METHYLATION OF GUANIDOACETIC ACID BY HOMOCYSTINE PLUS CHOLINE WITH RAT LIVER SLICES

Sirs:

The methylation of guanidoacetic acid by liver slices is accelerated by methionine; choline, under these conditions, exerts no significant accelerating effect.[1] In view of the fact that homocystine plus choline can replace methionine for growth,[2] and of the isotope experiments which proved the transfer *in vivo* of the methyl groups of choline to creatine,[3] it has been suggested, from indirect evidence, that the pathway of the methyl group to creatine is more direct from methionine than from choline.[4] More specific evidence is desirable, especially as neither homocystine nor homocysteine has been identified in animal tissues.

In experiments with rat liver slices designed to obtain such evidence it was found that *dl*-homocystine plus choline accelerates the methylation of guanidoacetic acid as effectively as does *dl*-methionine. Homocystine is ineffective without choline, as is choline without homocystine.

dl-Homocystine and choline are more effective than *dl*-homocysteine and choline.[5]

These observations are the first, as far as we are aware, on the utilization, outside the whole animal, of the methyl groups of choline in the formation of creatine.

They furnish direct evidence that homocystine can function as a carrier of the methyl groups of choline in the methylation of guanidoacetic acid; and as it is more effective in this respect than homocysteine, the actual carrier is probably closer to homocystine than to homocysteine. The occurrence of homocystine or homocysteine *in vivo* remains to be demonstrated.

The above observations do not answer the question whether the formation of methionine is an obligatory antecedent to the methylation of guanidoacetic acid by homocystine plus choline. The immediate methyl donor to guanidoacetic acid may be methionine or a derivative of it or of methylated homocystine. We hold this question open because of observations (unpublished) on the inhibition of the methylation of guanidoacetic acid by oxidation inhibitors, *e.g.* KCN, As_2O_3, and As_2O_5.

[1] Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **132**, 559 (1940).

[2] du Vigneaud, V., Chandler, J. P., Moyer, A. W., and Keppel, D. M., *J. Biol. Chem.*, **131**, 57 (1939).

[3] Simmonds, S., Cohn, M., Chandler, J. P., and du Vigneaud, V., *J. Biol. Chem.*, **149**, 519 (1943).

[4] du Vigneaud, V., *Harvey Lectures*, **38**, 39 (1942–43).

[5] We wish to thank Professor Vincent du Vigneaud for his generous gift of the *dl*-homocystine and *dl*-homocysteine used in these experiments.

Methylation of Guanidoacetic Acid by Rat Liver Slices

The results are expressed as $Q_{creatine} \times 100$. All solutions contained 1.5 mg. per cent of guanidoacetic acid.

Experiment No.	Guanidoacetic acid alone	Guanidoacetic acid plus					
		dl-Methionine (6.25 mg. per cent)	*dl*-Homocystine (6.25 mg. per cent)	*dl*-Homocysteine (6.25 mg. per cent)	Choline chloride (39 mg. per cent)	*dl*-Homocystine (6.25 mg. per cent) + choline chloride (39 mg. per cent)	*dl*-Homocysteine (6.25 mg. per cent) + choline chloride (39 mg. per cent)
1	1.45	5.28	1.85	2.04	2.04	5.77	2.68
2	1.72	3.19	1.61		1.87	3.46	1.50
3	1.06	3.00	0.93	0.90	1.23	2.66	1.96
4	0.39	2.01	0.40		0.32	2.46	0.80

The table summarizes some typical results. The values in the appropriate controls are omitted.

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