

same time more accurate than the ordinary acidic methods.

When oximes of ketoacids were hydrolysed ordinarily in an acidic medium, the liberated hydroxylamine was decomposed by various substances, including the liberated ketoacids. This interfering effect was eliminated by addition of 2,4-dinitrophenylhydrazine as well as by using the alkaline method (Table 3).

As the coexisting nitrite tends to decompose hydroxylamine and is colourized simultaneously, it is desirable to remove the nitrite beforehand. This was achieved when the test solution containing nitrite was kept for 20 min. with urea in an acidic solution before addition of 2,4-dinitrophenylhydrazine. In this case, it was necessary to increase the amount of Na-acetate to neutralize excess of acid. The results are shown in Table 4. This method is more convenient because oxime can be determined independently.

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Electrophoretic Studies of Vitamin B₁₂-factors. III

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Bernhauer *et al.* have recently isolated five different vitamin B₁₂-factors from digested sewage sludge^{1,2}. One of these factors, provisionally called "Factor III", has practically the same microbiological activity towards *E. coli* and *Lb. leichmannii* in the tube-assay as cyanocobalamin (vitamin B₁₂) and has strong activity towards *Ochromonas malhamensis*. The anti-

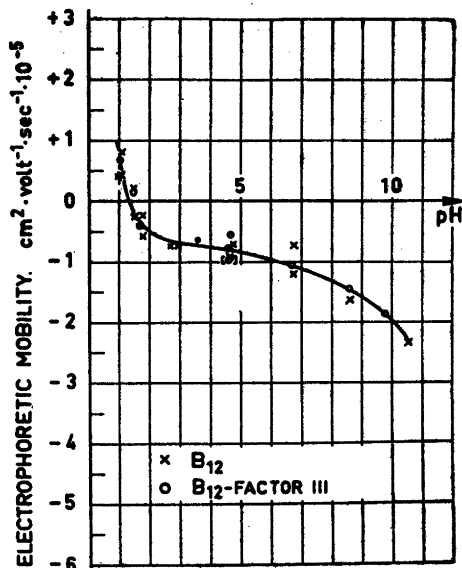


Fig. 1.

pernicious anemia activity of Factor III is similar to that of cyanocobalamin³. Factor III, however, differs from other vitamin B₁₂-factors with regard to *R_F*-values and distribution coefficients³. The absorption spectrum of Factor III closely resembles that of cyanocobalamin with the difference that the narrow band at 278 m μ is only faintly marked while a wide band at 295 m μ appears. Such a band has never been noticed before in any other vitamin B₁₂-factor. Factor III like cyanocobalamin itself is able to form aquo- and hydroxocomplexes as well as a cyano-complex containing more than one CN-group per molecule of Factor III.

A comparison between the effect of pH on the electrophoretic mobility of Factor III and of cyanocobalamin has been carried out in our laboratories.⁴ It was concluded at first that the electrophoretic properties of Factor III closely resembled those of cyanocobalamin, except at pH values between 1 and 2. On reinvestigating thoroughly the electrophoretic mobility of cyanocobalamin in this pH-region, we found that the isoelectric point of this compound is somewhat lower than pH 1.9, as was first stated, and occurs at pH 1.5. Factor III has its isoelectric point at the same pH-value. The variation of

electrophoretic mobility with pH for both Factor III and cyanocobalmin is shown in Fig. 1. It can be seen that the electrophoretic mobility-pH-curves of these two compounds are for all practical purposes identical.

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