THE ENZYMIC N-METHYLATION OF GLYCINE*

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Received August 24, 1960

The biological oxidation of the CH_3-group of methionine has been studied extensively (for bibliography see du Vigneaud, 1952). It has been suggested that this oxidation takes place via sarcosine (Horner and Mackenzie, 1950). The currently accepted belief (Mackenzie and Frisell, 1958) presumes that sarcosine is formed in a pathway involving the successive methylation of ethanolamine to choline, the oxidation of choline to betaine and the successive demethylation of this last compound. The second and third demethylations (of dimethylglycine to sarcosine and of sarcosine to glycine) are catalysed by specific dehydrogenases which simultaneously bring about the oxidation of the methyl group to "active C_1" fragments, which may in turn be further oxidised to CO_2. The importance of this pathway has never been clearly demonstrated and its quantitative significance is made dubious by two considerations. First, C^{14} from the CH_3-group of methionine appears as CO_2 more rapidly than that from the CH_3-group of choline or betaine (du Vigneaud, 1952, table xxii).

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* This work was supported by a research grant (A-3523 Nutr.) from the National Institute of Arthritis and Metabolic Disease, United States Public Health Service.

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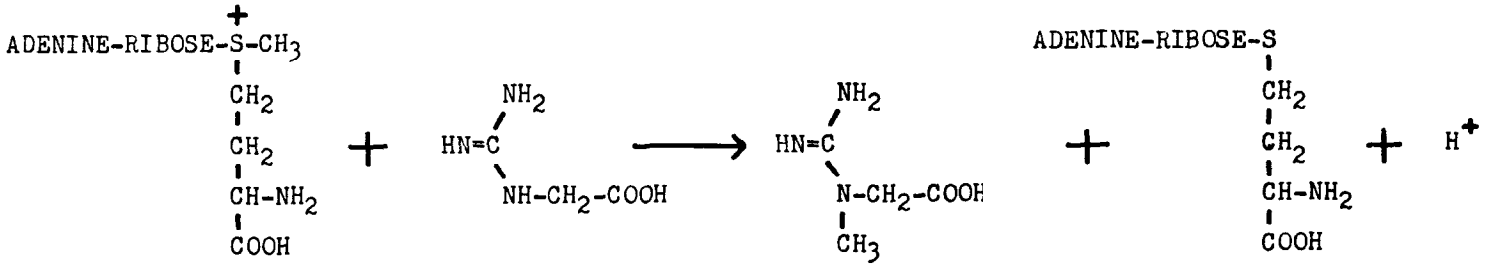
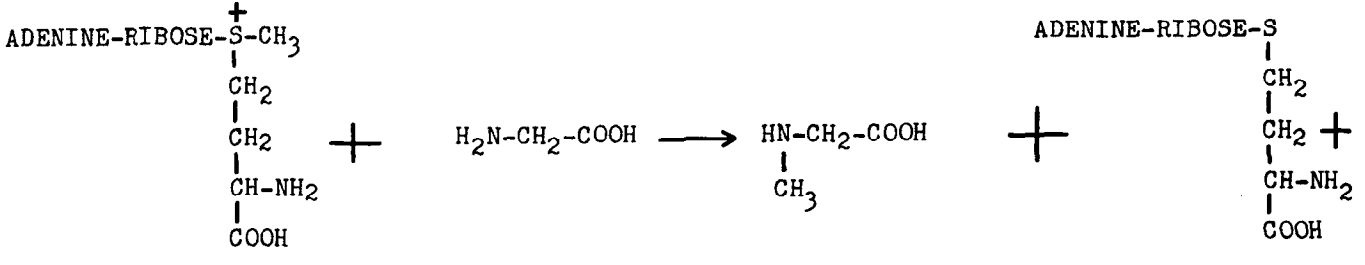


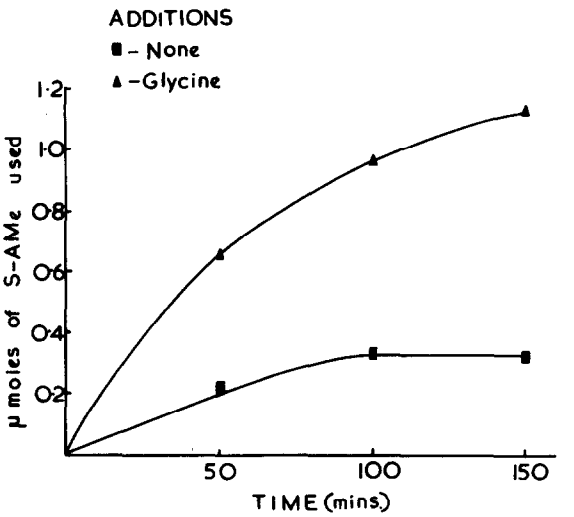
EQUATION I



EQUATION II

Second, recent studies on the biosynthesis of choline (Bremer and Greenberg, 1960) suggest that this pathway of sarcosine biosythesis and hence of methionine CH_3-oxidation would be very circuitous indeed.

The well known N-methylation of guanidoacetic acid (ureidoglycine) (Cantoni and Vignos, 1954) suggests another mechanism for the formation of sarcosine, namely, the N-methylation of glycine itself (compare Equations 1 and 2). This possibility has been explored by incubating S-adenosylmethionine with the dialysed supernatant fraction of guinea-pig liver homogenate. (The dialysis is necessary because of the large endogenous breakdown of S-adenosylmethionine in undialysed guinea-pig liver supernatant. (Blumenstein and Williams, 1960)). The disappearance of S-adenosylmethionine (measured according to Mudd, 1959) from such a system is greatly enhanced by the addition of glycine (Fig. 1).



The test system used contains 130 μmoles of tris buffer pH 8.0. 2.0 μmoles of S-adenosylmethionine prepared according to Stekol, Anderson and Weiss (1958), 20 μmoles of glycine (omitted from controls) and 1.0 ml. of dialysed supernatant from a homogenate of 40 gms. guinea pig liver in 60 ml. of 0.25 M sucrose, 0.02 M tris, pH 8.0. The final volume was 2.5 ml. Incubations were carried out at 38°C.

That the product of this reaction is indeed sarcosine is strongly supported by the following experiment: 2 μmoles of S-adenosylmethionine were incubated as above for 3 hours with 1 μmole of glycine-2-C^{14}. After deproteinization at 100°C for 10 minutes, the products were chromatographed on paper as described by Mackenzie and Frisell (1958). Only two radioactive spots were observed by radioautography, one corresponding to unchanged glycine and the other coincident with added sarcosine. About two-thirds of the glycine was transformed in molar correspondence with the disappearance of S-adenosylmethionine observed in these experiments. It would thus appear that no further methylation of sarcosine was occurring under these circumstances. The methylation of glycine to betaine has been reported in wheat seedlings (Barenscheen and von Valyi-Nagy, 1942), but the status of these experiments is somewhat uncertain (Cromwell and Rennie, 1954; Delwiche and Bregoff, 1958).

The enzyme responsible for this reaction has been detected in supernatant fractions from the livers of guinea-pig, rat, rabbit, and mouse. It is present in much lower concentrations, if at all, in analogous preparations from calf, pig, lamb and chicken livers. Both the biological distribution of the enzyme and preliminary observations during the ammonium sulphate fractionation of guinea-pig liver supernatant suggest that glycine methylpherase activity is a property of a new enzyme and not an extension of the substrate specificity of guanidoacetic acid methylpherase.

The existence of the reaction reported here provides a more direct route for the rapid oxidation of the methyl group of methionine and a more satisfactory explanation for the observed phenomena than those hitherto suggested.

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