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Quantitative Enzymic Conversion of Cocarboxylase (Vitamin B₁-Pyrophosphate) to Free Thiamin.

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Recently Lohmann and Schuster¹ reported that thiamin (vitamin B₁) may exist in nature as a pyrophosphoric acid ester to constitute cocarboxylase, the coenzyme of carboxylase. Thiamin itself cannot function as the coenzyme, although the action of pure cocarboxylase *in vitro* is strongly stimulated by the addition of the pure vitamin.² However, pigeon curative tests have shown that cocarboxylase is practically as equally effective as free thiamin. A number of workers³⁻⁶ have subsequently reported that in the thiochrome method for determining thiamin the cocarboxylase forms a thiochrome but this is not extracted by isobutanol and hence cannot be estimated.⁴ For this reason analyses by this method of preparations rich in cocarboxylase (such as yeast), give values decidedly less than those obtained by biological assay.⁶ Cocarboxylase may be hydrolyzed¹ to the monophosphoric acid ester of thiamin by 1 N HCl solution, but the latter ester is resistant to acid hydrolysis. Alkaline hydrolysis will completely remove the second phosphoric acid grouping but inasmuch as there is a concomitant destruction of the end-product (thiamin) such a procedure is impractical for analytical purposes. Levine⁶ has observed that, when aqueous yeast extracts are allowed to stand at room temperature exposed to the atmosphere for a period of 48 hours with no preservative added, there is a conversion of the cocarboxylase present to free thiamin. He attributed this change to the activity of a yeast phosphatase. Kidney phosphatase^{1, 5, 7} has been used as means for hydrolyzing the coenzyme to the free vitamin. However, with such a preparation the conversion does not appear to be quantitative.

* Upjohn Fellow in Clinical Research, 1937-1939.

¹ Lohmann, K., and Schuster, P., *Biochem. Z.*, 1937, **294**, 188.

² Ochoa, S., *Nature*, 1938, **141**, 831.

³ Kinnersley, H. W., and Peters, R. A., *Biochem. J.*, 1938, **32**, 677.

⁴ Roth, H., *Biochem. Z.*, 1938, **297**, 52.

⁵ Cerecedo, L. R., and Hennessy, D. J., *Abstr. Am. Chem. Soc., Div. Biol. Chem.*, September, 1938.

⁶ Levine, H., unpublished data.

⁷ Tauber, H., *J. Biol. Chem.*, 1938, **123**, 499.

In the present study an extension of our chemical method⁸ for the estimation of thiamin in biological materials has been applied to an investigation of the conversion of cocarboxylase to thiamin. We have found that both cocarboxylase and the monophosphoric acid ester of thiamin† react with diazotized p-amino acetophenone to yield colored solutions but that these pigments are not extracted by xylene. This is comparable to the findings recorded with the thiochrome method for determining thiamin. Tests conducted on a number of yeast extracts have not indicated that bacteria play a part in the conversion of cocarboxylase to thiamin, but that this change results from the action of a phosphatase which is liberated from the cell during the drying of the yeast. Thus, aqueous solutions obtained by initial alcoholic extraction of yeast or aqueous extraction at 70°C contain cocarboxylase in amounts comparable to that originally present in the yeast powder, and these amounts are not changed by subsequent bacterial contamination. The aqueous extraction of the yeast powder *at room temperature* gives solutions which will completely hydrolyze not only its own cocarboxylase but also added phosphorylated thiamin. The incubation of an aqueous suspension of the whole yeast powder under aseptic conditions, with toluene as a preservative, results in a complete hydrolysis of the cocarboxylase present. The free thiamin may then be quantitatively extracted by heating one-half hour at 70°C at a slightly acid pH in an atmosphere of nitrogen and removed from the insoluble residue by centrifugation. Chloroform completely prevents the conversion of cocarboxylase to free thiamin.

The pH optimum for the hydrolysis of cocarboxylase is rather closely restricted to that of 4.5. The enzyme is very active over a wide range of temperature, from 10 to 55°C, with the optimum at 45°C. At 70°C the enzyme activity is irreversibly destroyed. Under the optimal conditions given the reaction is complete in 12 hours. Yeast preparations or extracts, devoid of soluble protein, have not been found to show enzyme activity. Methods for the isolation of the undenatured protein fraction of the active yeast powder have yielded products free of thiamin but still capable of hydrolyzing, though not completely, the phosphorylated vitamin extracted from the yeast powder and also the synthetic monophosphoric acid ester.

The incubation of our active yeast powder with a number of

⁸ Melnick, D., and Field, H., Jr., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 723.

† We are indebted to Drs. Joseph L. Melnick and Kurt G. Stern, of the Laboratory of Physiological Chemistry, Yale University, for a generous supply of the synthetic crystalline monophosphoric acid ester of thiamin.

vitamin B₁ concentrates, such as extracts of yeast, rice polish and wheat germ, has enabled us to convert all of the vitamin present into the form by which it may be determined chemically. By difference between the thiamin values obtained before and after hydrolysis, the concentration of the vitamin in the phosphorylated form may be estimated. In the samples assayed we have found some yeast preparations to contain as much as 75% of its thiamin in this form, a rice polish extract 17%, and a wheat germ preparation 10%. Obviously, in the preparation of any dried yeast powder or extract the ratio of phosphorylated to free thiamin in the final product is dependent upon the conditions associated with the preparations. If during the process conditions are such as to allow the phosphatase present to act, a much smaller ratio will be obtained. Our findings of an autohydrolysis of the phosphorylated thiamin in aqueous suspensions of dried yeast powders support the earlier findings of Auhagen⁹ that autolyzed yeast loses its cocarboxylase activity.

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Cortical Hormone-Like Action of Progesterone and Non-Effect of Sex Hormones on "Water Intoxication."*

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Gaunt and Hays have shown that crystalline progesterone maintains life and excellent health in adrenalectomized ferrets, whereas the estrogens are toxic and testosterone non-beneficial.¹ These findings probably account for the survival of pseudopregnant adrenalectomized animals.² † To determine if other species would respond

⁹ Auhagen, E., *Biochem. Z.*, 1933, **258**, 330.

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¹ Gaunt, R., and Hays, H. W., *Science*, in press.

² Literature cited by Gaunt, R., and Hays, H. W., *Am. J. Physiol.*, in press.

† Dr. G. W. Thorn reports a confirmation of the life-maintaining action of progesterone in adrenalectomized dogs (personal communication).