An Enzymic Assay for the Determination of Millimicrogram Quantities of B_{12}-Coenzyme[1]

Dioldehydrase (1), an enzyme which catalyzes the conversion of 1,2-propanediol to propionaldehyde in the presence of B_{12}-coenzyme,[2] can be used to assay the coenzyme quantitatively. The assay is based upon the fact that the amount of propionaldehyde produced is directly proportional to the amount of B_{12}-coenzyme present. The lower limit of the assay is 0.001 μg of B_{12}-coenzyme. Vitamin B_{12} and hydroxocobalamin, when present at twice the level of B_{12}-coenzyme, do not interfere with the assay. Other B_{12} derivatives are not expected to interfere, although they have not actually been tested.

Assay Procedure. The following reagents are used: 0.2 *M* potassium phosphate buffer, pH 8.0, dioldehydrase (1), 10–12 units/ml 0.01 *M* K_2HPO_4-2% 1,2-propanediol, and the reagents required for the aldehyde assay of Böhme and Winkler (2). The assay is carried out in 15 × 125 mm test tubes. To each tube is added 0.2 ml buffer and 0.6 ml B_{12}-coenzyme solution to be assayed or standard B_{12}-coenzyme solution. The test tubes are kept in a 37° bath. After temperature equilibration is reached, the reaction is started by the addition of 0.2 ml enzyme solution and allowed to proceed for 30 min. To terminate the reaction, 0.1 ml 2 *N* HCl is added. The 2,4-dinitrophenylhydrazine reagent (1.0 ml), used in the aldehyde determination, is then pipetted directly into the reaction mixture and the aldehyde determination is completed as described (2).

With each assay at least two B_{12}-coenzyme standards and two blanks are included. One blank contains no B_{12}-coenzyme, and the other contains the unknown coenzyme solution but no enzyme. The second blank is generally negligible. All assays are carried out in the dark. Flashlights are used to provide sufficient light to carry out the necessary operations.

The results of a typical assay are shown in Fig. 1. The enzyme used was a highly purified preparation of dioldehydrase. Less highly purified preparations have been used and were equally suitable.

Other Vitamin B_{12}-coenzymes, adenyl-cobamide coenzyme or benz-

[1] This work was supported by Grant No. GM-012633, National Institutes of Health, USPHS. Contribution No. 418 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts.

[2] The following trivial names are used: B_{12}-coenzyme = 5,6-dimethylbenzimidazolylcobamide-5′-deoxyadenosine; hydroxocobalamin = 5,6-dimethylbenzimidazolylcobamide hydroxide.

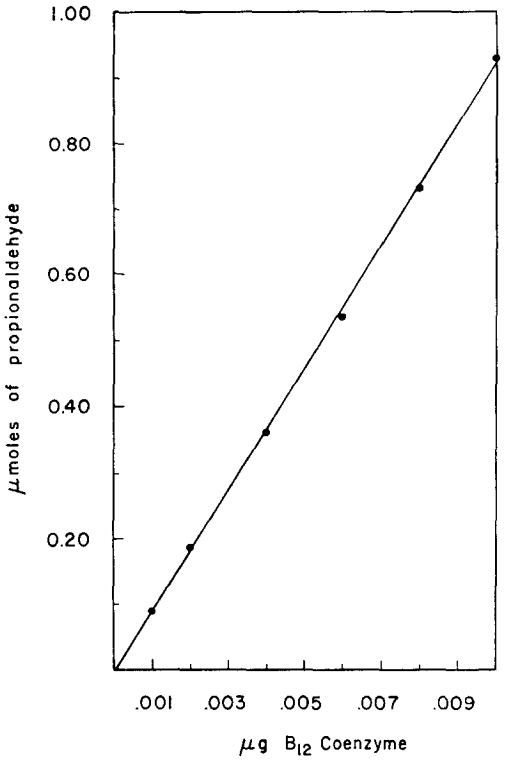

FIG. 1. Assay of B_{12}-coenzyme. Assay conditions as described in the text. Optical densities were determined in a Klett colorimeter with filter No. 54. Abscissa represents total amount of B_{12}-coenzyme in assay tube.

imidazolyl-cobamide coenzyme, activate dioldehydrase as efficiently as B_{12}-coenzyme (3). It is probable, therefore, that the assay described here is also suitable for these coenzymes. However, since they were not available to us, they were not tested in the assay system described here.

Application of Method. The method has been used extensively in this laboratory to assay the B_{12}-coenzyme content of mixtures of cobamides obtained from chemical synthesis or from enzymic reactions in which relatively pure enzymes were used. In all cases the mixtures analyzed were, as a result of preliminary purification or through dilution prior to assay (generally 500 to 1000-fold), free from salts and other materials that would interfere in the assay. The main sources of potential inhibition of the assay system were other cobamides. These, however, as was pointed out above, do not interfere under the conditions employed. The feasibility of the assay has also been tested with crude extracts from non mammalian sources. In this case, 1 vol of an aqueous suspension of

the material to be assayed was added to 10 vol of boiling 95% ethanol and boiled with stirring for 5 min. The solid was removed and re-extracted with half the volume of ethanol of the original extraction. The ethanol extract was then brought to dryness under reduced pressure. The residue was taken up in water and an aliquot assayed for coenzyme content. The following are typical results we obtained: *Aerobacter aerogenes* (ATCC 8724) 0.5 μg/gm wet cells; *Clostridium tetanomorphum* (ATCC 3606) 8.5 μg/gm wet cells; *Hydra littoralis* (starved 24 hr) 0.12 μg/ml packed hydra. Control experiments showed that in no case was any material present in these extracts which interfered with the assay. These assays were carried out primarily to establish that this assay procedure can be used with bacterial extracts of the type employed here. No effort was made to establish whether the extraction procedure quantitatively extracts all the coenzyme. The values obtained are not intended to provide quantitative information regarding the coenzyme content of the organisms examined. The feasibility of the assay, however, has been established.

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Received January 3, 1966

Interference in Nitrosonaphthol Method of Tyrosine Assay Due to Chloride

The spectrophotometric (3) and the fluorometric (5) procedures of quantitative tyrosine determination utilize the reaction between this amino acid and 1-nitroso-2-naphthol. The sensitivity of the fluorometric method (5.5×10^{-9} mole) is comparable to that obtained with an auto-