THE ISOLATION OF FACTOR ONE IN CRYSTALLINE FORM*

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After Birch and György (1) reported their studies on the chemical properties of vitamin B₆, it became uncertain, in the light of the multiple nature of vitamin B₆ (2), whether the properties reported were those of Factor 1 or Factor 2. Some of these chemical properties of vitamin B₆ were reinvestigated for their applicability to Factor 1. In agreement with the findings of Birch and György (1) for vitamin B₆ it was found that Factor 1 could not be precipitated by lead or mercuric salts but was readily precipitated by phosphotungstic acid. These properties of Factor 1 proved in a large measure to be the basis for its isolation in crystalline form.

Crystalline Factor 1 (3, 4) readily cleared up the acrodynia-like (5) dermatitis of rats with simultaneous resumption of growth. 5 micrograms of the crystalline material (3) fed daily caused an average daily gain in weight of 2.5 gm. over a period of 2 weeks. Although the method for isolating Factor 1 leaves much to be desired at the present time, it has yielded crystalline Factor 1 and it seems desirable to publish it at this time. The various steps of the method are now under investigation in the hope of rendering more certain and better the yield of crystalline Factor 1.

EXPERIMENTAL

Biological Methods—21 day-old rats were put on the basal Factor 1-deficient diet (2) for 10 days, after which they received

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daily 40 micrograms of riboflavin and 6 international units of thiamine.\textsuperscript{1} When the rats ceased to grow they were fed Factor 2 concentrate (2) prepared from rice bran extract.\textsuperscript{2} Such rats, after a gain of 20 to 30 gm. in weight, generally developed dermatitis and sooner or later began to decline in weight. At this stage they were ready for Factor 1 tests.

\textit{Isolation of Factor 1—}Fullers’ earth adsorbate\textsuperscript{3} from rice bran extract is extracted with a \( \text{Ba(OH)}_2 \) solution strongly alkaline to phenolphthalein and concentrated under reduced pressure of about 26 inches to remove volatile bases. After partial concentration, the \( \text{Ba(OH)}_2 \) is removed with \( \text{H}_2\text{SO}_4 \) and the extract concentrated to a thin syrup. This crude Factor 1 concentrate\textsuperscript{4} serves as the starting point for the fractionation of Factor 1. 10 volumes of ethanol are now added and the insoluble fraction containing considerable Factor 1 is removed. To the alcoholic extract solid \( \text{Ba(OH)}_2 \cdot 8\text{H}_2\text{O} \) is added until the solution is definitely alkaline to phenolphthalein, when a fine bulky precipitate comes out. Heat will aid this process. After standing overnight, the precipitate is filtered off and to the clear solution solid \( \text{HgCl}_2 \) is added in excess. The resulting precipitate is filtered off. The filtrate is concentrated to a small bulk and the precipitated mercuric salt filtered off. Water is added and the solution allowed to stand until the fine gummy precipitate coalesces and the solution clears. Alcohol is removed by distillation, barium with \( \text{H}_2\text{SO}_4 \), and mercury with \( \text{H}_2\text{S} \). The excess \( \text{HCl} \) in the solution is removed with freshly precipitated lead hydroxide. The solution should be faintly acid to Congo red. The lead is removed with \( \text{H}_2\text{S} \). Factor 1 is now precipitated with aqueous phosphotungstic acid without any addition of acid. The phosphotungstate is filtered off and recrystallized from water. This is accomplished by dissolving the phosphotungstate in a small amount of acetone.

\textsuperscript{1} The thiamine was a highly potent concentrate prepared for us by Dr. Elmer H. Stuart of The Lilly Research Laboratories, Indianapolis.

\textsuperscript{2} The rice bran extract is marketed by Vitab Products, Inc., Emeryville, California.

\textsuperscript{3} The fullers’ earth adsorbate was obtained from Vitab Products, Inc.

\textsuperscript{4} Crude Factor 1 concentrates were prepared for us in the laboratories of Eli Lilly and Company through the courtesy of H. W. Rhodehamel and in the laboratories of Vitab Products, Inc.
in which it is readily soluble and adding a large volume of water until no more milky precipitate comes out. The fine suspension of phosphotungstate is heated on the water bath, when it goes into solution. On cooling, a crystalline phosphotungstate appears in the form of square plates (Fig. 1). The crystalline phosphotungstate is decomposed with Ba(OH)$_2$, the barium phosphotungstate filtered off, and the excess barium removed with sulfuric acid. The concentrate is now reduced in vacuo to a thin syrup.

2 or 3 volumes of 95 per cent ethyl alcohol are added and then, on addition of acetone, the solution becomes cloudy. Acetone should be added till no more precipitate appears. On standing, Factor 1 will crystallize as rods or needles in rosettes or fan shapes on the sides of the flask, the bottom, and on the stirring rod. Fig. 2 shows the crystalline form of Factor 1.

Karrer, Laszt, and Verzar (6) have reported the cure of B$_6$ avitaminosis (acrodynia) with large doses of natural and syn-
Isolation of Crystalline Factor 1

thetic flavin phosphoric acid. Since crystalline Factor 1 is colorless, its chemical identity with flavin phosphoric acid is ruled out. Factor 1 apparently also differs from flavin phosphoric acid in that it does not seem to be adsorbed by lead sulfide (7). Further work is necessary to clarify this situation.

SUMMARY

A method for isolating Factor 1 in crystalline form is described.

BIBLIOGRAPHY

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