Table I shows that most aliphatic solvents have little effect upon the position of attack of a chlorine atom on 2,3-dimethylbutane. However, in the presence of aromatic solvents the chlorine atom is directed toward the tertiary hydrogen atoms, the effect increasing with the basicity of the aromatic hydrocarbon[7] and with a decrease in temperature.

Figure 1 demonstrates the effects of *t*-butylbenzene, benzene, and chlorobenzene on the relative reactivities of the tertiary and primary-hydrogen atoms of 2,3-dimethylbutane. Qualitatively, it is

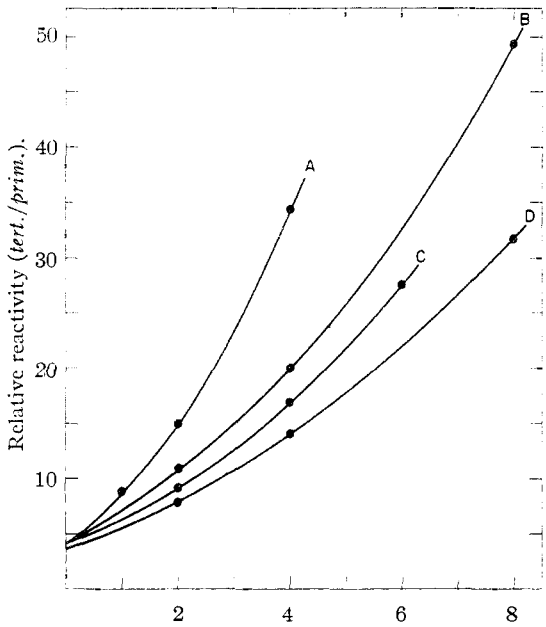
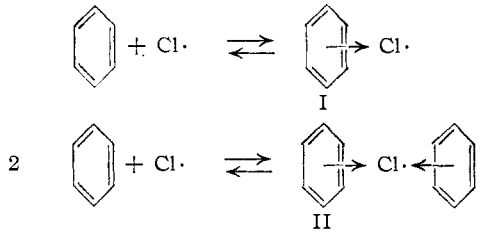


Fig. 1.—Relative reactivities of hydrogen atoms of 2,3-dimethylbutane in the presence of aromatic hydrocarbons: A, *t*-butylbenzene, 25°; B, benzene, 25°; C, chlorobenzene, 25°; D, benzene, 55°.

obvious that the attacking species is very selective at high concentrations of aromatic hydrocarbon. Quantitatively, the curves in Figure 1 fit the expression

$$\text{Rel. react. } (tert./prim.) = k + k'[\text{Ar}] + k''[\text{Ar}]^2$$

where Ar represents the aromatic hydrocarbon. An expression of this type can be derived if it is assumed that there are three types of chlorine atoms in an aromatic solvent, a free chlorine atom, I and II, and if I and II can attack only the *tert.*-hydrogen atoms.



The conclusion reached in regard to the complexing of free radicals by aromatic solvents calls for reappraisal of many homolytic substitutions, particularly those involving competition reactions. Complexing may also be important in other homolytic reactions, such as vinyl polymerization.[3,8] The study of solvent effects in free radical reactions is being extended to other solvents, substrates and free radicals.

(7) H. C. Brown and J. D. Brady, THIS JOURNAL, **74**, 3570 (1952).

(8) W. H. Stockmeyer and L. H. Peebles, Jr., *ibid.*, **75**, 2278 (1953); G. M. Burnett and H. W. Melville, *Disc. Faraday Soc.*, **2**, 322 (1947).

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THE ROLE OF PUTRESCINE AND METHIONINE IN THE ENZYMATIC SYNTHESIS OF SPERMIDINE IN *ESCHERICHIA COLI* EXTRACTS

Sir:

The polyamines, spermidine and spermine, are widely distributed in natural materials, but little is known of their biosynthesis.[1] Previous isotopic work from this laboratory, with growing cultures of *Escherichia coli*, *Aspergillus nidulans*, and *Neurospora crassa*, has indicated that putrescine[2] and methionine[3] supplied the four- and three-carbon moieties, respectively, of the polyamines.

We are now able to demonstrate the synthesis of spermidine $[NH_2(CH_2)_3NH(CH_2)_4NH_2]$ from putrescine $[NH_2(CH_2)_4NH_2]$ in cell-free extracts of *E. coli*. These preparations have been partially purified, and the reaction has been shown to require L-methionine, adenosine triphosphate (ATP), and Mg^{++} (Table I). The isotope of 1,4-C^{14}-putrescine and of 2-C^{14}-methionine, but not of $C^{14}H_3$-methionine or of $C^{14}OOH$-methionine, was incorporated into spermidine.

Preliminary data indicate that three steps are involved in this reaction

$$\text{ATP} + \text{L-methionine} \xrightarrow{Mg^{++}} \text{compound I} \xrightarrow[-CO_2]{Mg^{++}}$$

$$\text{compound II} \xrightarrow{\text{putrescine}} \text{spermidine}$$

(1) In the first step ATP and methionine form compound I. In the presence of 0.01 *M* NaCN this accumulates, and can be purified by chromatography. On the basis of preliminary data, compound I is believed to be adenosylmethionine. It contains approximately one equivalent of methionine (as shown by the incorporation, in separate experiments, of the isotope from 2-C^{14}-methionine, $C^{14}H_3$-methionine, and $C^{14}OOH$-methionine), one equivalent of adenine (absorption maximum at 260 mμ) and no phosphorus. The R_f of compound I is very similar to that reported[4] for adenosylmethionine [0.12 in ethanol 80, HAc 5, H_2O 15; 0.07 in butanol–acetic acid–H_2O]. Compound I can serve as a methyl donor with nicotinamide methylpherase[4] [60% yield], in the absence of ATP, Mg^{++}, and methionine.

(1) For a literature review see: S. M. Rosenthal and C. W. Tabor, *J. Pharmacol. Exp. Therap.*, **116**, 131 (1956).

(2) H. Tabor, S. M. Rosenthal and C. W. Tabor, *Federation Proc.*, **15**, 367 (1956).

(3) R. C. Greene, *ibid.*, **16**, 189 (1957).

(4) G. Cantoni, *J. Biol. Chem.*, **204**, 403 (1953); G. Cantoni in "Methods in Enzymology," S. P. Colowick and N. O. Kaplan (eds.), Academic Press Inc., New York, N.Y., Vol. II, 254, 257 (1955); Vol. III, 600 (1957).

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).

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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).