Enzymatic Preparation of D.P.N. H_2 and Determination of D.P.N. *

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Methods have been described for both chemical and enzymatic determination of D. P. N.[1-3]. In their work on yeast alcohol dehydrogenase, Negelein and Wulff used the enzyme to prepare reduced D. P. N.[4]. They were, however, not able to reduce more than 85 % of the coenzyme.

Reduction with dithionite has been widely used but has the disadvantage that it is very difficult to free the solution completely from sulphite and sulphate, which will interfere with many enzyme reactions. The determination of D. P. N. with dithionite is not possible when alcohol, acetone and pyridine are present, since these substances will give a cloudy solution with dithionite. The method is also time consuming when many determinations have to be done; new buffers must be prepared each time.

The kinetics of the animal alcohol dehydrogenase shows [5] that an alkaline pH will be favourable for the complete reduction of D. P. N. This offers a means for the rapid determination of a certain amount of D. P. N.**

The following method for the preparation and determination of reduced D.P.N. is based upon the fact that in the presence of an excess of alcohol, at a pH of 10, all the D. P. N. will be reduced by alcohol dehydrogenase. T. P. N. will not interfere since the enzyme only reacts with D.P.N.[6]

Preparation of dihydro-D.P.N. The D.P.N.H_2 is prepared as follows: About 20 mg of D.P.N., usually about 55 % pure, is dissolved in water and the pH brought to 10—11 with dilute sodium hydroxide. Then 0.5 ml of absolute alcohol and about 5 mg of enzyme are added. During the reduction the pH is kept constant by the addition of a few drops of dilute sodium hydroxide.

After all the D.P.N. has been reduced the solution is heated to about 90° C to denature the enzyme. The solution is then dried under reduced pressure.

Determination of D.P.N. To a 1.0 cm cuvette containing 0.1 *M* NaOH-glycine buffer pH 10, a suitable amount of D.P.N. solution is added (about 10 to 80 gamma of pure D.P.N.). Then 0.1 ml of absolute alcohol and 1 to 2 mg of enzyme are added. If heavy metals or other enzyme inhibitors are suspected in the coenzyme solution, more enzyme is added. After about 10 to 15 minutes the reaction reaches completion. The difference in the extinction coefficient (log I_0/I) at 340 mμ before and after the addition of the enzyme multiplied by 118 gives percent D.P.N.[7].

We have compared this method with the chemical procedure described by Le Page [3] and complete agreement has been found.

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** The enzyme is commercially available (A. B. Astra, Södertälje, Sweden).