

(7) L. Warren and J. G. Flaks, *ibid.*, **15**, 379 (1956).

(8) J. M. Ravel, R. E. Eakin and W. Shive, *J. Biol. Chem.*, **172**, 67 (1948).

(9) G. R. Greenberg and E. L. Spilman *ibid.*, **219**, 411 (1956).

The role of glutamine in the 1-carbon transfer reaction is yet to be elucidated.

MEDICINAL CHEMICAL RESEARCH SECTION — N. H. SLOANE
RESEARCH DIVISION — W. F. BARG, JR.
AMERICAN CYANAMID COMPANY — E. BOGGIANO
LEDERLE LABORATORIES — B. COULOMB
PEARL RIVER, NEW YORK — B. L. HUTCHINGS

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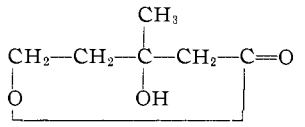
THE UTILIZATION OF β-HYDROXY-β-METHYL-δ-VALEROLACTONE IN CHOLESTEROL BIOSYNTHESIS

Sir:

Wolf, *et al.*,[1] have shown recently that the structure of the growth-promoting factor for *Lactobacillus acidophilus* (ATCC 4963) discovered by Skeggs, *et al.*,[2] and obtained by Wright, *et al.*,[3] is β-hydroxy-β-methyl-δ-valerolactone



The structural similarity of this lactone to β-hydroxy-β-methylglutaric acid prompted us to investigate its possible role in cholesterol metabolism. To this end we have studied cholesterol synthesis in cell-free rat liver homogenates using the technique described by Bucher[4] and modified by Rabinowitz and Gurin.[5]

Material prepared from distillers' solubles, as well as synthetic DL samples, suppress the incorporation of 1-C^{14}-acetate into cholesterol (expts. 1, 2, Table I). Since such an effect is open to several interpretations, β-hydroxy-β-methyl-δ-valerolactone (DVA) labeled in the 2-position with C^{14} was synthesized[6] and used in a second experiment. The lactone-C^{14} was incorporated into cholesterol to such a degree as to indicate preferential utilization of the 2-carbon of the lactone over the 1-carbon of acetate for sterol synthesis (expt. 2, Table I).

In the light of these observations, and the experiments of Rabinowitz and Gurin,[5] of Bloch, *et al.*,[7] and of Rudney,[8,9] indicating that β-hydroxy-β-methylglutaric acid (HMG) and β,β-dimethylacrylic acid (DMA) may be incorporated into cholesterol, experiments were conducted to determine the relative efficiency of HMG, DMA and DVA in contributing isotope for cholesterol biosynthesis.[10] The results of one of these experiments are shown in Table II.

(1) D. E. Wolf, C. H. Hoffman, P. E. Aldrich, H. R. Skeggs, L. D. Wright and K. Folkers, THIS JOURNAL, **78**, 4499 (1956).

(2) H. R. Skeggs, L. D. Wright, E. L. Cresson, G. D. E. MacRae, C. H. Hoffman, D. E. Wolf and K. Folkers, *J. Bact.*, in press.

(3) L. D. Wright, E. L. Cresson, H. R. Skeggs, G. D. E. MacRae, C. H. Hoffman, D. E. Wolf and K. Folkers, THIS JOURNAL, in press.

(4) N. L. R. Bucher, THIS JOURNAL, **75**, 498 (1953).

(5) J. L. Rabinowitz and S. Gurin, *J. Biol. Chem.*, **208**, 307 (1954).

(6) Prepared for us by Dr. C. S. Miller of this laboratory.

(7) K. Bloch, L. C. Clark and I. Harary, *J. Biol. Chem.*, **211**, 687 (1954).

(8) H. Rudney, THIS JOURNAL, **76**, 2595 (1954).

(9) H. Rudney, *ibid.*, **77**, 1698 (1955).

(10) We wish to acknowledge our indebtedness to Dr. S. Gurin, Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia, and to Dr. J. L. Rabinowitz, Radioisotope Unit, Veterans Administration Hospital, Philadelphia, for generous gifts of both natural and radioactive HMG and DMA.

TABLE I

INCORPORATION OF ACETATE AND OF β-HYDROXY-β-METHYL-δ-VALEROLACTONE (DVA) INTO CHOLESTEROL IN RAT LIVER HOMOGENATES

Each flask contained 5 ml. of liver homogenate, and 1 mg. each of ATP and DPN. Labeled or non-labeled substrates were added as indicated. Final volume was 9.5 ml. Gas phase was 95% O_2–5% CO_2. In a given experiment all flasks contained aliquots of the same liver preparation. Incubation was carried out at 37° for 4.5 hours. Cholesterol was isolated and counted as the digitonide.

Expt.	Substrates NaOAc	Substrates DVA	Activity added total c.p.m. × 10^5	Recovered cholesterol c.p.m./mg. C
1	0.16 mg., 1-C^{14}	....	8.7	1,280
	0.16 mg., 1-C^{14}	80 units[a] concentrate	8.7	570
2	0.10 mg., 1-C^{14}	....	1.15	4,800
	0.10 mg., 1-C^{14}	0.92 mg., synthetic, non-labeled	1.15	2,180
	0.10 mg. non-labeled	0.92 mg., 2-C^{14}	1.15	28,400

[a] One unit of activity has since been shown to be equivalent to 0.010 mg. of DL synthetic material.

TABLE II

ACTIVITY OF HMG, DMA, AND β-HYDROXY-β-METHYL-δ-VALEROLACTONE (DVA) IN CHOLESTEROL BIOSYNTHESIS BY RAT LIVER HOMOGENATES

Protocol as in Table I. All flasks contained aliquots from one pool of liver homogenate. "Corrected" values are calculated to DVA as standard. Each compound was added at the level of 6 μM. per flask. Total cholesterol estimated by a modification of the method of Abell, *et al.*[11]

Substrates	Activity added total c.p.m. × 10^4	C^{14} found c.p.m./mg. C.	Cholesterol recovered C^{14} corrected c.p.m./mg. C	Total mg./flask
3′-C^{14}-HMG	33.0	400	139	1.61
4-C^{14}-DMA	4.9	1,350	3,170	1.64
2-C^{14}-DVA	11.5	33,700	33,700	1.77

These data indicate that only 0.16% of the isotope of 3′-C^{14}-HMG and 3.8% of the radioactivity of 4-C^{14}-DMA are incorporated into cholesterol. In contrast, 43.4% of the isotope of 2-C^{14}-DVA appears in the sterol. If the utilization by liver tissue is restricted to only one of the optical isomers of synthetic factor (as is the case with *Lactobacillus acidophilus*), then it appears that virtually all of the biologically available DVA-C^{14} must have been transferred to sterol. It has been reported that while some HMG can be utilized *in toto* for the synthesis of β-hydroxyisovaleric acid (and presumably cholesterol), a considerable amount of HMG undergoes cleavage to acetate and acetoacetate.[12,13] On the other hand, the great extent of conversion of the C^{14} carbon of DVA into cholesterol suggests that the major pathway of cholesterol biosynthesis from this compound is direct, as opposed to cleavage to smaller molecules.

It thus appears that β-hydroxy-β-methyl-δ-va-

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(13) J. L. Rabinowitz, *ibid.*, **77**, 1295 (1955).