It is not easy to anticipate whether the balance between quantum-mechanical delocalization energy and the compression energy necessary to force classical structures (like the "Kekule" structures of type X) into the same geometry will indeed place species VI–VIII in the category of homo-aromatic mesomeric compounds. While quantum-mechanical delocalization energies in cases VI–VIII will be much less than in cases III–V, we probably can expect compression energies to be less also. The balance between compression energy and quantum-mechanical delocalization energy in cases VI–VIII appears to be sufficiently analogous to the one for I, that the observed results with I warrant experiments to test for the occurrence of homo-aromatic structures VI–VIII. These are being undertaken.

Examples of homoconjugation have been discussed previously, such as the homoallylic[3a] cation, the *anti*-7-norbornenyl cation[3b] (termed a bishomocyclopropenyl cation by Roberts[3c]) and the "planar pseudo-aromatic structure" for tropilidene visualized by Doering.[4] The latter example could be called monohomobenzene. Cases VI–VIII discussed above differ from these by having complete equivalence of the classical contributing structures. Perhaps "perhomo-aromatic" is a useful term for VI–VIII.

(3) (a) *E.g.*, M. Simonetta and S. Winstein, THIS JOURNAL, **76**, 18 (1954); (b) S. Winstein, M. Shatavsky, C. Norton and R. B. Woodward, *ibid.*, **77**, 4183 (1955); (c) W. G. Woods, R. A. Carboni and J. D. Roberts, *ibid.*, **78**, 5653 (1956).

(4) W. E. Doering, *et al.*, *ibid.*, **78**, 5448 (1956).

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REQUIREMENT FOR FLAVIN COENZYME IN THE ENZYMATIC SYNTHESIS OF METHIONINE IN VITRO[1]

Sir:

Several investigators have studied the biosynthesis of the methyl group of methionine in cell-free systems with serine or formaldehyde as donors and homocysteine as acceptor of the one-carbon fragment.[2-6] In extracts of certain mutants of *Escherichia coli*, cofactor requirements were shown previously for pyridoxal phosphate, tetrahydrofolic acid, adenosine triphosphate, DPNH[7] and vitamin B_{12}.[2,3,8] Recently in this laboratory a requirement for FAD or FMN has been demonstrated.

The effect of the flavin coenzymes has been shown with a system consisting of these three purified enzyme fractions obtained from *E. coli* mutant 113-3,[9] which requires vitamin B_{12} or methionine for growth: (1) serine hydroxymethylase, (2) a vitamin B_{12}-containing enzyme[3,8] and (3) a fraction partially purified by ammonium sulfate precipitation and chromatography on adsorbents. The vitamin B_{12}-containing enzyme was purified about 60-fold by means of ammonium sulfate fractionation and then by adsorption and elution from calcium phosphate gel and by chromatography on diethylaminoethyl cellulose and hydroxylapatite.

The enzymatic system carried out methionine synthesis in the presence of the protein fractions and all of the indicated cofactors (Table I). The addition of reduced flavin compounds to incubation mixtures obviated the requirement for DPNH when incubation was carried out under hydrogen.

TABLE I

All vessels contained per ml.: potassium phosphate buffer, *p*H 7.2, 50–100 μmoles; L-serine, 5–10 μmoles; L-homocysteine, 10 μmoles; pyridoxal phosphate, 0.25 μmole; adenosine triphosphate, 5 μmoles; Mg^{++}, 10 μmoles; tetrahydrofolic acid, 0.5 μmole; serine hydroxymethylase; B_{12}-containing enzyme; and third enzyme fraction. To this basic system these additions were made when indicated: DPNH, 2 μmoles; oxidized FAD, 0.16 μmole; reduced FAD or FMN (catalytic hydrogenation with 30% palladium on charcoal), 0.2 μmole. Incubation was for 2 or 3 hours at 37°. Methionine was assayed microbiologically with *Leuconostoc mesenteroides* P 60.

Expt.	Additions	Gas phase	Methionine synthesized mμmoles
A	DPNH, oxidized FAD	N_2	71
	DPNH	N_2	11
	None	N_2	7
B	Reduced FMN	H_2	592
	Reduced FAD	H_2	388
	Filtered catalyst suspension (without flavin compounds)	H_2	42
C	Reduced FMN	H_2	236
	Reduced FMN	He	34

It is believed that the high values for methionine synthesis obtained were due to regeneration of reduced flavin by hydrogen gas catalyzed by traces of palladium which escaped filtration. Incubation under helium resulted in very little methionine formation. These preliminary results suggest that the requirement for pyridine dinucleotide in methionine biosynthesis can be explained by its role in reducing the flavin component of the system.

(1) This work was supported in part by grants from the National Science Foundation and the Greater Boston Chapter of the Massachusetts Heart Association.

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(7) Abbreviations used are: DPNH, reduced diphosphopyridine nucleotide; FAD, flavin adenine dinucleotide; FMN, riboflavin-5-phosphate.

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(9) B. D. Davis and E. S. Mingioli, *J. Bacteriol.*, **60**, 17 (1950).

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IDENTIFICATION OF A STEROL WITH ACRASIN ACTIVITY IN A SLIME MOLD

Sir:

Acrasin is a chemotactic hormone produced by individual amoeboid cells of the slime mold, *Dictyostelium discoideum*.[1,2,3] In response to a

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(3) B. M. Shaffer, *Nature*, **171**, 957 (1953).