The Participation of the Two Non-Equivalent C-5' Hydrogens of B_{12}-Coenzyme in the Catalytic Process[1]

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In the course of our studies of the mechanism of action of cobamide-coenzyme we observed that all of the tritium from chemically synthesized DBCC-C-5'(^{3}H)[2] is transferred to the reaction product (Frey et al., 1967). Similarly, addition of chemically synthesized DBCC-C-5'(^{3}H) to ribonucleotide reductase results in the release of all the tritium to the solvent (Beck et al., 1966). These results are surprising since the two C-5' hydrogens of the coenzyme are not sterically equivalent and one might expect that approximately 50% of the tritium from the synthetic coenzyme should participate in the enzymatic reaction. The fact that all of the tritium is transferred indicates that the two non-equivalent hydrogens participate in the reaction, and suggests that an intermediate

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[2] The following abbreviations will be used: DBCC = dimethylbenzimidazolyl-cobamide coenzyme; DBCC-C-5'(^{3}H) = DBCC containing tritium at the C-5' position.

occurs in which the two hydrogens are equivalent[3]. The results available so far, although indicative, are not conclusive. It is possible, in view of the complexity of the substrate, that the $NaBH_4(^3H)$ reduction which was used to introduce tritium into the C-5' position of the coenzyme, was highly stereospecific and leads to the predominant formation of one of the two possible isomers. If the isomer, which is produced, is the one that transfers tritium to the reaction product, then total tritium transfer would not require the participation of both C-5' hydrogens. Since the question as to whether or not both hydrogens at the C-5' position can participate in the enzymatic reaction is of extreme importance in arriving at a detailed mechanism of action of cobamide coenzyme, we decided to carry out experiments which would lead to an unambiguous answer to this question. These experiments are reported here and have led to the conclusion that both hydrogens at the C-5' position of the cobamide coenzyme can participate in the enzymatic reaction.

Equilibration of the α-Hydrogens of Propionaldehyde with the C-5' Hydrogens of DBCC in the Presence of Dioldehydrase - It has been previously shown (Frey *et al.*, 1967) that hydrogen (tritium) exchange takes place between the C-5' hydrogens of the coenzyme and the α-hydrogens of propionaldehyde. Both α-hydrogens of propionaldehyde must be able to participate in the exchange since dioldehydrase catalyzes the formation of both (R) and (S) α-deuteropropionaldehyde (Zagalak *et al.*,

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[3] It is also possible that the reaction is non-stereospecific. In view of the well established high degree of stereospecificity of enzymic reactions, this possibility is highly unlikely.