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Electrophoretic Studies of Cobalamins. I

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The present paper deals with electrophoretic experiments on vitamin B_{12} (cyanocobalamin) and on the vitamin B_{12}-cyanide complex (di- or tricyanocobalamin).

EXPERIMENTAL

The apparatus employed was of Tiselius' type with the improvements designed by Svensson [1]. The experiments were run in preparative cells at 0° C. Buffer solutions having an ionic strength of 0.1 and covering a pH range from 1.1 to 10.55 were used. Weighed amounts of crystalline vitamin B_{12} (Cobemin, Merck & Co. Inc.) were dissolved in the buffer solutions to give a concentration of about 100 μg/ml. The electrophoretic mobility was calculated from the content of vitamin in the different cells, which was determined spectrophotometrically, using the maxima at 550 and 361 mμ. For every estimation of vitamin B_{12} the spectrum in the neighbourhood of these peaks was measured to make certain that no change of the molecule had taken place during the experiment, *e. g.* conversion to hydroxo- or aquocobalamin. For more extreme pH-values, *i. e.* pH 1.1, 1.8 and 10.55, the vitamin B_{12} content of the cells was also determined microbiologically, using *E. coli* 113—3[2] as test organism in the agar cup plate method. The results of these microbiological estimations always agreed with those obtained spectrophotometrically.

It was observed that in some experiments, especially at high pH-values, both upper chambers contained vitamin B_{12}, as if the vitamin dissociated into two oppositely charged components. This confusing phenomenon — which may be electrokinetic in nature — could be avoided if the electrophoresis was carried out in a sugar gradient, which was accomplished by dissolving the vitamin in a buffer solution containing 2 % glucose, pushing the cells into position for electrophoresis and allowing the sugar to diffuse into the upper chambers. The diffusion of glucose, which is much faster than that of vitamin B_{12} could be observed by the ordinary optical method. The current was turned on after 5 hours and the movement of vitamin B_{12} could then also be observed optically — despite the low concentration — as the light refractive index curve of the not migrating glucose was overlapped by the red colour of the vitamin and the movement of the coloured front could be observed. When the vitamin B_{12} boundary had to move in a glucose gradient a definite electrophoretic movement was established *.

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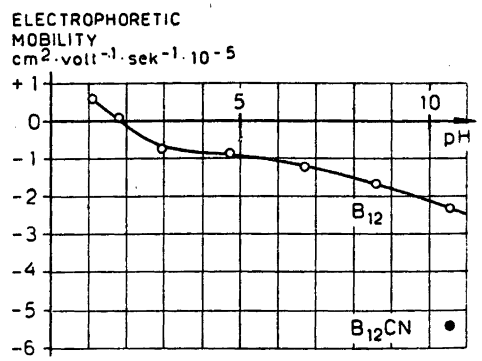

Fig. 1. Electrophoretic mobility of vitamin B_{12} (cyanocobalamin) at different pH-values and of the vitamin B_{12}-cyanide complex (di-or-tri-cyanocobalamin) at pH 10.55.

RESULTS

Fig. 1 shows the curve obtained by plotting the mobility values for vitamin B_{12} against pH. The vitamin B_{12} molecule moved towards the anode in experiments where the pH was 2.95 or higher, but towards the cathode at the experiments run at pH 1.1 and 1.8. The isoelectric point appears to be near pH 1.9. Vitamin B_{12} recrystallized once (run at pH 2.95 and 10.55), three (pH 1.1 and 6.75) and five times (pH 4.75) from aqueous acetone gave results which were in agreement with those observed with the original B_{12}-preparation (Cobemin, Merck).

The mobility of the cyanocobalamin at pH 7 ($\mu = 0.1$) is $1.3 \cdot 10^{-5}$ cm^2 volt^{-1} sek^{-1}. Due to diffusion, accurate mobility figures are difficult to obtain with a compound having a comparatively low molecular weight and a low mobility.

The vitamin B_{12}-cyanide complex was run in a buffer solution of potassium cyanide alone which had a pH of 10.55 and an ionic strength of 0.01. The solution of vitamin B_{12} in this buffer showed the characteristic maxima at 578 and 368 mμ, both before and after the electrophoresis. The mobility of the B_{12}-cyanide compound was found to be $5.4 \cdot 10^{-5}$ cm^2 volt^{-1} sek^{-1}.

DISCUSSION

Brink *et al.*[3] have shown that vitamin B_{12} is a polyacidic base, as revealed by potentiometric titration in glacial acetic acid solution. They also stated that the basic groups were too weak to be detected when the compound was titrated in aqueous solution. Alicino [4] observed that vitamin B_{12} could form with perchloric acid a salt, having the composition $B_{12} \cdot 6\ HClO_4$, indicating the presence of six basic groups in the vitamin. Recently H. Schmid, A. Ebnöther and P. Karrer [5] reported that 5—6 molecules of ammonia per molecule of vitamin B_{12} could be obtained on catalytic reduction in hydrochloric acid of vitamin B_{12}. The results of the present investigation in which vitamin B_{12}

was observed to move towards the cathode at low pH values provides further evidence for the existence of weak basic group(s) in the molecule.

Vitamin B_{12} is generally referred to as a neutral compound [6-8]. As the vitamin appears to have an isoelectric point at pH 1.9, this is strictly true only at this pH value. The acidic group or groups that were observed when the electrophoresis was carried out at pH values higher than 1.9 are probably comparatively weak as judged from the mobility values. Lester Smith *et al.*[7] report no change with pH (varying from 2 to 9.5) in the partition coefficients of vitamin B_{12} between benzyl alcohol and water, which could mean that the acidic groups are too weak to influence measurably the partition of the compound between these two solvents. The failure of one of us and other authors [9,10] to demonstrate any definite electrophoretic mobility of vitamin B_{12} by paper electrophoresis may possibly be explained by the same reason.

I II

Fig. 2. Formula I represents vitamin B_{12} (cyanocobalamin). Formula II the vitamin B-cyainde complex (di-cyanocobalamin).

Bush *et al.*[8] have proposed the formula I (Fig. 2) for the cyanocobalamin. In this formula the „zeros denote a neutral group or groups coordinated to cobalt by dative bonds". It is evident that these O-groups, whether occurring together with the minus-groups in a single molecular aggregate or not, represent groups that can ionize, being basic or acidic depending on the pH. Cooley *et al.*[11] suggest that introduction of a second cyanide group into the cyanocobalamin leads to displacement of the N_3 of the 5,6-dimethylbenzimidazole from the coordination with cobalt. The vitamin B_{12}-cyanide complex formed can thus be written as shown in formula II (Fig. 2), where a second CN^--group replaces one of the O-groups rendering the molecule as a whole negative. Consequently the vitamin B_{12}-cyanide complex behaves as an acid [7]. This is in accord with the observed mobility values at pH 10.55 for the vitamin B_{12}-cyanide complex ($5.4 \cdot 10^{-5}$) as compared with that of the vitamin B_{12} itself ($2.4 \cdot 10^{-5}$) at the same pH. It should be mentioned, however, that Conn *et al.*[12] have provided polarographic evidence for the uptake of two cyanide molecules per molecule of vitamin B_{12} in a buffer consisting of 0.1 *M* sodium cyanide and 0.1 *M* lithium borate and having a pH of 10.99.

SUMMARY

Electrophoretic studies of vitamin B_{12} (cyanocobalamin) at pH values varying from 1.1 to 10.55 revealed both acidic and basic groups and showed that the vitamin has an isoelectric point near pH 1.9. The mobility of vitamin B_{12} was observed to be $1.3 \cdot 10^{-5}$ cm^2 $volt^{-1}$ sek^{-1} at pH 7.0. The vitamin

B_{12}-cyanide complex showed a mobility value of $5.4 \cdot 10^{-5}$ cm^2 $volt^{-1}$ sek^{-1} at pH 10.55 whereas the mobility value for vitamin B_{12} at this pH was $2.4 \cdot 10^{-5}$ cm^2 $volt^{-1}$ sek^{-1}.

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