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 COMMUNICATIONS TO THE EDITOR
 

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**THE FORMATION OF S-ADENOSYLHOMOCYSTEINE IN ENZYMIC TRANSMETHYLATION REACTIONS<sup>1</sup>**
*Sir:*

It has been assumed for many years that homocysteine might be formed biologically in transmethylation reactions from methionine.<sup>2</sup> Recent studies have shown that enzymatic activation is a prerequisite to the transfer of the methyl group of methionine.<sup>3</sup> The product of this enzymatic activation is S-adenosylmethionine (AMe).<sup>4,5</sup> It follows, therefore, that adenosylhomocysteine, S-(5'-desoxy-adenosine-5')-homocysteine, rather than homocysteine should be the primary product resulting from transmethylation involving methionine.<sup>6,7</sup> This deduction has now been verified experimentally and the present communication describes the enzymatic preparation of adenosylhomocysteine.

The enzyme GA methylpherase,<sup>8</sup> which catalyzes the reaction



was used for the preparation of adenosylhomocysteine. Guanidinoacetic acid (120  $\mu\text{m}$ .) and AMe (90  $\mu\text{m}$ .) labeled with S<sup>35</sup> were incubated for 150 minutes in phosphate buffer (0.05 M, pH 7.4) and BAL (0.002 M) with 70 units (80 mg.) of a partially purified preparation of GA methylpherase. The incubation was terminated by addition of trichloroacetic acid. Unreacted AMe was removed from the protein filtrate by precipitation with ammonium reineckate. After removal of the excess reineckate, the supernatant containing ASR, was made 0.02N with respect to HCl and passed through a small column of Norite A (500 mg.); the Norite was then washed with 100 ml. of water. The filtrate and washings which contained no radioactivity were discarded. The Norite column was then eluted with four 50-ml. portions of aqueous pyridine (10%). The first two eluates, which contained over 90% of the total counts, were pooled, freed from pyridine and concentrated under reduced pressure. This material in addition to ASR contained creatinine, guanidinoacetic acid and traces of pyridine. These contaminants were removed by descending chromatography on Whatman no. 1 paper (solvent system EtOH, acetic and water, 75:5:20).

(1) Aided by grants from the Williams-Waterman Fund and the American Cancer Society.

(2) L. W. Butz and V. du Vigneaud, *J. Biol. Chem.*, **99**, 135 (1932).

(3) G. L. Cantoni, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1952, Vol. 2, p. 129.

(4) These abbreviations will be used: AMe for S-adenosylmethionine, *i.e.*, active methionine; ASR, S-adenosylhomocysteine; ATP; adenosinetriphosphate; GSH, reduced glutathione, GA, guanidinoacetic acid; BAL, British Anti-Lewisite, *i.e.*, Dimercaptopropanol.

(5) G. L. Cantoni, *J. Biol. Chem.*, **204**, 403 (1953).

(6) J. Baddiley, G. L. Cantoni and G. A. Jamieson, *J. Chem. Soc.*, 2662 (1953).

(7) D. W. Woolley, *Nature*, **171**, 323 (1953).

(8) G. L. Cantoni and P. T. Vignos, Jr., *J. Biol. Chem.*, **209**, 647 (1954).

Examination of the chromatogram revealed only one area exhibiting radioactivity, ultraviolet absorbency and ninhydrin positive reaction. This area was cut out, and eluted with water. On the basis of adenine content 29  $\mu\text{m}$ . of purified ASR was obtained. The purified material behaved as a single substance when chromatographed with several different solvents; it had an ultraviolet absorption spectrum characteristic for adenine nucleosides, with a maximum at 260 m $\mu$ ; for each mole of adenine it contained one mole of pentose; it gave a positive reaction with the ninhydrin test and with the nitroprusside test for methionine.<sup>9</sup> Finally, the purified material was found to be identical with synthetic adenosylhomocysteine, the preparation of which will be described in a separate communication.<sup>10</sup>

The behavior of ASR in various biological systems is under investigation in this laboratory.

DEPARTMENT OF PHARMACOLOGY  
WESTERN RESERVE UNIVERSITY  
SCHOOL OF MEDICINE  
CLEVELAND, OHIO

G. L. CANTONI  
EDUARDO SCARANO<sup>11</sup>

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(9) T. E. Mcarthy and M. X. Sullivan, *ibid.*, **141**, 871 (1941).

(10) J. Baddiley and G. A. Jamieson, in press.

(11) Fulbright Fellow.

**FLAVIN MONONUCLEOTIDE-DEPENDENT MENADI-  
ONE REDUCTASE COUPLED TO CYTOCHROME C  
REDUCTION IN CELL-FREE EXTRACTS OF *Achromobacter fischeri*<sup>1</sup>**
*Sir:*

A menadione reductase linked to DPN<sup>2</sup> has been demonstrated by Wosilait and Nason<sup>3</sup> in cell-free extracts of *E. coli* and other organisms including *Achromobacter fischeri*. These workers were unable to demonstrate a flavin requirement with their system. A requirement for FAD or FMN in the menadione reductase system of cell-free extracts of *Streptococcus faecalis* has been demonstrated by M. I. Dolin (personal communication). We observed that both cytochrome c and menadione in small amounts will inhibit cell-free bacterial luminescence, the results suggesting that menadione might mediate hydrogen transfer between flavoprotein and cytochrome c. Since FMNH<sub>2</sub> is required for bacterial luminescence,<sup>4,5</sup> the nature of cytochrome c and menadione inhibition could be a competition with the luminescent pathway for the hydrogens of the flavoprotein. The function of menadione as proposed would be important because of the vitamin K

(1) Work performed under United States Atomic Energy Commission Contract No. W-7405-eng-26.

(2) DPN = diphosphopyridine nucleotide; FAD = flavin adenine dinucleotide; FMN = flavin mononucleotide; FMNH<sub>2</sub> = reduced flavin mononucleotide; BAL = British antilewisite (2,3-dimercaptopropanol).

(3) W. D. Wosilait and A. Nason, *J. Biol. Chem.*, **203**, 785 (1954).

(4) B. L. Strehler, E. N. Harvey, J. J. Chang and M. J. Cormier, *Proc. Natl. Acad. Sci. U.S.A.*, **40**, 19 (1954).

(5) W. D. McElroy, J. W. Hastings, V. Sonnenfeld and J. Coulombe, *Science*, **118**, 385 (1953).