VI. THE USE OF COLLOIDAL FERRIC HYDROXIDE SOL FOR ADSORBING VITAMINES B AND D¹.

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EDDY, KERR and WILLIAMS [1924] have described the preparation and isolation of vitamine D (yeast-growth-promoting vitamine) using the following method. An aqueous extract of yeast, freed from proteins by coagulation, was concentrated to suitable strength, the p_H of the solution adjusted to 4.7 and one volume of a colloidal ferric hydroxide sol added. The ferric hydroxide was prepared according to the method described by Nelson and Kerr [1924]. It was claimed that under such conditions impurities are eliminated without impairing the activity of vitamine D. After freeing the liquid from this first precipitate and adjusting the reaction to p_H 5.3, 4 volumes of the same iron sol were added; it was stated that almost all the vitamine D could be obtained in this way. In looking over the curves of adsorption of vitamine D at various hydrogen ion concentrations one is struck, however, by the divergence of the results of the third parallel experiment.

Before going into the actual isolation of vitamine D we have made a thorough study of the method described. As a starting point we have used aqueous and alcoholic extracts of yeast, wheat bran and rice polishings. We do not wish to cast the slightest doubt on the results of Eddy, Kerr and Williams, but only to point out that their method did not yield good results in our hands. In our study we have extended our procedure to vitamine B and we have studied the adsorption of both substances at various hydrogen ion concentrations. Our results suggest perhaps that the method of precipitation of vitamins B and D by means of colloidal iron sol yields results which are not constant and which cannot be relied on.

As a starting material we prepared aqueous extracts of yeast, and aqueous and alcoholic extracts of wheat bran and rice polishings, in which the vitamine D content has been determined by the method of Funk and Dubin [1920]. This method has been uniformly used throughout the whole investigation. For the determination of vitamine B we used pigeons which had been placed in vitamine B equilibrium by the method of Funk and Paton

¹ In this paper the author uses his own system of nomenclature, which is not that usually adopted in this country. (Ed.)

[1922] and Collazo and Funk [1924, 1925]. In this way the amount in cc. of each preparation given per day was determined which sufficed to keep the animals at constant weight on a diet of polished rice. By substituting the original solution by the filtrates obtained after precipitation with colloidal ferric hydroxide sol, the loss or gain in weight of pigeons per 100 g. of weight and per unit of vitamine given was taken as an index of the vitamine content. This method has been found to give quite reliable results. In our investigation we did not limit ourselves to the two hydrogen ion concentrations but we have used a whole scale from p_H 4.0 to 5.5 which has been adjusted both by the colorimetric method of Sørensen and by the gas chain method. Aside from these systematic adsorption studies we have repeated the work of Eddy, Kerr and Williams on the isolation of vitamine D by keeping exactly to their method. Here also we were unable to obtain a noticeable precipitation of vitamine D. After these negative results we tested whether this method could be adapted for the preparation of vitamine B. By using larger volumes of colloidal ferric hydroxide sol we did not succeed in throwing down more than 30 % of the vitamine B present originally in the solution. Somewhat similarly irregular results were obtained by Levene and van der Hoeven [1925] using silica gel. The amount of vitamine B adsorbed varied from 41 to 65 % and the authors remark that the degree of adsorption depends on the presence of casual impurities, in their case probably of phosphates. Our results can be seen from the appended figures.

DISCUSSION OF THE RESULTS.

Vitamine D.

1. *Yeast.* In case of purified aqueous extract of yeast some of the vitamine D was adsorbed in the vicinity of p_H 5.3, whilst on a second precipitation the smallest amount of vitamine D was found in the filtrate of p_H 4.4 (see Fig. 1).

2. *Wheat.* In case of wheat the first precipitation gave a slight diminution of vitamine D at p_H 5.0, whilst the second precipitation gave the best adsorption in the vicinity of p_H 4.6 (Fig. 2).

3. *Purified alcoholic extracts of rice polishings.* Two experiments made with the same extract (shown in Figs. 3 and 4) gave a great deviation of results. The first precipitation gave a considerable loss of vitamine D, whilst the second gave an increased vitamine activity as measured by growth of yeast. In the second experiment (Fig. 4) the results were more regular and the vitamine D was mostly precipitated at p_H 4.6.

4. *Aqueous extract of rice polishings.* Here only the result of the second precipitation was recorded and, as we see from Fig. 5, practically no effect was noticed.

If we summarise the results of the experiments here recorded and others that we have made during our investigation we are unable to find that the

colloidal ferric hydroxide sol has any specific adsorbing power for vitamine D. Moreover our curves tend to show that the reading of the results is very much influenced by precipitation of inhibitory substances for yeast growth which are more or less adsorbed at various p_H values. One may find perhaps that the growth of yeast increased not because some of the vitamine D was adsorbed, but by elimination of some growth-inhibiting substances, whilst in other cases we might find an apparent diminution of vitamine D by the mere fact that the inhibitory substances happened to remain in solution. The method in any case was a failure in our hands, as the iron precipitates decomposed by the method by Eddy, Kerr and Williams failed to yield any vitamine D.

Fig. 1. Influence of hydrogen ion concentration on the adsorption of vitamine D by colloidal ferric hydroxide sol. Aqueous yeast extract used.

The upper short line indicates the activity of the extract before treatment, whilst the lower short line is the control with yeast cells without adding the extract. The continuous curve is the result of the first treatment with a single volume of the colloidal solution whilst the interrupted curve is the result of the second precipitation with four volumes of the same adsorbent.

Fig. 2. Same as fig. 1 using aqueous wheat bran extract.

Vitamine B.

Being unsuccessful with the preparation of vitamine D by the adsorption method, we turned our attention to vitamine B, which according to our previous findings is much more easily adsorbed than vitamine D. To study this question we have used the same extracts as above but we have investigated only the effect of the first precipitation. Working with alcoholic extracts of rice polishings the method was without value, the filtrate containing as much vitamine B as the original solution (Fig. 6). Somewhat better results were obtained with aqueous yeast extract. Here some adsorption of the substance could be recorded at p_H 5.1 but in the best case adsorption only to the extent of 30 % could be obtained. With an aqueous extract of rice polishings two minimal points could be recorded, one at p_H 4.2 and the second at p_H 5.0.

Fig. 3.

Fig. 3. Same as fig. 1 using alcoholic extract of rice polishings.

Fig. 4.

Fig. 5.

Fig. 5. Effect on vitamine D activity of one single addition of four volumes of colloidal ferric hydroxide sol to an aqueous extract of rice polishings.

Fig. 6.

Fig. 6. Effect of addition at various hydrogen ion concentrations of one volume of colloidal ferric hydroxide sol on the activity of vitamine B measured by the action of filtrates on pigeons.

The curves indicate the loss or gain in the weight of pigeons calculated on 100 g. of body weight and 100 cc. of each particular extract. The calculation of the amount of extract given is of importance because each bird received a dose which corresponded to its individual vitamine requirement.

———— aqueous extract of rice polishings.
 - - - - - " " wheat bran.
 - . - . . alcoholic extract of rice polishings.
 aqueous extract of yeast.

A similar effect was found by using an aqueous extract of wheat. Increasing the amount of colloidal ferric hydroxide sol to 4 volumes did not yield any more favourable results and the preparation of vitamine B from the precipitates obtained was on the whole unsuccessful.

SUMMARY.

The adsorption of vitamine D using the method of Eddy, Kerr and Williams (colloidal ferric hydroxide sol) gave unreliable results in our series of experiments. This method applied to preparation of vitamine B gave also negative and inconsistent results. It is probable that the degree of adsorption depends on the presence of casual impurities in the extracts.

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