TABLE I

ENZYMATIC SYNTHESIS OF SPERMIDINE FROM 1,4-C^{14}-PUTRESCINE

The complete system contained C^{14}-putrescine dihydrochloride[a] (0.12 μmole, 8,000 c.p.m.), L-methionine (1 μmole), ATP (2.5 μmoles), $MgSO_4$ (30 μmoles), *E. coli* enzyme (10 mg. of an ammonium sulfate fraction), and triethanolamine buffer (*p*H 8.1) in a final volume of 0.3 ml. Incubation time was 3 hr. at 37°. Spermidine was isolated by Dowex 50 (H) and Amberlite XE-64 (H) chromatography by a modification of the methods previously reported (ref. 1).

	Spermidine,[b] c.p.m.
Complete system (C^{14}-putrescine, ATP, Mg^{++}, L-methionine, enzyme)	5070
No ATP	140
No Mg^{++}	220
No L-methionine	160
D-Methionine[c] instead of L-methionine	120
Compound I instead of ATP and L-methionine[d]	5700
Adenosyl-L-methionine instead of ATP and L-methionine[d]	6460

[a] Synthesized by catalytic reduction (in ethanol–H_2SO_4) of C^{14}-succinonitrile, formed from $NaC^{14}N$ and bromopropionitrile. We wish to thank Drs. H. Bauer and E. May for help with these syntheses. [b] For convenience isotopic assays usually were used. In other experiments the spermidine formed was assayed with dinitrofluorobenzene, demonstrating net synthesis. Further identification of the spermidine formed was attained by rechromatography and by recrystallization, with carrier, to constant specific activity. [c] 1 μmole of D-methionine. [d] The incubation mixtures in these two experiments contained buffer, $MgSO_4$, and enzyme (as indicated above), 0.12 μmole of C^{14}-putrescine (8,000 c.p.m.), and 0.17 μmole of compound I or adenosyl-L-methionine in a final volume of 0.3 ml. Control incubation mixtures without enzyme showed no synthesis of spermidine.

TABLE II

DECARBOXYLATION OF C^{14}OOH-DL-METHIONINE AND OF C^{14}OOH-COMPOUND I

The complete system in experiment A contained putrescine (0.12 μmole), $MgSO_4$ (30 μmoles), *E. coli* enzyme (5 mg. of an ammonium sulfate fraction), ATP (2.5 μmoles), triethanolamine buffer (*p*H 8.1), and C^{14}OOH-DL-methionine in a final volume of 0.3 ml. The complete system in experiment B contained the same additions of $MgSO_4$, enzyme and buffer plus C^{14}OOH-compound I in a final volume of 0.3 ml. Incubation time was 45 minutes at 37°.

	$C^{14}O_2$, c.p.m.
A. With C^{14}OOH-DL-methionine[a] (0.13 μmole, 40,000 c.p.m.)	
Complete system (putrescine, ATP, Mg^{++}, C^{14}OOH-methionine, enzyme)	1044
No putrescine	1146
No ATP	30
No Mg^{++}	24
Complete system + NaCN (3 μmoles)[b]	54
B. With C^{14}OOH-compound I (0.003 μmole, 800 c.p.m.)	
Complete system (Mg^{++}, C^{14}OOH-compound I, enzyme)	378
No Mg^{++}	98

[a] C^{14}OOH-DL-methionine was prepared by a modification of the non-isotopic synthesis of R. Gaudry and G. Nadeau, *Can. J. Research* **26B**, 226 (1948). [b] In the presence of NaCN compound I accumulated. Larger amounts of compound I were made with 2.6 μmoles of C^{14}OOH-DL-methionine (700,000 c.p.m.), 50 μmoles of ATP, 600 μmoles of $MgSO_4$, 60 moles of NaCN, *E. coli* enzyme (100 mg. of ammonium sulfate fraction) in a volume of 6 ml. Compound I was purified by Dowex 50 (H) or XE 64 (H) chromatography, or by paper chromatography.

(2) In the second step compound I undergoes enzymatic decarboxylation (Table II) in the presence of Mg^{++}. ATP is not necessary for this reaction, although, for the formation of $C^{14}O_2$ from C^{14}OOH-labeled methionine, both ATP and Mg^{++} are essential. 0.01 *M* NaCN markedly inhibits decarboxylation; putrescine is not required. Compound II has been partially purified by chromatography, but has not been separated from compound I.

(3) Either compound I or adenosylmethionine,[5] in the presence of putrescine and the enzyme, forms spermidine in the absence of ATP or methionine (Table I). The remainder of the molecule would be expected to form thiomethyladenosine[6]; some preliminary evidence for a product of this type has been obtained.

Experiments with intact *E. coli* demonstrate that the synthesis of spermidine represents an important pathway for the metabolism of methionine. A three-carbon transfer is involved, comparable to the methyl-transfer of Cantoni. Further work is in progress on the purification of the respective enzymes and products, and on the role of other substrates as three-carbon donors and acceptors.

(5) S-Adenosyl-L-methionine was prepared according to the method of Cantoni (ref. 4), using ATP, L-methionine, and a rabbit liver enzyme.

(6) F. Schlenk and R. L. Smith, *J. Biol. Chem.*, **204**, 27 (1953).

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH — HERBERT TABOR
PUBLIC HEALTH SERVICE — SANFORD M. ROSENTHAL
U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
BETHESDA, MARYLAND — CELIA W. TABOR

RECEIVED MAY 1, 1957

THE CRYSTAL STRUCTURE OF SODIUM PEROXIDE

Sir:

From single-crystal X-ray diffraction work on sodium peroxide, a unit cell has been deduced which indexes the powder pattern more satisfactorily than does the unit cell reported in the literature.[1] A single crystal of Na_2O_2 was found among the fragments from a cooled melt of high purity Na_2O_2 contained in a crystalline MgO crucible. From the Laue patterns taken it can be shown that sodium peroxide is hexagonal, belonging to Laue group 6/mmm. Single crystal rotation patterns and powder patterns taken with Cu Kα X-radiation yield the repeat distances $a = 6.22 \pm 0.01$ Å. and $c = 4.47 \pm 0.01$ Å. Interplanar spacings calculated from these dimensions agree well with those observed on a powder pattern taken with a camera of 114.6 mm. radius.

Since no systematic absences are found in the reflections, the space groups P622, P6mm, P$\bar{6}$2m, P$\bar{6}$m2 and P6/mmm remain as possibilities, from those having Laue symmetry 6/mmm. Density measurements on Na_2O_2(2.2 to 2.8 g./cc.) favor a unit cell containing three formula units (Na_2O_2), which gives a calculated density of 2.60 g./cc. Spatial considerations favor only the structure (of

(1) F. Feher, *Angew. Chem.*, **51**, 497 (1938).

symmetry $P\bar{6}2m$) with the following approximate positions: Oxygen: $0,0,p$; $0,0,-p$; $1/3,2/3,q$; $2/3,1/3,q$; $2/3,1/3,-q$; Sodium: $r,0,1/2$; $0,r,1/2$; $-r,-r,1/2$; $s,0,0$; $0,s,0$; $-s,-s,0$; where $p = 0.167$, $q = 0.333$, $r = 0.724$, $s = 0.366$. Intensities calculated from these parameters are consistent with estimated powder and rotation intensities.

This structure has been deduced independently by Klemm and Foppl.[2]

In this structure, the peroxide ions lie parallel to the c-direction, one at the corner and two within the unit cell. Each peroxide ion is in contact with nine sodium ions, three about the middle and three about each end. A sodium ion of 0.98 Å. radius, and a peroxide ion regarded as a cylinder of radius 1.29 Å. and length 1.49 Å. with caps of radius 1.29 Å. are consistent with this structure and with other crystallographic data.[3,4]

There appears to be no direct correlation between this hexagonal unit cell and the tetragonal unit cell reported by Feher[1] with dimensions $a = 6.65$ Å., $c = 9.91$ Å., and $Z = 8$. The fact that the ASTM Index lists for Na_2O_2 at least 15 powder lines (of the 29 listed) either not observed or of significantly greater intensity than observed for pure Na_2O_2 is readily explained as due to the presence of impurities, principally NaOH, in the material which gave the ASTM pattern.

We wish to acknowledge the support of a du Pont Summer Fellowship (R.L.T.) and of funds from the Wisconsin Alumni Research Foundation in this work.

(2) W. Klemm, private communication, 1957.
(3) S. C. Abrahams and J. Kalnajs, *Acta Cryst.*, **7**, 838 (1954).
(4) T. Moeller, "Inorganic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1952, p. 140.

DEPARTMENT OF CHEMISTRY
DEPARTMENT OF GEOLOGY — RICHARD L. TALLMAN
UNIVERSITY OF WISCONSIN — JOHN L. MARGRAVE
MADISON, WISCONSIN — STURGES W. BAILEY

RECEIVED APRIL 22, 1957

ACETOLACTATE, AN EARLY INTERMEDIATE IN VALINE BIOSYNTHESIS[1]

Sir:

Strassman, *et al.*,[2,3] and Adelberg[4] have proposed schemes for the conversion of pyruvate to L-valine and of α-ketobutyrate to L-isoleucine. Each scheme involved a condensation of acetaldehyde and the appropriate keto acid followed by a pinacol rearrangement to account for the distribution of C^{14} in tracer studies. As pointed out by Adelberg,[4] the first step in L-valine biosynthesis might be a ketol (as first proposed by Strassman, *et al.*[2]) or an aldol condensation, since either reaction would be consistent with the data. Evidence that the initial step is acetolactate formation (ketol condensation) is presented here.

A valineless mutant of *Escherichia coli*, strain M4862,[5] accumulates α-acetolactic acid when grown in media supplemented with limiting amounts of L-valine. Extracts prepared by sonic oscillation of cells of this mutant, harvested when acetolactate accumulation was marked, were observed to produce at least ten times as much α-acetolactate from pyruvate as did an extract of the parent *E. coli* strain W (Table I, Expt. 1). The formation of acetolactate was inhibited by L-valine.

A similar valine sensitive system was also formed by a wild type *Aerobacter aerogenes*, strain 1033, when grown in minimal medium containing L-histidine as the sole carbon source (chosen so as to assure absence of the classical carboligase[6] system). The same strain grown in nutrient broth with 1% glucose exhibits virtually no carboligase activity at *p*H 8 but is extremely active when tested at *p*H 6 (Table I, Expt. 2). L-Valine does not inhibit this enzyme.

TABLE I

ACETOLACTATE FORMATION BY BACTERIAL EXTRACTS

		Moles/10 min. Without L-valine		With L-valine	
Expt.	Extract	Acetoin	Aceto-lactate	Acetoin	Aceto-lactate
1	*E. coli* Strain W *p*H 8.0	0.04	0.14	..	..
	E. coli Strain M4862 *p*H 8.0	.05	1.74	0.01	0.28
2	*A. aerogenes* 1033 *p*H 8.0	<0.01	<0.01	<0.01	<0.01
	(Broth grown) *p*H 6.0	1.47	<0.01	1.52	<0.01
	A. aerogenes 1033 *p*H 8.0	<0.01	.06	<0.01	<0.01
	(Histidine grown) *p*H 6.0	<0.01	<0.01	<0.01	<0.01

Assay system contained 10^{-2} M pyruvate, 20 μg. cocarboxylase, 2×10^{-3} M Mg^{++} and cell-free extract equivalent to 25 mg. wet weight of cells in one ml. 0.1 M phosphate buffer. Ten minutes incubation at 37° in air. Reaction stopped with $Zn(OH)_2$. Acetoin and acetolactate determined by method of Westerfeld[7] before and after decarboxylation[6] of β-ketoacids with 1.8 N H_2SO_4. In a separate experiment the acetolactate was isolated and converted to the bis-phenylhydrazone of diacetyl. The melting point was 258–259° (uncorrected) and there was no depression upon mixing with the same derivative prepared from synthetic α-acetolactic acid.

If acetolactate is an intermediate in L-valine biosynthesis, the inhibition of its formation by L-valine could be the feedback loop by which L-valine controls its own biosynthesis.[8] That acetolactate is very likely on the pathway in question is indicated by the following observations: (1) acetolactate labeled in the β-methyl position was incorporated into L-valine and L-leucine of *E. coli* pro-

(1) Supported by Grant RG-4015 of the United States Public Health Service and by funds from the Eugene Higgins Trust.
(2) M. Strassman, A. J. Thomas and S. Weinhouse, THIS JOURNAL, **75**, 5135 (1953).
(3) M. Strassman, A. J. Thomas, L. A. Locke and S. Weinhouse, *ibid.*, **76**, 424 (1954).
(4) E. A. Adelberg, *ibid.*, **76**, 4241 (1954).
(5) H. E. Umbarger and E. A. Adelberg, *J. Biol. Chem.*, **192**, 883 (1951).
(6) E. Juni, *ibid.*, **195**, 715 (1952).
(7) W. W. Westerfeld, *J. Biol. Chem.*, **161**, 495 (1945).
(8) E. A. Adelberg and H. E. Umbarger, *ibid.*, **205**, 475 (1953).