repeated after they had become diabetic. In 37 experiments performed upon 14 dogs before and after pancreatectomy, we obtained definite evidence of the intestinal absorption of insulin in 35% of 14 experiments upon normal dogs and in 92% of 23 experiments upon diabetic dogs. In all experiments the insulin pinacol tablets were inserted into the intestinal loop and the dosage of insulin employed was 50 units. The readings in Table I are typical of the results obtained.

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Use of Phenol in Application of Prebluda-McCollum Reagent for Determining Vitamin B_1.

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Recently Prebluda and McCollum[1] reported that Vitamin B_1 reacts in alkaline solution with diazotized *p*-amino acetophenone to yield a water-insoluble, purple-red compound. Subsequently, we found[2] that xylene quantitatively extracts this pigment and that the xylene layer lends itself to colorimetric evaluation. This procedure, coupled with our adsorption and subsequent elution technics using synthetic zeolite,† appears highly specific for the determination of the vitamin.

Ten cc of a thiamin hydrochloride solution at a pH of 7.0 are pipetted into a 50 cc centrifuge bottle. This is followed by the addition of 20 cc of the Prebluda-McCollum reagent. After 24 hours at room temperature 2 cc of xylene are added and the mixture shaken vigorously for 1½ minutes. After centrifugation the color in the xylene layer is compared in a micro-colorimeter with a standard similarly treated. Subsequently, in studies involving the use of phenol for extracting the vitamin from saturated salt solutions, recoveries greater than 100% were consistently obtained due to the presence of trace quantities of phenol in the final concentrates, which

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1 Prebluda, H. J., and McCollum, E. V., *Science*, 1936, **84**, 488; *J. Biol. Chem.*, 1938, in press.

2 Melnich, D., and Field, H., Jr., *J. Biol. Chem.*, 1938, Proc., lxxxiii.

† "Decalso," kindly furnished by the Permutit Company, New York, N. Y.

escaped ether extraction. Other organic hydroxy compounds, such as ethyl alcohol and pH indicators, also increase the sensitivity of the reaction.

In order to investigate the behavior of phenol in this respect, a series of determinations were made in which varying amounts of phenol were added to the vitamin solutions, each 10 cc in volume and containing a total of 100 gamma of thiamin hydrochloride. No phenol was added to the standard solution. Although phenol, like some other organic compounds, reacts with the reagent to yield a colored solution, only the red pigment due to the coupled vitamin has been found to be extracted by the organic solvent. The results of these determinations are presented in Fig. 1.

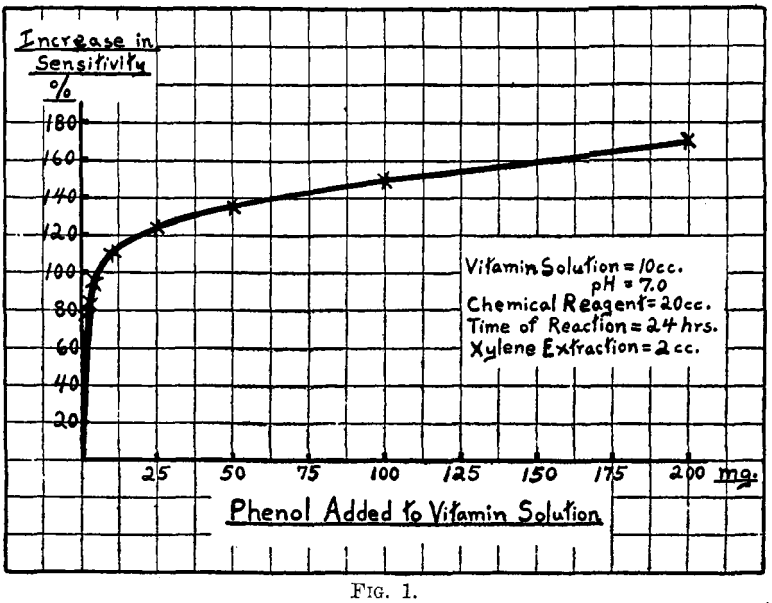

FIG. 1.

The use of phenol to increase the sensitivity of the reaction for the chemical determination of thiamin (vitamin B_1). A total of 100 gamma of thiamin hydrochloride was used in each test.

It is apparent from the curve in Fig. 1 that small quantities of phenol increase greatly the sensitivity of the reaction but with larger amounts of phenol the intensity of the color in the xylene layer becomes relatively constant. Thus small amounts of phenol, which may be present in the final solutions, have a negligible effect on the reaction when 50 mg of phenol have been added. In the presence of very large amounts of phenol (300 mg or more) the reaction fails unless larger quantities of the Prebluda-McCollum reagent are used.

With the addition of the 50 mg quantity of phenol accurate determinations were obtained on solutions ranging from 0.4 to 5 times the standard, in this case also the 10 gamma per cc solution of thiamin hydrochloride. For amounts more than 2 times the standard, greater quantities of xylene were used in order to yield solutions the color intensities of which were comparable to the standard. Without the phenol it had previously been necessary to employ a standard more closely approximating the test solution in concentration, and in some cases a previously determined reference curve had to be used in order to obtain the true values.

In these estimations of the thiamin concentrations in the presence of phenol, there was such an increase in the sensitivity of the reaction that the xylene layer was intensely pigmented. Accordingly, a micro-method was devised, using three-tenths quantities of vitamin solution, phenol and reagent, but the same volume of xylene to extract the reaction product. By this procedure it is possible to determine accurately the vitamin concentration in solutions containing as little as 10 gamma total of thiamin hydrochloride.

In addition to increasing the sensitivity of the chemical method for estimating the Vitamin B_1 concentration, the addition of phenol to the vitamin solution eliminates errors in apparent recoveries which are otherwise produced by varying the volume of the vitamin solution or the amount of the chemical reagent, allows the use of one standard solution despite very wide differences in the concentrations of the vitamin, exerts an appreciable protective action on the vitamin in solutions at a pH just alkaline to litmus, and finally eliminates the effects of trace substances which are capable of increasing the sensitivity of the reaction and which may be present in the final concentrate.

In the present paper all tests described were conducted on pure aqueous solutions of thiamin hydrochloride.‡ This reaction can not be applied in its present form to biological materials unless quantitative procedures for the preparation of suitable concentrates are first devised. This phase of the problem is being studied.

‡ We are indebted to the Upjohn Company, Kalamazoo, Mich., for furnishing us with a generous supply of crystalline vitamin B_1 hydrochloride.