anisidine hydrochloride. We presumed that the spot which should be red seemed yellow owing to the scarceness in quantity of the sugar.

Thanks are due to Dr. Yosito Sakurai and Dr. Saburo Funahashi, professors at the University of Tokyo, for their interests in this work. A part of the expenditure was defrayed by the research fund donated by the Ministry of Education to S. K. (A study on

ribose as a constituent sugar of hemicelluloses, 1957).

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Received September 9, 1958

[Bull. Agr. Chem. Soc. Japan, Vol. 22, No. 6, p. 437~438, 1958]

Studies on Vitamin B₁₂-Synthesizing Enzyme. Part I. Isolation of an Enzyme Preparation from *Streptomyces olivaceus*.

Sir:

It is widely known that *Streptomyces* produces vitamin B₁₂, and the existence of a synthetic enzyme in the cells has been expected. We have therefore attempted studies on the isolation of the enzyme from *Streptomyces olivaceus* PD 61, and activity of the enzyme preparation was tested for the biosynthesis of vitamin B₁₂ in vitro, in a synthetic medium (pH 7.2) containing 140 γ of Cobalt-porphyrin (Na salt), 30 γ of 5,6-dimethylbenzimidazole, 30 γ of D-ribose, 48 γ of DL-threonine, 20 γ of diammoniumhydrogenphosphate, 20 γ of potassium cyanide and 6 γ of ATP per ml.

Streptomyces olivaceus was first cultured in 80-ml of Dulaney medium in the range of 28-32°C for seven days under shaking. Then, the cells were collected by centrifugation and washed with distilled water repeatedly. The cells thus obtained were treated with a Raytheon Sonic Oscillator for thirty minutes, and the sediment (1.49 g) was washed with 15 ml of 10% sodium chloride. The insoluble portion (1.39 g) was subsequently treated with 14 ml of 0.1% sodium hydroxide. To the alkali-

insoluble fraction (1.27 g) was added 13 ml of 70% ethylalcohol, and 10 ml of the alcohol-soluble fraction was digested at 37°C with 500 mg of diastase and a few drops of toluene. After incubation for 45 hours, the digest was treated with 90 ml of barbiturate buffer (pH 7.2), and the mixture was centrifuged. The upper-layer was saturated with ammonium sulphate. After treatment for one hour, the precipitate was sedimentated on a centrifuge. The resulting product was suspended in 10 ml of barbiturate buffer, pH 7.2 and dialysed through cellophane membrane against running water for three days. Twenty-five ml of the dialysed solution was freeze-dried over a mixture of dry-ice and acetone, yielding 7 mg of enzyme preparation in amorphous powder.

The enzyme preparation prepared in this manner was subsequently tested for its vitamin B₁₂-producing activity in vitro by application of the ordinary tube method. Determination was carried out at 28°C using 10 ml of the synthetic medium (pH 7.2) described above each tube containing 2 mg of the enzyme preparation. The amount of vitamin B₁₂ pro-

TABLE I

Components of Reaction Medium				Total Amount of Vitamin B ₁₂ produced, γ per liter	
Enzyme preparation, mg/tube	ATP, γ /tube	KCN, γ /tube	Factors*	Euglena method	Ochromonas method
2	60	200	+	4.86	0.98 (1.92)***
2	—	200	+	—	0.58
2	60	—	+	—	0.56
—	60	200	+	0.52	0.02
—	—	—	—	0	0
2**	60	200	+	0	—

* Factors except ATP and KCN from the complete reaction medium.

** Enzyme preparation inactivated by treatment with alkali (100', 30 min).

*** This value means the highest one obtained in three tests.

duced in a period of one hour was accurately estimated by microbioassay with both *Euglena gracilis* and *Ochromonas malhamensis*. The results obtained are shown in Table I.

The above results may indicate that the enzyme preparation isolated from *Streptomyces olivaceus* possibly activates the synthesis of vitamin B₁₂. Further studies on the properties of the enzyme are now in progress.

We wish to express our sincere thanks to Prof. S. Funahashi, Prof. K. Iwamoto, Mr. T. Suzuki, Prof. T. Kobayashi, Dr. A. Yoshida, Mr. H. Sakai and Dr. S. Kinoshita for their kind advice. This work was aided in part by a grant from the Scientific Research

Fund, Ministry of Education, to which the authors are indebted.

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Received September, 6, 1958.

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