XCII. NOTE UPON THE PREPARATION OF CRUDE COCARBOXYLASE FROM VITAMIN B₁ BY YEAST

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LOHMANN & SCHUSTER [1937, 1, 2] isolated cocarboxylase from yeast, and proved that this was a pyrophosphoric ester of vitamin B₁. Wishing to obtain crude carboxylase from small amounts of yeast before the details of their complete paper [1937, 2] appeared, we found that baker's yeast aerated in phosphate solution (containing pyrophosphate) with addition of synthetic vitamin B₁ chloride hydrochloride readily gave cocarboxylase. Subsequently pyrophosphate proved to be unnecessary. The cocarboxylase was extracted by heating the yeast and was precipitated from the extract by neutral lead acetate following Auhagen. It seems clear therefore that it is the combined form of this vitamin, first described by Kinnersley & Peters [1928] as constantly precipitable by neutral lead acetate. Since the preliminary account of our work [1937], there have appeared papers by Euler & Vestin [1937] and Tauber [1937] claiming synthesis with dried yeast, and by Stern & Hofer [1937] reporting a very small synthesis with phosphorus oxychloride.

We are describing our latest methods for preparing these crude solutions because they may be of value to others. The pyrophosphoric ester of the vitamin can be detected by the formation of blue fluorescence by treatment with dilute ferricyanide in alkaline solution; this blue fluorescence does not pass into an *iso*- or *n*-butyl alcohol layer. Unlike vitamin B₁ also, as stated by Lohmann and Schuster, it gives no formaldehyde *azo*-reaction.

EXPERIMENTAL

Requirements. Baker's yeast 10 g.; *M*/10 phosphate buffer (KH₂PO₄, Na₂HPO₄), pH 6.2, 15 ml.; glucose 100 mg.; vitamin B₁ 2-7 mg.

Method. The materials are shaken in a conical flask in a bath at *c.* 28° while O₂ passes, the vitamin B₁ being added last. After 1½ hr. 100 mg. glucose are added, and the reaction is stopped after 2½ hr. The flask is cooled while the O₂ stream continues, the contents are quickly centrifuged in previously cooled tubes and the remaining yeast is washed with ice-cooled water. The yeast is mixed with a minimum volume of ice-cooled water and added gradually to a convenient volume of boiling water sufficiently slowly to prevent appreciable cooling. The suspension is now centrifuged and washed with hot water. To the extract (30 ml.) after cooling 5 ml. 25 % neutral lead acetate are added and the lead precipitate is collected by centrifuging, suspended in 7-8 ml. water and treated with H₂S for 15 hr. From this stage onwards where possible cooling upon ice is used to minimize decomposition in the acid solutions. PbS is removed by centrifuging, the centrifugate filtered (volume about 6 ml.), adjusted to *c.* pH 5.5 by addition of NaOH and the H₂S removed by aeration. The cocarboxylase

present can be estimated approximately on 0.02 ml. by conversion into thiochrome form, though there is usually more fluorescence than cocarboxylase activity, owing presumably to the simultaneous presence of the monophospho-compound. The solution kept at 0° gradually loses activity. 0.05 ml. of the solution has about the same activity with washed yeast as some 3.5 γ Lohmann's pure cocarboxylase.

Notes. 1. The pH is approximately 6.5; trials at other reactions have led to no improvement.

2. The amounts have been increased to 200 g., 50 g. per flask in similar proportions, but the yield has been rather decreased.

3. The phosphorylation of about 1 mg. vitamin B₁ by 10 g. yeast is about the best so far reached.

4. The result is better if the yeast is fresh. We have not succeeded with dried yeast.

5. Special tests have not been made to decide whether the sugar addition is necessary; in many experiments amounts from 15 to 100 mg. sugar have been added initially in addition to the subsequent amount.

6. The method is based upon some 30 separate experiments.

SUMMARY

A method is described for the preparation of crude cocarboxylase from synthetic vitamin B₁.

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